Neuroimaging in obsessive-compulsive and related disorders: investigation of the frontal-striatal and limbic circuits

van den Heuvel, O.A.

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Odile A. van den Heuvel

The studies described in this thesis were performed at the VU University medical center (PET Center and MR department) and GGZ Buitenamstel (outpatient department for anxiety disorders), Amsterdam, The Netherlands.

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Neuroimaging in obsessive-compulsive and related disorders: investigation of the frontal-striatal and limbic circuits

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. T. Sminia,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der Geneeskunde
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De Boelelaan 1105

door

Odile Antoinette van den Heuvel

geboren te Saverne, Frankrijk

promotoren:	prof.dr. R. van Dyck prof.dr. A.A. Lammertsma
copromotoren:	prof.dr. H.J. Groenewegen dr. D.J. Veltman

voor Ada

Working in the field of neuroimaging is just like looking for a flower in a meadow.

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Outline of the thesis

The main objectives of this thesis were to investigate whether dysfunctions of the frontal-striatal and limbic circuits are basic to the emotional and cognitive processes underlying the symptomatology of obsessive-compulsive disorder (OCD) and to determine the disorder-specificity of these abnormalities. To address these issues, task related changes in regional cerebral blood flow (rCBF) and blood oxygenation level dependent (BOLD) signal were measured using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), respectively. In order to investigate the psychopathological processes of the disorder, three experimental paradigms were designed addressing three different mental states: 1) emotional perception during provoked contamination fear, 2) higher-order cognitive function during executive performance, and 3) the interaction between emotional and cognitive processes during a paradigm addressing attentional bias. Brain activation patterns in unmedicated OCD patients were compared with those in both healthy control subjects and patients with related disorders, such as panic disorder (PD) and hypochondriasis.

As will be described in part I, **chapters 2 and 3**, various neuroimaging techniques are available for investigating neuropsychiatric disorders. Each modality has its own specific applicability in the search for the underlying mechanisms of the various mental processes of interest. Both strengths and limitations of these techniques should be taken into account in choosing a modality for a specific experiment. As in most symptom provocation experiments, in the present thesis, oxygen-15 labeled water ($H_2{}^{15}O$) and PET were used to visualize the neuronal correlates of contamination fear. Due to the radioactive half-life of 2 minutes, the interscan interval must be at least 8 to 10 minutes. Acquisition of subsequent PET scans (each of about 90 seconds) in this typical on-off schedule is useful to capture relatively slow processes such as induced symptomatic states. Also, the obligatory interscan intervals enable recovery from the provoked emotional response between experimental conditions.

In contrast to symptom provocation studies, most neuropsychological paradigms require the higher temporal resolution of fMRI. Functional MRI enables separate analysis of a large amount of subsequent events, i.e. short mental states in a specific experimental condition. A whole brain echo-planar imaging (EPI) acquisition generally takes 2 to 3 seconds. During a neuropsychological task of about 15 minutes, usually 200 to 450 images can be acquired. In this way, fMRI is a powerful technique to compare relatively fast task related changes in BOLD signal, both within and between study groups of interest.

Chapters 2 and 3 provide recent overviews of the existing neuroimaging literature in OCD and PD, respectively. These reviews contain both early studies and recent findings, incorporating the experimental results that are described more extensively in part III of this thesis. Therefore, these chapters should be seen as state-of-the-art presentations of the field at the present time rather than at the start of the experimental work described in this thesis (2000). These reviews clearly show the rapid developments within this field and they further illustrate the frontal-striatal and limbic hypothesis.

Before describing OCD specific experiments, part II of this thesis addresses two methodological issues that required attention prior to analyzing patient data.

During the analyses of the PET data, it became clear that subject motion could introduce significant artifacts. In fact, in a first-pass analysis, inaccurate attenuation correction due to motion induced misalignment between transmission and emission scans appeared to be responsible for (erroneous) task related changes in regional activity concentrations. To further investigate this issue an alternative method for attenuation correction, the image registration (IR) method, was implemented and evaluated. Both in phantoms and in humans, the accuracy of the IR method was compared with that of other methods for attenuation correction. The results are given in **chapter 4**.

In case of fMRI an almost infinite reservoir of neuropsychological tasks can be used for investigating various emotional and/or cognitive processes. Most of these tasks have been developed for application in healthy control subjects. An important methodological issue is to adjust these neuropsychological paradigms to psychiatric study populations. Comparison of task-related activation patterns between different groups requires a sensitive paradigm that specifically addresses the function of interest and incorporates a correction for differences in task performance. A self-paced, event-related and parametric design allows for flexibility in response, separate analysis of false and correct events, and comparisons between subjects or groups at each task level. This results in increased comparability across groups. In **chapter 5**, the design of a parametric self-paced pseudo-randomized event-related version of a planning task is presented.

Part III of this thesis describes the background and results of the experiments performed in OCD patients. This part addresses the neuronal correlates of normal and pathological, emotional and cognitive processes during three induced mental states. Task related activation patterns in medication-free OCD patients are compared with those in both healthy and psychiatric control groups.

In order to visualize the neural correlates of the symptomatic state in OCD patients with contamination fear, a $H_2{}^{15}O$ PET symptom provocation experiment was performed. This study, as a replication of earlier provocation designs, was meant to contribute to a better understanding of previous findings that were inconsistent due to various methodological concerns. A randomized block design with standardized visual presentations of ‘dirty’ and ‘clean’ surroundings was used to compare the task-related changes in rCBF in a homogeneous group of medication-free OCD patients with those in healthy control subjects. The results are given in **chapter 6**.

Although dysfunctions in the frontal-striatal system seemed to be implicated in the symptomatic state of OCD, little was known about its role in the accompanying cognitive impairments. Therefore, one objective was to investigate the flexibility of the frontal-striatal system in OCD during a higher-order executive, but emotionally neutral, task. A parametric fMRI version of a planning task was used to increase the specificity of the experimental effects of interest and to control for differences in task performance between groups. Task-related changes in BOLD signal were measured in medication-free OCD patients, and compared with those in healthy control subjects. In **chapter 7**, findings and implications are presented and discussed.

A third experimental approach to unravel the altered functioning of the frontal-striatal and limbic systems in OCD is through the investigation of emotional interference processes. Attentional bias to disease related information is considered to be

pathogenetically important in anxiety disorders. The critical process of 'gating' (i.e. selectively attend to relevant stimuli, whilst simultaneously ignoring irrelevant competing stimuli) has been linked to frontal-striatal function. The other element, emotional appraisal of information, has been linked to limbic function. Using an fMRI version of the cognitive and emotional Stroop task, the neuronal correlates of attentional bias in OCD, PD and hypochondriasis were investigated. This comparison between related disorders might contribute to a better understanding of the disorder-specificity of the abnormalities. The results are described in **chapter 8**.

The last chapter of this thesis (**chapter 9**) discusses strengths and limitations of the present findings. In addition, it addresses implications for future research in this field.

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Neuroimaging in obsessive-compulsive disorder

Remijnse PL, van den Heuvel OA, Veltman DJ

Submitted

Abstract

Obsessive-compulsive disorder (OCD) is a neuropsychiatric condition that is characterized by intrusive anxiety-provoking thoughts (obsessions) and subsequent repetitive behaviors (compulsions). Neurobiological models of OCD have emphasized the mediation of clinical symptoms by aberrant frontal-striatal circuits in addition to dysfunctional neurotransmitter interactions, i.e. serotonergic-dopaminergic and glutamatergic-serotonergic. These models were mainly based on preclinical animal and laboratory reports as well as lesion studies and early imaging paradigms. Recently, a neurobiological model for normal human emotion perception has been described, proposing distinct ventral and dorsal processing systems corresponding with differential levels of emotion perception. This construct appears relevant for elucidating the pathophysiology of OCD, as OCD is classified as an anxiety disorder.

In the present paper we present a comprehensive review of the neuroimaging literature in OCD with the aim of critically appraising the support provided for current pathophysiological models, as well as relating these findings to constructs of normal emotion perception. Traditionally, neuroimaging designs in OCD included structural studies in search of morphological abnormalities, resting state measurements of cerebral blood flow and metabolism, and symptom provocation designs. Over the last few years, sophisticated functional neuroimaging paradigms have been introduced using cognitive and/or emotional paradigms; also, radioligands have become available to assess receptor distribution and affinity. These recent study designs are more hypothesis-driven and better respect the heterogeneity of OCD, increasing their sensitivity and specificity for elucidating the pathophysiological substrate of this disorder.

Results of imaging studies point to the need for adjustments in current models with respect to the involvement of frontal-striatal circuits in OCD. In line with recent insights into the neurobiology of emotion processing, a ventral-dorsal dissociation may be distinguished. Moreover, data from pediatric OCD patient samples have commenced to shed light on developmental characteristics of OCD. Furthermore, longitudinal designs from early into later life, in combination with pre-post treatment comparisons, are of great value for assessing the state-trait duality. Finally, the use of multimodal study designs holds great promise for the near future.

Introduction

Obsessive-compulsive disorder (OCD) is a neuropsychiatric condition that is characterized by intrusive anxiety-provoking thoughts (obsessions) and subsequent repetitive behaviors (compulsions)[1]. Lifetime prevalence rates of this often debilitating disorder as found in recent epidemiological studies range from 0.5%[2] to 2.9%[3] in the general population.

The role of neurobiological factors in the etiology of OCD was first suggested by descriptions of obsessive-compulsive behavior in patients with subcortical disorders, e.g. Huntington's disease[4], Sydenham's chorea[5], and pallidal lesions[6], and in patients with frontal lobe lesions[7]. Based on such observations, as well as on more recent functional neuroimaging data, several neurobiological models for OCD have been proposed[8-10]. Although differing on details, all these models emphasize the mediation of OCD symptoms by hyperactivity of frontal-striatal circuitry. In brief, cortical regions, mainly the orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC), and striatal structures are presumed to be captured in a hyperfunctional, self-perpetuating loop[9]. In this hyperactive circuit, the prefrontal regions putatively subserve enhanced 'error detection', corresponding to the patients' characteristic sense that 'something is wrong'. The striatum is supposedly engaged in abnormal 'gating', relating to the selection and generation of aberrant behavioral patterns on the basis of erroneous information from cortical areas. Moreover, the dysfunctional striatum may allow the emergence of repetitive behavioral patterns that have a reinforcing function in alleviating distress caused by obsessional thoughts. Possibly, the striatum exerts this pathological function due to overactive dopaminergic projections to this structure[8;10]. Other neurotransmitters hypothesized to be involved in the pathophysiology of OCD are serotonin through its inhibitory interactions with dopamine, and glutamate by means of its excitatory role in frontal-striatal connections and its interactions with serotonin[11].

Although these models stress the involvement of para-limbic regions such as OFC and ACC in the pathophysiology of OCD, they do not attribute a role to key limbic structures, i.e. the insula and amygdala. This is surprising given the fact that OCD is considered an anxiety disorder and the amygdala is assumed to be the central component of fear processing[12]. A recent neurobiological model for human emotion processing[13;14] proposed several levels of emotion perception with corresponding neuronal correlates: first, the identification of the emotional significance of a stimulus and, subsequently, the production of an affective state. These subprocesses were assumed to be associated with a *ventral* neuronal system consisting of the amygdala, insula, ventral striatum and ventral regions of the prefrontal and cingulate cortices. Second, the regulation of the affective state was hypothesized to be represented by a *dorsal* neuronal pathway, i.e. hippocampus and dorsal regions of prefrontal and cingulate cortices. The authors used this model to describe affective symptoms in four main psychiatric disorders[14]. However, OCD was not among these disorders, leaving a lacuna to be filled.

In this article, we intend to contribute to the existing literature on the pathophysiology of OCD by reviewing all available neuroimaging papers in OCD, categorized according to the applied technique or paradigm. First, we summarize the results of structural studies, after which we discuss resting state functional imaging studies. Next, symptom provocation

designs are discussed followed by cognitive paradigms and, finally, ligand and magnetic resonance spectroscopy (MRS) studies. In each paragraph, we aim to not only review the results but also critically appraise methodological issues, and discuss the possibilities and limitations of the imaging technique or paradigm at stake for unraveling the neurobiology of OCD. A final discussion will integrate the findings, discuss the implications for the above-mentioned emotion processing model[13] and neurobiological models of OCD, and provide some directions for future research.

Structural imaging studies

Findings of structural imaging studies in OCD are summarized in Table 2.1. The first studies suggesting brain abnormalities in OCD were based on either *qualitative* evaluation of computed tomography (CT) scans, or measurements of whole brain volume and ventricle-to-brain ratios VBR[15;16]. Some years later, Luxenberg et al.[17] used CT for measurements of the striatum in a small sample of early-onset male OCD patients and healthy controls, reporting decreased bilateral caudate volumes in OCD. Subsequent volumetric studies in OCD, which were mainly based on magnetic resonance imaging (MRI) data, restricted their data analysis to the striatum. Variability of neuroanatomical criteria for delineation of regions of interest (ROIs), used in most of these studies[18-26], may partly explain inconsistent results obtained in these studies. Whereas results of Robinson et al.[21] supported the finding of decreased striatal volumes, also normal[18;20;22-26] or even increased[19;27] striatal volumes have been reported.

More recent MRI studies expanded the number of ROIs or performed whole brain data acquisition and analysis. These studies, however, revealed similarly inconclusive results. Concerning the ventral regions of the prefrontal cortex, including the ACC, increased[26;28;29] as well as decreased[27;30;31] volumes have been reported. Thalamic volumes were increased in medication-naïve pediatric OCD patients, normalizing after paroxetine treatment[32], as well as in adult OCD[29]. Both enlarged and diminished volumes were also found for temporal lobe structures such as the amygdala and hippocampus[28;30;33;34].

An alternative method of volumetric analysis of MR images is voxel-based morphometry (VBM)[35]. In VBM, structural images are segmented on a voxel-by-voxel basis and subsequently warped to anatomical standard space, so that individual volumetric differences will appear as differences in gray matter density. Potential confounds in VBM include differences in white matter/gray matter contrast between groups, and misclassification of voxels due to partial volume effects[36]. On the other hand, time-consuming drawing of ROIs, with its often low interrater reliability, can be avoided; moreover, VBM is a useful technique to assess subtle differences in gray matter concentrations at a microstructural level. So far, only 2 studies have used VBM in OCD[29;37]. Comparing 25 OCD patients with 25 healthy control subjects, Kim et al.[29] found increased gray matter densities in left OFC, left thalamus and bilateral hypothalamus in OCD patients. Gilbert et al.[37] investigated the relationship between age and gray matter concentrations in 21 children with OCD and 15 control subjects. A negative correlation was found in the dorsolateral prefrontal cortex (DLPFC) in controls, reflecting a physiological

Table 2.1. Structural imaging studies in OCD

Authors (public. date)	*Technique*	*Subjects*	*Main findings*	*Methodological comments*
Gilbert et al. (2004)[37]	MRI	21 pediatric OCD vs 15 co	Negative correlation between age and gray matter concentration in DLPFC in controls, not in OCD.	VBM
Pujol et al. (2004)[27]	Whole brain MRI	72 OCD vs 72 co	Decreased volume of medial PFC, OFC and L insulo-opercular region, increased volume bilateral ventral striatum and anterior cerebellum.	
Szeszko et al. (2004)[26]	MRI	23 pediatric OCD vs 27 co	Decreased volume L pallidum and increased gray matter volume of bilateral ACC.	ROI method (caudate, putamen, pallidum, ACC), comorbid diagnoses
Szeszko et al. (2004)[33]	Whole brain MRI	11 pediatric OCD vs 11 co (before vs after paroxetine)	At baseline (drug-naive): asymmetry of amygdala (L>R) in OCD, not in co. Post vs pre-treatment: decreased volume of L amygdala, correlation with paroxetine dosage.	Limited sample size
Choi et al. (2004)[31]	MRI	34 OCD vs 34 co	Decreased volume L anterior OFC, correlated with decreased performance on Rey-Osterrieth Complex Figure test.	ROI method (OFC), educational bias
Kwon et al. (2003)[34]	MRI	22 OCD vs 22 co vs 22 schizophrenia	Decreased volume bilateral hippocampus (in both OCD and schizophrenia), increased volume L amygdala (only in OCD).	ROI (hippocampus, amygdala, thalamus) instead of whole brain
Kim et al. (2001)[29]	MRI	25 OCD vs 25 co	Increased gray matter density in L OFC, L thalamus and bilateral hypothalamus, decreased in L cerebellum and cuneus.	VBM
Gilbert et al. (2000)[32]	MRI	21 pediatric OCD vs 21 co (before vs after paroxetine)	At baseline: increased thalamic volume. After paroxetine treatment: normalization of thalamic volume, correlated with reduction in symptom severity.	ROI (thalamus), treatment effect based on limited sample size (N=10)
Szeszko et al. (1999)[30]	MRI	26 OCD vs 26 co	Decreased volumes of bilateral OFC and amygdala in OCD; hemispheric asymmetry of hippocampal-amygdalar complex in co, not in OCD.	ROIs instead of whole brain.

Table 2.1 (continue)

Authors (public. date)	*Technique*	*Subjects*	*Main findings*	*Methodological comments*
Rosenberg et al. (1998)[28]	MRI	21 pediatric OCD vs 21 co	Increased volume ACC, normal volumes DLPFC, posterior cingulate, amygdala, hippocampus and temporal cortex. Negative correlation (trend) between age and ACC volume in controls, not present in OCD.	ROI method (OFC, DLPFC, ACC, post. cingulate, temporal cortex, hippocampus, amygdala)
Bartha et al. (1998)[25]	MRI / MRS	13 OCD vs 13 co	Normal striatal volumes, but decreased levels of N-acetylaspartate in L striatum.	Limited sample size, ROI method (striatum)
Stein et al. (1997)[24]	MRI	13 OCD vs 12 co vs 17 trichotillomania	Normal striatal volumes, but positive correlation between striatal volume and performance on neuropsychological tests (Rey-Osterrieth Complex Figure test and Stroop test).	Limited sample size, ROI method (striatum)
Rosenberg et al. (1997)[23]	MRI	19 treatment-naïve pediatric OCD vs 19 co	Decreased volumes of putamen, normal caudate volumes, larger third ventricles, inverse correlation between volume of putamen and OCD symptom severity.	ROIs (striatum, PFC, ventricles)
Jenike et al. (1996)[38]	MRI	10 female OCD vs 10 female co	Decreased total white matter, but increased total cortex and operculum volumes.	Limited sample size
Aylward et al. (1996)[22]	MRI	24 OCD vs 21 co	Normal caudate volume.	ROI method (striatum)
Robinson et al. (1995)[21]	MRI	26 OCD vs 26 co	Decreased bilateral caudate volumes.	ROIs (striatum, PFC, ventricles)
Stein et al. (1993)[20]	CT	8 OCD(+HSS) vs 8 OCD(+LSS) vs 8 co	Increased ventricular volumes in OCD(+HSS) compared to OCD(+LSS) and controls. Normal caudate volumes.	ROIs (striatum, ventricles)
Scarone et al. (1992)[19]	MRI	20 OCD vs 16 co	Increased volume of R caudate nucleus.	ROI method (striatum), medicated patients.
Kellner et al. (1991)[18]	MRI	12 OCD vs 12 co	Normal caudate volume.	Limited sample size, ROIs (striatum, ACC, corpus callosum region), medicated patients
Garber et al. (1989)[144]	MRI	32 OCD vs 14 co	Prolonged T_1 relaxation time in right frontal cortex, R>L asymmetry of OFC	Medicated patients

Table 2.1 (continue)

Authors (public. date)	*Technique*	*Subjects*	*Main findings*	*Methodological comments*
Luxenberg et al. (1988)[17]	CT	10 male early-onset OCD vs 10 male co	Decreased bilateral caudate volumes.	ROI method (striatum, ventricles), limited sample size, age biased.
Behar et al. (1984)[16]	CT	17 adolescent OCD vs 16 co	Ventricular enlargement.	VBR, comorbid major depression
Insel et al. (1983)[15]	CT	10 OCD vs 10 co	Normal VBR, normal R-L asymmetry, no cortical atrophy.	VBR

Table 2.1, 2.2, 2.3 and 2.4: All main findings reported for OCD versus controls, unless indicated otherwise.
Abbreviations: ACC = anterior cingulate cortex, BD = bipolar disorder, BDI = Beck Depression Inventory, bil = bilateral, BT = behavioral therapy, CBT = cognitive behavioral therapy, CPT = continuous performance task, CT = computed tomography, CGI = Clinical Global Impression scale, co = controls, DLPFC = dorsolateral prefrontal cortex, ECD = ethyl-cisteinate-dimer, FDG = fluorodeoxyglucose, GAF = Global Assessment of Functioning, HMPAO = hexamethyl propyleneamine-oxime, IFG = inferior frontal gyrus, inf = inferior, L = left, lat = lateral, MDD = major depressive disorder, med = medial, OCD = obsessive compulsive disorder, OCD(+HSS) = OCD patients with high soft signs scores, OCD(+LSS) = OCD patients with low soft signs scores, OFC = orbitofrontal cortex, PD+A = panic disorder with agoraphobia, PET = positron emission tomography, PFC = prefrontal cortex, post = posterior, PTSD = posttraumatic stress disorder, R = right, rCBF = regional cerebral blood flow, ROI = regions-of-interest, SPECT = single-photon emission computed tomography, SSRIs = selective serotonin reuptake inhibitors, sup = superior, Tc-exametazime = Technetium-exametazine, temp = temporal, VBM = Voxel based morphometry, VBR = ventricle-to-brain ratio, VLPFC = ventrolateral prefrontal cortex, vs = versus, Xe = xenon, Y-BOCS = Yale-Brown Obsessive Compulsive Scale

age-related decrease in gray matter density. This correlation was absent in OCD patients, suggesting a neurodevelopmental abnormality.

During normal development of the brain neurons, dendrites, axons and synapses are first produced in excess, after which some are maintained while others are eliminated. This process of normally programmed cell death during brain development yields a decreasing ratio of gray to white matter. Increased gray matter density in specific regions of the frontal-striatal circuits in OCD patients compared with healthy control subjects may reflect a failure in synaptic proliferation or a defect in pruning processes. In 1996, Jenike and colleagues[38] were the first to report that cortex-to-white matter ratio was larger in OCD compared with controls. In addition to the search for subtle gray matter abnormalities, VBM might be used to detect white matter changes in the frontal-striatal pathways[39]. The investigation of normal and abnormal brain maturation in very young children with and without OCD may contribute to a better understanding of the role of neurodevelopmental processes in the pathophysiology of OCD. Longitudinal follow-up of these children into adolescence and adulthood enables visualization of natural history as well as the evaluation of long-term effects of treatment strategies.

To conclude, so far results of structural imaging studies in OCD have shown that morphological abnormalities, if present, are subtle. More refined analysis of whole brain images, including measurements of gray as well as white matter densities, is needed in homogeneous large samples. Accumulating evidence suggests that structural changes in OCD occur early in life, necessitating longitudinal follow-up of pediatric cohorts. Moreover, to understand the functional implications of structural changes, multi-modal imaging investigations are warranted. For instance, altered morphology may be related to an imbalance of interacting neurotransmitter systems[28]. Study designs combining MRS and VBM in pediatric OCD appear promising to investigate this issue. In addition, neuropsychological dysfunction and altered responsiveness of frontal-striatal circuits during functional MRI paradigms might be explained, at least partly, by subtle gray and/or white matter changes in frontal-striatal pathways. Therefore, results of functional imaging studies need to be corrected for possible morphological differences. Moreover, comparisons between OCD patients, their first-degree relatives and non-related control subjects may elucidate an underlying genetic predisposition. Finally, morphological characteristics, suggestive for neurodevelopmental abnormalities, may be used as endophenotypes for subsequent genetic investigations.

Resting-state studies

Resting-state studies aim to acquire data of basal brain metabolism or regional cerebral blood flow (rCBF) in patients while being in a neutral state. Resting-state studies have mainly been performed with the use of 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET) or Technetium-99m hexamethyl propyleneamine-oxime (99mTc-HMPAO) single photon emission computed tomography (SPECT), or inhalation of xenon-133 (133Xe) during SPECT. Results of these studies in OCD are summarized in Table 2.2.

Compared with healthy controls, these studies have frequently shown increased metabolism or rCBF for OCD patients in the OFC, either bilaterally[40-42], left-lateralized[43;44] or right-lateralized[45;46], and in bilateral[47;48] or left[44] ACC, left frontotemporal cortex[42], left insula[45] and right dorsal parietal cortex[41]. Also, elevated metabolism and rCBF have often been found in subcortical structures, such as bilateral[46;48-50] or right[42;44] thalamus, bilateral[40;43] or right[50] head of caudate nucleus, and bilateral putamen/pallidum[48;51]. These findings point toward hyperactive frontal-striatal circuitry in mediating OCD symptoms. This assumption is supported by frequently observed positive correlations between these metabolic alterations and symptom severity in OCD patients[41;45;46;52]. Also in line with this hypothesis is that functional correlations between these areas tend to diminish after successful treatment, as has been repeatedly demonstrated[53-55].

Conflicting findings have also been reported: *decreased* metabolism and rCBF have been found in the above mentioned structures as well, for the right OFC[52;56], left ACC[52], left lateral temporal cortex[56], left parietal cortex[45;56], bilateral[41] or right[56-58] caudate nucleus, bilateral putamen[57] and right[56] thalamus. Some of these functional abnormalities in this opposite direction were also correlated with clinical OCD rating scores[52;56]. However, metabolism and rCBF have been consistently found to be decreased in the bilateral dorsolateral/superior prefrontal cortex[56;58;59].

Differences between these studies with respect to the identified structures and to the direction of aberrant activity in OCD subjects may be due to several methodological issues, concerning both patient characteristics and technical (imaging) aspects. With regard to the former, the inclusion of OCD patients that used antiobsessional medication in some studies may have presented a confound[43;56;57;59]. Notably, in a recent pre-treatment to post-treatment functional neuroimaging study in OCD, the use of medication itself was found to induce brain metabolic alterations over time[60]. This implies that the concomitant use of medication in the above mentioned early resting-state studies might have confounded the results on cerebral metabolism or rCBF. Indeed, in several of these studies additional analyses were performed excluding medicated patients, which sometimes led to (slightly) different outcomes.

Another confounder in differentiating OCD and healthy subjects may have been the use of patient samples with concurrent major depression[43;50]. The exclusion of major depression in OCD for the comparison with controls is important, as depression rating scores in OCD patients have been found to be strongly correlated with decreased metabolism in right caudate, bilateral thalamus, bilateral amygdala and left hippocampus[49]. In support of this finding, Busatto et al.[52] demonstrated that diminished rCBF in right lateral OFC in OCD subjects relative to controls disappeared after excluding patients with concomitant major depression.

A final factor with regard to patient characteristics that may have skewed findings between patients and controls across resting-state studies is the inclusion of OCD groups differing on clinical subdimensions. These subdimensions are likely to be associated with distinct neurophysiological correlates[50;61;62]. However, most resting-state studies do not provide information on the composition of their patient samples in this respect, so that differences in patients' symptom dimensions across studies may have accounted for discrepant findings.

Table 2.2. Resting state imaging studies in OCD

Authors (public. date)	*Technique*	*Subjects*	*Main findings*	*Methodological comments*
Saxena et al. (2004)[50]	FDG PET	12 hoarding OCD vs 33 non-hoarding OCD vs 17 co	Increased metabolism in bil thalamus and R caudate in non-hoarding vs hoarding OCD and controls. In hoarding OCD: decreased metabolism in post cingulate vs normals, decreased metabolism in bil dorsal ACC vs non-hoarders.	ROI and voxel-by-voxel, comorbid depression in hoarding and non-hoarding OCD groups
Lacerda et al. (2003)[46]	HMPAO SPECT	16 OCD vs 17 co	Increased rCBF in R sup and R inf PFC and bil thalamus. Positive correlation CGI and R inf PFC	ROI
Nakatani et al. (2003)[51]	Xe-CT	31 OCD (before and after CBT) vs 31 co	Increased rCBF in bil putamen/pallidum. After successful CBT: decrease in rCBF in R caudate nucleus, correlated with functional improvement (GAF score).	ROI, no rescanning of control group
Kang et al. (2003)[67]	FDG PET	10 OCD (before vs after SSRI)	Follow-up of Kwon et al. (2003). Decreased rCBF in bil OFC, R hippocampus and R putamen, increased rCBF in post (parietal-occipital) regions.	Voxel-by-voxel, no control group, medication as a confounder not excluded
Kwon et al. (2003)[45]	FDG PET	14 OCD vs 14 co	Increased metabolism in R OFC and L insula, decreased metabolism in parietal-occipital cortex. Positive correlation metabolism in L putamen, R hippocampus and R parietal cortex with Y-BOCS scores.	Voxel-by-voxel
Saxena et al. (2002)[60]	FDG PET	25 OCD vs 25 MDD vs 16 OCD+MDD (before vs after paroxetine) vs 16 co	Follow-up of Saxena et al. (2001). In OCD responders: decreased metabolism in R caudate and R putamen. In OCD (responders+non-responders): decreased metabolism in R VLPFC, bil OFC and thalamus.	ROI and voxel-by-voxel
Saxena et al. (2001)[49]	FDG-PET	27 OCD vs 27 MDD vs 17 OCD+MDD vs 17 co	In OCD+MDD: decreased metabolism in L hippocampus. In OCD: increased metabolism in bil thalamus. In all OCD patients: negative correlation between depression scores and activity in subcortical structures.	ROI and voxel-by-voxel
Hoehn-Saric et al. (2001)[66]	HMPAO SPECT	16 OCD+MDD (before vs after sertraline or desipramine)	At baseline: increased rCBF in PFC, ACC and basal ganglia in eventual responders vs. non-responders. Decreased rCBF after treatment diffusely in PFC in responders, not in non-responders.	Voxel-by-voxel, no control group, post-treatment changes not controlled for changes in depression severity

Table 2.2 (continue)

Authors (public. date)	*Technique*	*Subjects*	*Main findings*	*Methodological comments*
Alptekin et al. (2001)[42]	HMPAO SPECT	9 OCD vs 6 co	Increased rCBF in bil OFC, R thalamus and L fronto-temporal cortex.	ROI, limited sample sizes
Busatto et al. (2000)[52]	ECD SPECT	26 OCD vs 22 co	Decreased rCBF in R lateral OFC and L dorsal ACC. Y-BOCS scores positively correlated with L lat OFC and R med OFC activity.	Voxel-by-voxel, 8 OCD subjects with concomitant MDD
Saxena et al. (1999)[65]	FDG PET	20 OCD (before vs after paroxetine)	Decreased metabolism in R anterolateral OFC and R caudate in responders vs. non-responders. Lower pretreatment metabolism in L+R OFC predicted greater improvement in OCD severity with treatment.	ROI
Brody et al. (1998)[70]	FDG PET	27 OCD in prediction study; 18 treated with BT, 9 with fluoxetine	Higher pretreatment metabolism in L OFC associated with greater clinical improvement in BT group, yet worse outcome in medication group.	ROI, small N in medication group, no random assignment to treatment groups
Lucey et al. (1997)[58]	HMPAO SPECT	15 OCD vs 15 PD+A vs 16 PTSD vs 15 controls	Extension of Lucey et al. (1995). Reduced rCBF in bil sup frontal cortices and R caudate for OCD and PTSD compared to PD and controls. BDI scores (highest in PTSD and OCD) correlated negatively with caudate metabolism.	ROI, BDI ratings not excluded as possible confounder for caudate hypometabolism.
Schwartz et al. (1996)[54]	FDG PET	9 OCD (also combined with 9 OCD from Baxter et al., 1992) after CBT	Replication from Baxter et al. (1992): decrease in L+ R caudate after successful BT, correlated with improved Y-BOCS scores on left side. Before treatment: significant correlations between OFC/Cd/thalamus on the right side.	ROI, no control group
Lucey et al. (1995)[56]	HMPAO SPECT	30 OCD vs 30 controls	Lower rCBF in R inf frontal cortex (including some of the OFC), R+L sup frontal cortex (including DLPFC), R caudate, L lat temp, L parietal and R thalamus. Lower rCBF in R inf frontal cortex correlated with Y-BOCS scores.	ROI, small number of patients medicated (N=8)
Perani et al. (1995)[48]	FDG PET	11 OCD (9 of whom rescanned after SSRIs) vs 15 controls	Pretreatment: increased activity in cingulate, putamen and thalamus. Pre- to post-treatment: decrease in cingulate metabolism, correlated with improved Y-BOCS scores.	ROI, no rescanning of controls

Table 2.2 (continue)

Authors (public. date)	*Technique*	*Subjects*	*Main findings*	*Methodological comments*
Rubin et al. (1995)[55]	Xe inhalation and HMPAO SPECT	8 OCD pre- and post-treatment (clomipramine) vs 8 co	Follow-up of Rubin et al. (1992): For HMPAO pre- to post-treatment: decline in R dorsal parietal, L postero-frontal, bil OFC activity, persistent decrease in bil caudate	ROI, no rescanning of controls, confounding medication effect not excluded
Edmonstone et al. (1994)[57]	Tc-exemetazime SPECT	12 OCD vs 12 MDD vs 12 controls	Decreased uptake in bil putamen and R caudate in OCD vs other groups. Inverse relationship between anxiety ratings and reduced basal ganglia uptake.	ROI, medicated subjects
Baxter et al. (1992)[53]	FDG PET	9 OCD before and after fluoxetine or BT vs 9 co	Significant decrease in R caudate metabolism in responders to fluoxetine and BT, correlated with decrease in Y-BOCS scores for medication group.	ROI, limited sample size, no direct comparisons between patients and controls
Swedo et al. (1992)[64]	FDG PET	13 OCD after drug treatment	Follow-up of Swedo et al. (1989). Decreased metabolism in R+L OFC after therapy. Lower caudate metabolism at baseline predicted better long-term outcome. Change in R OFC correlated with improved OCD symptom scores.	ROI, no control group rescanned
Rubin et al. (1992)[41]	Xe inhalation and HMPAO SPECT	10 OCD vs 10 co	Increased uptake of HMPAO in R dorsal parietal, bil OFC and L postero-frontal. Decreased uptake in bil caudate. Positive correlation between rCBF and symptom severity.	ROI, no rating of depressive symptoms in patients
Hoehn-Saric et al. (1991)[68]	HMPAO SPECT	6 OCD after therapy with fluoxetine	Extension of Machlin et al. (1991). Decrease in relative medial frontal uptake after successful therapy, accompanied by a significant decrease in OCD symptoms.	ROI, control group not rescanned, depressive symptoms not rated
Machlin et al. (1991)[47]	HMPAO SPECT	10 OCD vs 8 co	Increased rCBF in relative medial frontal uptake (including PFC regions and ACC), negatively correlated with Hamilton Anxiety scores. No difference for OFC.	ROI, only prefrontal areas assessed
Benkelfat et al. (1990)[63]	FDG PET	8 OCD before and during clomipramine while performing CPT	Follow-up of Nordahl et al (1989). Decreased metabolism in R OFC and L caudate during successful treatment, correlated with improved OCD severity ratings.	ROI, no rescanning of control group
Martinot et al. (1990)[59]	FDG PET	16 OCD vs 10 co	Decreased absolute metabolism in whole brain, OFC, DLPFC, medial frontal PFC, temp cortex, striatum, thalamus. Decreased relative metabolism in DLPFC.	ROI, partly medicated patient sample.

Table 2.2 (continue)

Authors (public. date)	*Technique*	*Subjects*	*Main findings*	*Methodological comments*
Baxter et al. (1989)[145]	FDG PET	14 OCD vs 10 MDD vs 10 OCD+MDD vs10 BD vs 6 mania vs 12co	OCD=controls > MDD=BD in L + R DLPFC activity. OCD+MDD < OCD in L DLPFC activity.	ROI, only DLPFC assessed
Swedo et al. (1989)[44]	FDG PET	18 OCD vs 18 co	Increased absolute metabolism L+ R prefrontal, L orbital frontal, L premotor, R inf temp, R thalamus, L+R ACC. Increased relative metabolism in R prefrontal and L ACC.	ROI, no anatomic specification of 'prefrontal' regions
Baxter et al. (1988)[40]	FDG PET	10 OCD vs 10 co	Increased metabolism in bil hemispheres, bil heads of caudate, bil orbital gyri, bil orbital/hem ratio.	Except for OFC, only absolute metabolic rates reported
Baxter et al. (1987)[43]	FDG PET	14 OCD (10 restudied after treatment with trazodone) vs 14 MD vs 14 co	Increased absolute uptake in bil heads of caudate nuclei, bil orbital gyri, and increased relative uptake in L orbital gyrus, in OCD vs controls. After successful treatment: further increase in R caudate/hem ratio in OCD.	Comorbid MD in OCD group

Next to patient inclusion factors, imaging methodologies differ across resting-state studies and thus may also explain divergent findings. Such differences are the reporting of absolute versus relative metabolic or rCBF rates, the use of ROIs versus voxel-by-voxel analysis, the various ways of outlining neuroanatomical structures, and the determination of sites, sizes and shapes of the applied ROIs. A clear example of how such factors may influence comparability of results between studies has been provided by Aylward and colleagues[22]. These authors investigated if previously reported differences between OCD and healthy subjects in caudate metabolism[40;43;48;59] merely reflected the use of absolute versus relative metabolic rates. They concluded that differences between OCD and normal subjects disappeared after correcting caudate metabolic rates for differences in whole brain glucose uptake.

Changes in activity after treatment

Apart from cross-sectional designs, resting-state studies have been conducted longitudinally in OCD patients, i.e. at baseline and following treatment, either with antidepressant medication or cognitive behavioral therapy (CBT). These studies point towards a decrease in baseline-elevated metabolism or rCBF in the OFC[55;60;63-67], ACC[48;66;68], caudate nuclei[51;53;54;60;63;65;67], putamen[60;67] and thalamus[60]. These changes were found both after medication and CBT. Importantly, these neuronal differences at follow-up were significantly greater in responders compared with non-responders[60;65;66] and/or were associated with decreases in OCD symptom severity[48;53-55;63;64;68] or in general functioning[51]. However, discrepant findings with regard to post-treatment metabolic rates in OCD patients have also been reported, such as a further increase in pretreatment elevated right caudate metabolism after trazodone[43], an increase in right putamen metabolism[63] and a decrease in bilateral caudate glucose uptake[55] both before and after clomipramine treatment.

Despite these few discordant findings, the overall impression is that improvement of OCD symptoms is accompanied by a normalization of frontal-striatal circuit activity. Two methodological issues need to be pointed out, however. First, except for two studies[53;60], in these longitudinal designs the control group was not rescanned, so that treatment effects were potentially confounded with time (order) effects. However, session to session variability for metabolic or rCBF alterations is likely to be small, as shown for HMPAO SPECT[69] and FDG PET[53] in healthy subjects. Second, in some of these longitudinal studies, the use of medication[55;67] or the improvement in comorbid depression[66] cannot be excluded as a confounder for post-treatment brain activity alterations.

Finally, resting-state studies have been used to predict treatment response. Swedo et al.[64] found that lower pre-treatment metabolism in right OFC and right ACC predicted better short-term outcome on clomipramine (2 months later), whereas lower left caudate activity predicted better long-term response outcome (over 1 year). Based on analyses from previously published data[53;54], Brody and colleagues[70] demonstrated that higher pre-treatment activity in left OFC was associated with more improvement in the CBT group, yet less improvement in the fluoxetine-treated group. Similarly, Saxena et al.[65] found bilateral lower OFC pretreatment metabolism to be a good predictor for paroxetine response. Thus, remarkably consistent findings show that decreased activity in OCD-associated brain regions at baseline is related to a better outcome after medication

treatment, whereas the opposite (i.e. increased activity at baseline) may be true for CBT. An exception is the study of Hoehn-Saric and colleagues[66] in which higher pre-treatment rCBF in OFC, basal ganglia and cingulate areas was associated with better clinical outcome after medication. This discrepant finding may be attributed to the use of an OCD patient sample with comorbid depression.

In summary, resting-state studies have indicated that OCD pathophysiology is mediated by hyperactivity in frontal-striatal circuits, involving the OFC, caudate nucleus, putamen and thalamus. Interestingly, a recent meta-analysis that weighed effect sizes for regional CBF and metabolism differences between OCD and control groups across resting-state functional studies, showed that significant hyperactivity in OCD was actually limited to the orbital gyrus and the head of the caudate nucleus[71]. Although the importance of such quantitative assessments of functional abnormalities in OCD cannot be underestimated, the very nature of such analyses restricts the inclusion to comparable paradigms, thereby leaving out a substantial number of valid yet incompatible studies. The functional abnormalities described in the current review appear to be state-related, as brain activity in these structures tends to normalize upon successful treatment with medication or CBT. The finding of elevated resting-state activity in various brain regions in OCD may have implications for other functional neuroimaging designs in this disorder, in particular symptom provocation or cognitive activation paradigms. In such designs, brain activity during a baseline condition is often subtracted from an experimental condition, and these differences are subsequently compared between OCD and control groups. However, if baseline activity differs between groups as indicated by resting-state studies, these interaction effects are more difficult to interpret.

A related issue when considering these resting-state imaging data, is what constitutes the actual nature of ‘rest’ in OCD. It may be incorrect to regard this state as an asymptomatic one, since OCD is characterized by *tonic* rather than *phasic* symptomatology, as opposed to other anxiety disorders, e.g. panic disorder. If indeed ‘resting-state’ is not equivalent to ‘symptom-free’ in OCD, again this has implications for the interpretation of abnormalities found in other functional imaging paradigms (e.g. symptom provocation or cognitive activation), as determining the exact nature of ‘mind states’ in each of these paradigms is currently impossible.

Symptom provocation studies

Another strategy to differentiate between state and trait aspects of OCD is represented by symptom provocation designs. By alternating rest and symptom states within a session, the role of time (order) effects, as discussed in the previous paragraph, can be ruled out. However, studies using symptom provocation in OCD have yielded mixed results, as summarized in Table 2.3. Overall, the most consistent finding is provocation-induced increased activation of various frontal-striatal regions, such as the OFC[61;72-79], ACC[73;75], striatum[72-74;78;79], and thalamus[72;78;79]. In contrast to the *increased* activation in *ventral* prefrontal regions, OCD patients showed *decreased dorsal* prefrontal activation during the symptomatic state[72;80]. In addition to the frontal-striatal involvement, activation of middle

temporal lobe structures, such as the amygdala, also has been demonstrated during the provoked state[72;74;75;80]. Moreover, OCD patients with predominant contamination fear showed increased activation in the insular cortex, implicated in disgust perception[61;74;77].

Inconsistencies in results may again be explained by methodological issues, concerning technical aspects of imaging (e.g. imaging modality, ROIs versus whole brain data acquisition and analyses, correction for motion artifacts), patient selection (i.e. medication status, comorbidity, disorder sub-dimensions and sample size), and stimulus paradigms (i.e. randomized versus off-on design, tactile versus visual, and idiosyncratic versus standardized stimuli). Many provocation studies used tactile stimuli ('dirty' vs. 'clean'), which necessitated ritual behavior (i.e. hand washing), interrupting the scan sessions. Since in these studies subjects, once provoked, remained symptomatic for up to several hours, a balanced order paradigm (intended to control for order effects) was precluded[73-76;81].

In contrast to most OCD symptom provocation studies investigating contamination fear in *'washers'*, Cottraux and colleagues[82] included OCD patients with prominent *checking* rituals. OCD patients as well as controls showed increased orbitofrontal perfusion during the provoked compared with the neutral state. In addition, provocation-induced rCBF in the basal ganglia was higher in controls compared with OCD patients. Our own results, based on a comparison between eleven medication-free contamination-feared OCD patients and ten healthy control subjects, also showed decreased caudate activation in OCD patients compared with controls, during provocation of contamination fear[80]. Since most studies, which report increased provocation-induced caudate activation, lack a healthy control group[72;73;79], it cannot be excluded that activation of the basal ganglia reflects normal processing of emotional information or ritualistic behavior, also present in healthy volunteers. Moreover, in OCD patients, the recruitment of the frontal-striatal circuit seems to be even sub-optimal compared with controls[80;82]. Therefore, it seems likely that the failure of the frontal-striatal circuit in OCD patients to control the processing of negative disease related stimuli results in an inadequate fear response.

This fear response involves limbic activation, which was reflected by the activation of the amygdala in our study[80]. Whereas we found that left amygdala activation was stable over time during the provoked states, right amygdala showed a sensitization effect, positively correlated with scores of subjective distress and obsessionality. In contrast to our PET results, many provocation experiments in OCD have observed amygdala involvement only in small subgroups and/or at lower statistical thresholds[74;75], or not at all[61;73;77-79;82]. This inconsistency may be explained partly by the use of ROIs excluding the amygdala[79;82], limited statistical power of small sample off-on designs[73;74], use of medication[61;78], and differences in stimulus paradigm. Although the amygdala receives multimodal sensory information, projections from visual processing areas are particularly prominent[83]. Therefore, salient visual stimuli may give rise to amygdala activation more easily than tactile or auditory stimuli. The use of antidepressants is likely to suppress amygdala function[84]. Amygdala involvement in OCD during the symptomatic state is in agreement with studies using fear paradigms in normal controls[85-90], supporting the role of the amygdala as a key structure in evaluating the behavioral significance of external stimuli and fear responses[91]. Since amygdala activation has also been found during symptom

provocation in patients with posttraumatic stress disorder[92] as well as during CCK challenge in healthy volunteers[93], activation of limbic structures during symptom provocation in OCD may reflect an inadequate, but nonspecific, fear response. As mentioned before, this fear response seems to be secondary to a failure of the frontal-striatal circuit to control the processing of negative (e.g. contamination-related) stimuli.

OCD is a clinically heterogeneous disorder. Factor-analytic studies have consistently identified at least 4 stable symptom dimensions: contamination/washing, aggressive/checking, hoarding, and symmetry/ordering[94;95]. As mentioned in the previous paragraph, different neuronal mechanisms may underlie the heterogeneous symptoms of the various OCD dimensions. In two symptom provocation studies, differentiation between OCD subcategories has been investigated[61;78]. Phillips and colleagues[61] compared 'checkers' and 'washers' using standardized visual stimuli. Similar to the results of Cottraux et al.[82], 'checkers' as well as normal control subjects showed increased activation of the frontal-striatal regions during the provoked versus neutral state. In contrast, insular activity was observed in 'washers'. A methodological drawback of this study, restricting the between-subtype comparison, is the fact that only washing-relevant, no checking-relevant stimuli, were presented during the provocation.

Recently, Mataix-Cols and colleagues[78] also investigated the neuronal differentiation between OCD dimensions, using general aversive, washing-related, checking-related, hoarding-related, and neutral visual stimuli in a heterogeneous group of OCD patients. In contrast to Phillips et al.[61] who allocated their *patients* to mutually exclusive subgroups, Mataix-Cols and colleagues compared different *stimulus* sub-types within one heterogeneous group of OCD patients. Whereas ventral regions of the frontal-striatal circuits were activated during washing-related provocation, checking-related stimuli induced recruitment of dorsal regions of these circuits. Unfortunately, no symptom subtype by stimulus subtype interaction analysis was described. In future research, it would be interesting to combine the two strategies, thus comparing both patient symptom dimensions (e.g. using the Padua Inventory-Revised[96]) and stimulus sub-type within the same study design.

Two studies have been performed to investigate the correlation between pre-treatment response during symptom provocation and the efficacy of treatment with selective serotonin reuptake inhibitors (SSRIs). Rauch and colleagues[76] found in a small sample of contamination-feared OCD patients lower bilateral orbitofrontal and higher bilateral posterior cingulate activation to be predictive of a better response to fluvoxamine. Hendler et al.[97], using SPECT, investigated a larger group of OCD patients with mixed symptoms. Before treatment, responders showed less provocation-induced activation in the dorsal ACC than non-responders, although these results might have been biased by differences in comorbid depressive symptoms. An additional post-hoc ROI analysis showed that prospective responders also had significantly more pre-treatment provocation-induced activation in the right caudate nucleus than non-responders. Following 6 months of sertraline treatment, symptom provocation led to increased activation in left anterior temporal cortex in responders, but not in non-responders. Whether the results reflect biological subtypes of the disorder with differential sensitivity to SSRIs, or just differences

Table 2.3. Symptom provocation studies in OCD

Authors (publication date)	*Technique/paradigm*	*Subjects*	*Main findings*	*Methodological comments*
van den Heuvel et al. (2004)[80]	$H_2{}^{15}O$ PET, visual stimuli (contamination vs neutral)	11 OCD (washers) vs 10 co	Increased activation in L amygdala and bil. extrastriate cortex, sensitization effect in R amygdala. In controls: increased activation in L DLPFC, OFC, L anterior temporal cortex, R caudate nucleus and bil. extrastriate cortex.	
Chen et al. (2004)[79]	MRI (PWI), idiosyncratic tactile stimuli (contamination vs neutral)	10 female OCD (washers)	Increased rCBF in bilateral OFC, R caudate nucleus and R thalamus.	No healthy control group, ROI method (OFC, caudate, thalamus)
Mataix-Cols et al. (2004)[78]	fMRI, visual stimuli (general aversive vs washing-related vs checking-related vs hoarding-related vs neutral)	16 OCD (mixed) vs 17 co	Increased activation in bil. ventromedial PFC and R caudate nucleus (washing-related stimuli), putamen, pallidum, thalamus and dorsal cortical regions (checking-related), L precentral gyrus and R OFC (hoarding-related), L occipito-temporal cortex (general aversive).	Comorbid diagnoses, medicated subjects
Shapira et al. (2003)[77]	fMRI, visual stimuli (general threat vs disgust vs neutral)	8 OCD (washers) vs 8 co	During disgust provocation increased activation in R insula, bil. inf. frontal gyrus, L parahippocampal gyrus, L post. cingulate cortex and L inf. occipital gyrus. No differences between OCD and controls during general threat provocation.	Limited sample size
Hendler et al. (2003)[97]	SPECT, idiosyncratic stimuli	26 OCD (mixed, 13 resp. vs 13 non-resp.) before vs after sertraline treatment	Before treatment: responders show less activation in R ACC than non-responders. In responders increased activation in L ant. temporal cortex after (compared to pre-) treatment.	Comorbid depression
Rauch et al. (2002)[76]	$H_2{}^{15}O$ PET, idiosyncratic tactile stimuli	9 OCD (washers) before vs after fluvoxamine treatment	Increased activation in R OFC during provocation. Lower bil. OFC and higher bil. post. cingulate activation predicted better treatment response, independent of symptomatic state at the time of PET data acquisition.	Limited sample size, off-on design

Table 2.3 (continue)

Authors (publication date)	*Technique/paradigm*	*Subjects*	*Main findings*	*Methodological comments*
Adler et al. (2000)[75]	fMRI, idiosyncratic stimuli	7 OCD (mixed)	Increased activation in bil. OFC, ant./lat. temporal cortex, R ACC and R med. temporal cortex (including hippocampus and amygdala).	Limited sample size, ROIs (OFC, ACC, basal ganglia, temporal regions), no control group, off-on design
Simpson et al. (2000)[81]	EEG, tactile stimuli (live vs imaginary exposure)	6 OCD (washers)	Increased activation of anterior relative to posterior brain regions during live exposure.	Limited sample size, medicated subjects, no control group, off-on design
Phillips et al. (2000)[61]	fMRI, visual stimuli (general disgust vs washing-related vs neutral)	14 OCD (7 washers vs 7 checkers) vs 14 co	In all subjects: increased activation in visual regions and insula during general disgust stimuli. During washing-related stimuli, increased activation in visual regions, insula and PFC in washers, and frontal-striatal regions in checkers and controls.	Medicated subjects
Breiter et al. (1996)[74]	fMRI, idiosyncratic tactile stimuli	10 OCD (mixed) vs 5 co	Increased activation in frontal-striatal regions, L insula and bilateral amygdala.	Medicated subjects, off-on design
Cottraux et al. (1996)[82]	$H_2{}^{15}O$ PET, idiosyncratic auditory stimuli (obsession vs neutral vs rest)	10 OCD (checkers) vs 10 co	No group x condition interaction effects reported. At rest: increased rCBF in superior temporal cortex in OCD. During provocation: increased rCBF in caudate nuclei in controls.	ROI method (prefrontal regions, caudate, putamen, thalamus, temporal cortex)
Rauch et al. (1994)[73]	$H_2{}^{15}O$ PET, idiosyncratic tactile stimuli	8 OCD (mostly washers)	Increased activation in bil. OFC, L ACC and R caudate nucleus.	Limited sample size, no healthy control group, off-on design
McGuire et al. (1994)[72]	$H_2{}^{15}O$ PET, idiosyncratic tactile stimuli (of hierarchic intensity)	4 OCD (washers)	Increased activation in R OFC, posterior cingulate, basal ganglia, thalamus and L hippocampus; decreased activation in R dorsal PFC and parietal-temporal region.	Limited sample size, no healthy control group

in pre-treatment symptom severity or comorbid depressive symptomatology awaits further investigation. In addition, since no placebo control group was included, it cannot be excluded that these neurophysiological changes reflect spontaneous improvement. Moreover, it would be interesting to know, if a therapeutic response to CBT may also be predicted by pre-treatment neuronal responses to symptom provocation.

Taken together, symptom provocation studies have implicated a number of regions in the pathophysiology of OCD. The results so far have provided evidence that frontal-striatal involvement reflects ritualistic behavior, even in normal subjects, whereas increased medial temporal activity (including the amygdala) has been associated with (pathological) anxiety. Provocation-induced anxiety may be associated with non-specific rCBF changes due to increases in vigilance and/or arousal that obscure the functional circuitry changes specific to the disease. For this reason, symptom induction in a scanning environment needs to be refined by the use of standardized, symptom- and dimension-specific stimuli, controlling for general emotional effects by including a generally aversive control condition. Although some evidence exists that different neuronal correlates underlie different OCD symptom dimensions, more research is warranted to further address the issue of heterogeneity, including analyses of covariance with scores of dimensional symptom subscales. In addition, to better understand the temporal order of neuronal effects during symptom provocation, region-specific changes in time have to be investigated, for example by simultaneous fMRI/EEG registration. Moreover, longitudinal designs and pre-post treatment measurements may provide more insight in the trait-state question. Finally, to enable correction for differences in morphology and resting state metabolism, combined data acquisition within the same subjects is needed.

Cognitive activation paradigms

The use of cognitive probes during functional neuroimaging is a highly promising tool for the study of neuropsychiatric disorders. These paradigms are sensitive and specific for unraveling their neuronal correlates, in that they allow the induction of hypothesis-driven and disorder-associated functional brain states of interest. OCD is associated with a number of cognitive deficits, as indicated by neuropsychological studies. Although results are far from consistent, impairments appear to stand out in the field of executive functioning and (nonverbal) memory, especially in the context of the need to apply organizational strategies to effectively complete such tasks[98;99].

So far, cognitive paradigms during functional neuroimaging in OCD have been relatively sparse and have been used to investigate different, often unrelated cognitive domains (see Table 2.4). In one of the first of such designs, Rauch and colleagues[100], using $H_2{}^{15}O$ PET, showed a lack of bilateral striatal activation during successful *implicit* learning in OCD patients, in contrast to healthy subjects. Instead, patients recruited (para)hippocampal regions that normally serve the processing of *explicit* learning and memory. This finding was replicated in a small (N=6) OCD sample during fMRI instead of PET scanning[101]. It was argued that (para)hippocampal brain areas are engaged during task performance in OCD to compensate for frontal-striatal dysfunction. This hypothesis was

Table 2.4. Cognitive activation paradigms in OCD

Authors (public. date)	*Technique/Paradigm*	*Subjects*	*Main findings*	*Methodological comments*
van den Heuvel et al. (in press)[106]	fMRI, cognitive and emotional Stroop task (attentional bias)	16 OCD vs 15 PD vs 13 hypochondriasis vs 19 co	Increased recruitment of posterior regions during cognitive interference; during OCD-related emotional interference: increased activation of VLPFC, dorsal ACC, putamen and amygdala activation vs controls.	Patients and controls not matched for age and gender, no hypochondriasis-related word stimuli
van den Heuvel et al. (in press)[105]	fMRI, Tower of London task (planning)	22 OCD vs 22 co	Decreased frontal-striatal functioning, i.e. diminished activations in DLPFC, caudate nucleus and putamen; increased activitiy in ACC, VLPFC, parahippocampal.	Patients and controls not matched for educational level
van der Wee et al. (2003)[107]	fMRI, n-back task (working memory)	11 OCD vs 11 co	Increased activation in ACC at all levels of task difficulty in OCD, and normal involvement of DLPFC, premotor regions and parietal cortex.	Both samples only women
Ursu et al. (2003)[108]	fMRI, Continuous Performance task (response conflict)	11 OCD vs 13 co	Increased activation in ACC during high-conflict trials and error trials in OCD that correlated positively with symptom severity (trend).	Comorbid anxiety disorders, medicated subjects, ROI analysis
Pujol et al. (1999)[104]	fMRI, Word Generation task	20 OCD vs 20 co	Increased task-related activation of L lateral PFC and impaired suppression of this activation during the following rest condition. Inverse correlations for 'activation signal' and positive correlations for 'remaining activation' with total Y-BOCS score.	No controlled task performance, ROI (L lateral PFC) instead of whole brain analysis
Rauch et al. (1997)[100]	$H_2{}^{15}O$ PET, Serial Response Time task (implicit learning)	9 OCD vs 9 co	Implicit learning without recruitment of bil striatum (present in controls), yet activation of bil (para)hippocampal regions (absent in controls).	Limited sample size, both samples only women, interaction analysis only for striatum

confirmed in a neuropsychological study that showed a failure of implicit learning in OCD when subjects had to simultaneously perform an explicit memory task[102]. However, the reported differential activations between OCD subjects and controls by Rauch et al.[100;101] were only partly replicated in a recent, similar experiment during fMRI scanning[103].

Employing a verbal fluency paradigm, Pujol and colleagues[104] investigated left DLPFC function during fMRI. They noticed increased activation in this region for OCD subjects while performing the task, as well as elevated residual activation during a subsequent resting period. According to the authors, these functional alterations indicated enhanced responsiveness to cognitive challenge in the DLPFC, and diminished ability to suppress activation during rest in the same area. Differential activations in the DLPFC between OCD and healthy controls was also found in a recent study on planning capacity using the Tower of London task, conducted in our laboratory. In this experiment, we demonstrated that dorsal frontal-striatal circuitry was recruited to a lesser extent in OCD patients compared with controls[105]. Instead, patients activated the ACC, VLPFC, parahippocampal gyrus and dorsal brain stem. Results appear to reflect an inadequacy to appropriately engage the dorsal frontal-striatal circuit for planning in OCD, together with compensatory engagement of structures involved in maintenance of task rules in working memory (VLPFC), performance monitoring (ACC), intermediate-term memory capacity (parahippocampal gyrus) and arousal/effort (dorsal brain stem). The hypothesis of a compensatory increased effort in OCD patients compared with normal controls during task performance has received additional support from another recent cognitive activation study by our group [106]. Using a classical Stroop task, we found that posterior regions (fusiform area, parahippocampal gyrus, insula, brain stem, precuneus and parietal cortex) were increasingly activated in patients during cognitive interference (i.e. incongruent vs. congruent color naming). Moreover, in an emotional variant of this task, processing of OCD related versus neutral words during color naming was associated with enhanced activation of VLPFC, dorsal ACC, putamen and amygdala in OCD compared with controls. In comparison with other anxiety patients suffering from hypochondriasis and panic disorder, OCD subjects were unique in two ways. First, they exhibited neuronal effects during emotional interference (i.e. emotional versus neutral words) only for OCD related words, whereas hypochondriasis and PD patients reacted on both OCD- and panic related words. Second, patients with OCD recruited a predominantly *ventral* neuronal system for processing this disease-specific emotional material, as opposed to the other patient groups that also used a *dorsal* system for processing emotional cues. As noted in the introduction, these two activation pathways, i.e. a ventral and dorsal one, have recently been distinguished in human emotion processing. The former corresponds to amygdala, insula and ventral regions of the striatum, ACC and OFC, whereas the latter is composed of the hippocampus and dorsal regions of the ACC and PFC[13].

In a spatial working memory paradigm using the n-back task, van der Wee et al.[107] found diminished performance at the highest task load level although activity in regions normally found in spatial working memory, i.e. bilateral DLPFC and parietal cortex, was similar to control subjects in OCD at all load levels. However, in OCD subjects, the ACC was engaged to a greater extent at all load levels. The authors concluded that spatial working memory capacity is normal in OCD, but performance is impaired at the highest load level due to an interfering abnormal process originating in the ACC. Increased ACC

activity, not related to performance is in line with the results of Ursu et al.[108], who used a response conflict (continuous performance) task in OCD and normal subjects during fMRI. They found increased ACC activity both during errors and during correct trials encompassing high-conflict situations. Previously, increased *error-related* ACC activity had been found as heightened and prolonged error-negativity responses during false trials in an ERP paradigm, using a variant of the Stroop task[109]. However, the results of van der Wee et al.[107] and Ursu et al.[108] extend these findings by showing that increased ACC activity is also present during correct responses, across task paradigms. This may reflect an enhanced and dysfunctional action monitoring in OCD subjects, regardless of actual task performance. It may represent the neuronal substrate of the subjective feelings of doubt despite correct task performance that are so characteristic of this disorder.

In summary, the number of cognitive paradigms during functional neuroimaging in OCD up to this date is small. It can be expected, however, to increase rapidly over the next few years due to the opportunities these paradigms provide for elucidating the neuronal substrate of this disorder. The few experiments conducted so far have begun to shed light on various neurophysiological mechanisms involved in OCD. First, during cognitive challenge (both explicit and implicit), dorsal frontal-striatal responsiveness appears to be diminished in OCD, which is presumably compensated for by recruitment of posterior (e.g. medial temporal) brain regions. Second, OCD related emotional material may be processed primarily by ventral but not dorsal prefrontal pathways. This may indicate a lack of effortful regulation of affective states in OCD[13]. Third, increased and performance-unrelated ACC activity appears to be present across cognitive paradigms, putatively corresponding to enhanced, inadequate action monitoring in OCD. Further research is needed to investigate the neuronal substrate of cognitive deficits in OCD and the interactions between emotional and cognitive processing systems in OCD, i.e. between the limbic and dorsal frontal-striatal systems. In the selection of cognitive tasks, it is important that specific abnormalities in OCD as identified in recent or ongoing neuropsychological research be respected. For the interpretation of results from such designs, it is important to take into account findings from related imaging paradigms; for instance, the increased resting-state activity in brain regions such as the striatum in OCD may be responsible for a failure to recruit these areas with cognitive probes, i.e. may cause a ‘ceiling effect’.

MRS and ligand studies

MRS enables direct and non-invasive measurements of brain chemistry in vivo, and therefore this tool is specifically useful for child- en adolescent psychiatric research and longitudinal follow-up by repeated measurements without the possible risks involved when using ionizing radiation. Most MRS studies in OCD have been performed by Rosenberg and coworkers, investigating early-onset OCD in children and adolescents [110-117]. Compounds that can be measured by ^{1}H MRS include myoinositol (mI), choline (Cho), (phospho)creatine (Cr), glutamatergic compounds (Glx), and N-acetyl-aspartate (NAA). Increased Cho signal reflects increased levels of choline-containing compounds, such as acetylcholine. Changed Glx activity has been found to parallel altered brain glutamatergic

excitatory neurotransmission[118]. NAA is a marker for neuronal viability[119] and declines in neuronal tissue before neuronal loss is detectable by conventional MRI methods[120].

So far, MRS results concerning neurochemical changes in OCD have been inconsistent (see Table 2.5). An important methodological limitation of MRS is that data acquisition in most studies is limited to a single voxel or small volume in the striatum, thalamus or prefrontal cortex, restricting comparisons between studies. Decreased NAA signal in left[25] and right striatum[121] has been found in adult OCD, whereas striatal volumes were unchanged. This finding supports the idea that MRS enables early detection of decreasing cell density. In contrast to these studies, the results of Ohara and colleagues[122], based on medicated OCD patients, suggested normal viability of the neuronal cells in the putamen and pallidum. The caudate nucleus, however, was not included in the analysis. Differences in medication status cannot explain this inconsistency since Rosenberg and colleagues also found normal thalamic[113] and striatal NAA signal[111] in their medication-naive pediatric OCD patients. In addition, even increased NAA concentrations have been found in the DLPFC[114] of children with OCD. Moreover, absolute measures of the compounds showed that the reported reductions in right and left medial thalamic NAA/(Cho+Cr) and NAA/Cho ratios in pediatric OCD actually reflected an increase in Cho levels[110] rather than a decrease in NAA[113]. Therefore, the possible role of cholinergic dysfunction in the pathophysiology of OCD needs further elaboration.

Dysfunctional glutamatergic neurotransmission and altered serotonergic modulation of glutamatergic transmission at the level of the caudate nucleus may also be involved in the pathogenesis and maintenance of OCD symptoms[28;123]. Glutamate is the primary excitatory neurotransmitter in the frontal-striatal circuits and increased glutamatergic neurotransmission in specific regions of these circuits may contribute to obsessive-compulsive symptoms[123]. Glutamate receptor antagonists have been shown to reduce OCD-like behavior in mice[124]. Moreover, in a family-based association study, variants of the glutamate subunit receptor gene were associated with the susceptibility to develop OCD[125]. MRS results have provided additional evidence for a dysfunctional glutamatergic neurotransmission in OCD. Comparing 11 medication-naive pediatric OCD patients to 11 healthy control children, Rosenberg et al.[111] reported increased left caudate Glx concentrations in OCD. After 12 weeks of paroxetine treatment, Glx levels had normalized. Interestingly, adequate cognitive behavioral therapy did not induce a reduction in striatal Glx[116]. The disease-specificity of glutamatergic dysfunction in OCD still has to be addressed, since altered Glx concentrations in the ACC were found in OCD as well as in MDD[117].

In contrast to MRS studies, PET and SPECT ligand designs require radiolabeled high-affinity ligands to detect neurochemical differences between experimental groups. These techniques enable in vivo measurements of global and regional receptor density and affinity of the various interacting neurotransmitter systems. Although only a small number of ligand studies have been performed in OCD so far, future use of these techniques is promising. However, some important methodological issues have to be considered. First, the radiation risk limits its use in child and adolescent research, and in longitudinal follow-up designs. Second, since the spatial resolution of PET and SPECT is moderate at best, MRI co-registration is necessary for precise anatomical localization and correction for volumetric

differences between groups. Third, the functional mechanisms underlying decreased or increased ligand binding are not yet fully understood. Altered ligand binding can be explained by a number of factors, including 1) changes in endogenous neurotransmitter concentrations, 2) genetic modification of the receptor proteins, 3) morphological changes of specific brain regions and 4) altered receptor density due to up or down-regulation of the postsynaptic receptors. Moreover, changes in one neurotransmitter might be secondary to primary changes in another neurotransmitter system.

So far, ligand studies in OCD have mainly focused on 5-HT and DA. A possible role of dysfunctional brain serotonergic and dopaminergic neurotransmitter systems in the pathophysiology of OCD has been suggested a long time ago. The hypothesis that OCD involves a disturbance in 5-HT transmission was initially based on the clinical observation that the tricyclic antidepressant clomipramine is an effective psychopharmacological agent in OCD[126-128]. Since other tricyclic antidepressants are ineffective in OCD, it has been concluded that the efficacy of clomipramine is due to its potent serotonin reuptake inhibiting properties, similar to SSRIs. However, a substantial fraction of patients with OCD show no significant improvement in response to SSRIs alone, suggesting that an isolated disturbance in 5-HT function cannot fully account for the pathophysiology of OCD[129]. Clinical studies showed that the combination of a SSRI with an antipsychotic drug, antagonizing the DA receptor, is successful in a considerable amount of these SSRI-refractory cases[130;131].

Concerning the role of the monoaminergic systems in OCD, recent ligand SPECT and PET studies have given additional support for the hypothesis that both DA and 5-HT are involved in the modulation of obsessive-compulsive symptoms. Iodine-labeled [^{123}I]-2β-carbomethoxy-3β-(4-iodophenyl)tropane ([^{123}I] β-CIT) is a SPECT radiotracer with high affinity to monoamine transporters[132] and thus can be used to visualize the human central dopamine (DAT) and serotonin transporters (SERT) in vivo[133]. Although β-CIT lacks specificity within the monoamine transporter family, distinct brain regions were found to be related to DA and 5-HT binding: whereas striatal β-CIT uptake mainly reflects binding to DAT, in the other regions comprising the hypothalamus, thalamus, midbrain and pons, β-CIT uptake has been predominantly associated with SERT availability[134]. Alternative radiotracers used to visualize DAT and SERT are the SPECT-ligand iodine-labeled N-(3-iodopropen-2-yl)-2β-carbomethoxy-3β-(4-chlorophenyl)tropane ([^{123}I] IPT) and the PET-ligand C-11 labeled trans-1,2,3,5,6,10 β-hexahydro-6-[4-9methylthio)phenyl]pyrrolo-[2,1-a]-isoquinoline ([^{11}C] McN 5652), respectively.

So far, ligand studies focusing on SERT and DAT in OCD have produced conflicting results (see Table 2.5), probably due to methodological issues concerning differences in age, gender, medication history, comorbidity and/or genetic predisposition. Moreover, the sample sizes of these studies were limited. Pogarell et al.[135], using [^{123}I] β-CIT SPECT, found increased β-CIT binding in the midbrain and pons of medication-naive OCD patients, reflecting increased SERT availability. Midbrain β-CIT binding was highest in patients with childhood or adolescence onset of symptomatology, suggesting a more pronounced serotonergic dysfunction in early compared with late-onset OCD. In contrast to Pogarell et al.[135], other studies reported unchanged[136] or reduced[137] SERT availability, using [^{11}C] McN5652 PET and [^{123}I] β-CIT SPECT, respectively. Since SSRI treatment may lead to long-term decreases in SERT availability, inconsistencies may be explained by the fact

Table 2.5. Ligand and MRS studies in OCD

Authors (public. date)	*Ligand/Technique*	*Subjects*	*Main findings*	*Methodological comments*
Denys et al. (2004)[138]	[^{123}I] IBZM SPECT (D_2 receptor)	10 OCD vs 10 co	Decreased D_2 binding ratios in and reduced volume of L caudate nucleus.	SPECT-results not corrected for structural differences
Stengler-Wenzke et al. (2004)[137]	[^{123}I] â-CIT SPECT (SERT)	10 OCD vs 7 co	Reduced â-CIT binding in midbrain and upper brain stem (SERT).	Nonspecific binding â-CIT
Pogarell et al. (2003)[135]	[^{123}I] â-CIT SPECT (SERT and DAT)	9 OCD vs 10 co	Increased â-CIT binding in midbrain/pons (SERT), normal â-CIT binding in striatum (DAT), highest SERT availability in patients with early-onset OCD, negative correlation with depression scores, no correlation with OCD severity.	Nonspecific binding â-CIT, gender and age bias, no MRI co-registration.
Simpson et al. (2003)[136]	[^{11}C] McN 5652 PET (SERT)	11 OCD vs 11 co	Normal SERT availability in subcortical and limbic regions.	Nonspecific binding McN 5652
Kim et al. (2003)[140]	[^{123}I] IPT SPECT (DAT)	15 OCD vs 19 co	Increased IPT binding in R basal ganglia (trend for L basal ganglia)	
Rosenberg et al. (2004)[117]	^{1}H MRS (NAA, Cr, Cho, Glx)	20 pediatric OCD vs 14 pediatric MDD vs 14 co	Reduced Glx concentrations in ACC in pediatric OCD as well as in pediatric MDD compared to control children. Normal NAA, Cho and Cr.	OCD group significantly younger than MDD group and controls, measurements limited to ACC.
Russell et al. (2003)[114]	^{1}H MRS (NAA, Cr, Cho)	15 pediatric OCD vs 15 co	Increased NAA concentrations in L DLPFC, normal Cr and Cho concentrations.	
Smith et al. (2003)[115]	^{1}H MRS (Cho)	27 pediatric OCD vs 18 pediatric MDD vs 18 co	Increased Cho concentrations in bilateral medial thalamus in pediatric patients with OCD compared to controls and pediatric patients with MDD.	Measurements limited to bilateral medial and lateral thalamus.
Benazon et al. (2003)[116]	^{1}H MRS (NAA, Cr, Cho, Glx, mI)	21 pediatric OCD (before vs after CBT)	No significant pre-posttreatment change of Glx in L caudate nucleus.	Measurements limited to single voxel in L caudate nucleus.

Table 2.5 (continue)

Authors (public. date)	*Ligand/Technique*	*Subjects*	*Main findings*	*Methodological comments*
Rosenberg et al. (2001)[113]	^{1}H MRS (NAA, Cr, Cho)	11 pediatric OCD vs 11 co	Increased Cho in bilateral medial thalamus, normal Cr and NAA.	
Rosenberg et al. (2000)[111]	^{1}H MRS/MRI (NAA, Cr, Cho, Glx, mI)	11 pediatric OCD (before vs after paroxetine) vs 11 co	Increased Glx in L caudate nucleus in pediatric OCD compared to controls, normalizing after paroxetine treatment, positive correlation with reduction in OCD symptom severity scores.	Measurements limited to single voxel in L caudate nucleus, not placebo controlled.
Fitzgerald et al. (2000)[110]	^{1}H MRS (NAA, Cr, Cho)	11 pediatric OCD vs 11 co	Decreased NAA/(Cho+Cr) and NAA/Cho ratios in bilateral medial (not in lateral) thalamus.	Measurements limited to bilateral medial and lateral thalamus.
Ohara et al. (1999)[122]	^{1}H MRS (NAA, Cr, Cho)	12 OCD vs 12 co	Normal levels of NAA, Cho, and Cr in lenticular nucleus.	Medicated patients.
Bartha et al. (1998)[25]	^{1}H MRS/MRI (NAA, Cr, Cho, Glx)	13 OCD vs 13 co	Decreased NAA in L striatum and normal striatal volumes.	Analyses limited to small volume in L striatum.
Ebert et al. (1997)[121]	^{1}H MRS (NAA, Cr, Cho, Glx)	12 OCD vs 6 co	Decreased NAA in R striatum and ACC	Measurements limited to R striatum, ACC, occipital and parietal cortex.

All main findings reported for OCD versus controls, unless indicated otherwise.
IBZM = iodobenzamide, â-CIT = 2â-carbomethoxy-3â-(4-iodophenyl)tropane , McN 5652 = trans-1,2,3,5,6,10 â-hexahydro-6-[4-9methylthio)phenyl]pyrrolo-[2,1-a]-isoquinoline, IPT = N-(3-iodopropen-2-yl)-2â-carbomethoxy-3â-94-chlorophenyl) tropane, DAT = dopamine transporter, SERT = serotonin transporter, Cho = choline, Glx = glutamatergic compounds, NAA = N-acetyl-aspartate

that most OCD patients in the study of Pogarell et al. were medication-naive, in contrast to the patients in other studies.

Very recently, Denys and colleagues[138] performed a radiolabeled iodobenzamide ([^{123}I] IBZM) SPECT study, investigating D_2 receptor binding in 10 OCD patients and 10 healthy control subjects. Decreased D_2 receptor binding in the left caudate nucleus and a reduced left compared with right caudate volume was found in OCD. Decreased [^{123}I] IBZM binding might be explained by several underlying mechanisms. First, structural changes of striatal cells due to developmental abnormalities or degeneration processes might be present. Since left caudate volume was smaller in OCD patients compared with control subjects, this explanation cannot be ruled out. Second, reduced dopamine D_2 densities in OCD could also be the result of genetic variability, although this seems a less likely explanation for the observed hemispheric asymmetry. The authors suggested that the decreased [^{123}I] IBZM binding reflects postsynaptic dopamine D_2 receptor down-regulation by competition with high concentrations of endogenous dopamine. This third explanation is in agreement with the hypothesis of an imbalance between the direct and indirect pathways of the frontal-striatal circuits in OCD, due to increased inhibition of the indirect pathway by dopamine D_2[139]. Increased secretion and higher synaptic concentrations of endogenous dopamine may result from decreased inhibition of dopamine due to serotonergic dysfunction. The presence of increased endogenous dopamine concentrations in the striatum is supported by the [^{123}I] IPT SPECT results of Kim et al.[140], who found an increased DAT binding in the right basal ganglia and a tendency toward an increased DAT binding in the left basal ganglia. In contrast, Pogarell et al.[135] reported normal striatal ß-CIT binding in a smaller sample of mostly medication-naive OCD patients.

Future ligand studies in OCD should attempt to distinguish between changes in endogenous ligand concentrations and altered receptor density and/or affinity, for example by using double infusion protocols with high and low specific activity radiotracers. In addition, dopaminergic reactivity in OCD could be investigated with the aid of amphetamine pretreatment, similar to studies in schizophrenia[141].

In conclusion, results from MRS and ligand studies have revealed some evidence for changes in various interacting neurotransmitter systems in OCD, including serotonin, dopamine, glutamate and acetylcholine. The exact mechanism of the neurochemical imbalance and the interactions between different neurotransmitters needs further elaboration. The use of MRS in the follow-up of early-onset OCD patients to investigate glutamatergic and cholinergic changes, in combination with ligand serotonin and dopamine measurements in their later life, is likely to contribute to a better understanding of dysfunctions at a neurochemical level in OCD.

Summary and conclusions

In the present paper, we intended to critically review the neuroimaging literature in OCD. The most consistent finding emanating from various imaging modalities and paradigms is the role of frontal-striatal and limbic circuits in the pathogenesis and maintenance of the disorder. However, the functional meaning of altered activation patterns in these circuits

remains uncertain. The term 'frontal-striatal' appears to be too broad to capture the subtle alterations of brain functioning in OCD during the various emotional and/or cognitive paradigms investigated thus far. In addition, although OCD is classified in the DSM-IV as an anxiety disorder, the role of limbic regions such as the amygdala in the pathophysiology of OCD has received little attention in the neuroimaging literature. Moreover, the interactions between frontal-striatal and anterior medial temporal regions are still poorly understood.

A second general finding emerging from the data is the functional differentiation, which appears to be present between ventral and dorsal frontal-striatal regions. Resting state in OCD patients is associated with increased activity of the ventral parts of frontal-striatal circuitry - probably reflecting ongoing emotional and cognitive processing due to tonic symptomatology. During symptom provocation, activation of limbic structures (mainly the amygdala) and additional recruitment of ventral frontal-striatal regions may reflect the processing of salient information together with emotional responding. Furthermore, exaggerated responses (anxiety and/or distress) may, at least in part, be the result of insufficient suppression or top-down control by the dorsal frontal-striatal circuit. These neuroimaging results can now be related to the model of Phillips et al.[13]. As discussed in the introduction section, this model distinguishes three levels of emotion perception, relying on different parts of ventral and dorsal (prefrontal and temporal) regions: 1) the identification of the emotional significance of an external stimulus, 2) the generation of an affective state in response to the stimulus, and 3) the regulation of the affective state. At present it is insufficiently clear which processing subsystem is dysfunctional, or how abnormalities at these distinct levels interact in OCD; do these patients attribute exaggerated affective significance to disease related stimuli in the environment (level 1) or do they identify these cues appropriately, but subsequently develop a disproportional fear response due to a hypersensitive limbic system (level 2)? Or, alternatively, do these patients lack an adequate modulation of this initial fear response as a result of a dorsal dysfunction (level 3)? These questions will need to be addressed in future neurobiological research in OCD. Dysfunction of dorsal frontal-striatal circuitry might be secondary to abnormal maturation of gray and white matter during early childhood. Moreover, glutamatergic, serotonergic and/or dopaminergic dysfunction, resulting in an altered balance between these various neurotransmitter systems, are likely to interact with cerebral maturation processes. Therefore, more insight in normal brain maturation and its role in the characteristic, temporary compulsive-like behaviors in normal young children might contribute to OCD research [142]. Investigation of normal development might also provide an answer to the question whether OCD symptomatology primarily results from a cortical abnormality or primarily emanates from a subcortical dysfunction. The decreased responsiveness of the dorsal frontal-striatal circuit not only modulates emotional processing, but also has implications for cognitive, mainly executive, functioning. OCD patients show impaired performance on executive tasks, correlated with decreased dorsal frontal-striatal responsiveness and increased activation of posterior cortical and subcortical regions reflecting both compensatory mechanisms (e.g. effort) and heightened arousal. Cognitive function in OCD patients is also characterized by enhanced performance monitoring, associated with increased ACC recruitment.

Although the OFC and VLPFC have been implicated in decision making[143], so far no imaging studies using such a paradigm (e.g. a gambling task) have been performed in OCD. Moreover, fMRI paradigms might be useful to further investigate emotion-cognition interactions.

So far, almost all neuroimaging results in OCD have been based on comparisons between OCD patients and healthy control subjects. Concerning the issue of disorder-specificity, two questions arise: 1) is a neurophysiological differentiation possible between OCD subcategories, and 2) are the frontal-striatal and limbic activation patterns observed in functional imaging studies specific for OCD or is there an overlap with other anxiety disorders, mood disorders or even psychotic disorders? To answer these questions, two opposite approaches are needed: 'splitting', i.e. contrasting subcategories within OCD, and 'lumping', or grouping together of OCD patients with patients suffering from other neuropsychiatric disorders, by changing the focus to functional dimensions instead of DSM-categories.

Over the last two decades, imaging techniques have proven to be very useful in elucidating underlying neurophysiological and neurochemical mechanisms in a number of psychiatric disorders, including OCD, and the ongoing progress in technology makes these techniques even more promising in the near future. However, several issues related to important general methodological aspects in functional imaging, have to be considered. Probably the most urgent questions in this respect that still need to be addressed are: in which state is the patient at the moment of data acquisition and to what extent do researchers succeed in inducing and maintaining the intended emotional and/or cognitive state in subjects within an experimental setting? Functional MRI is blind to baseline differences, which may confound group-by-task comparisons, although a few studies have attempted to control for this problem by the use of parametric designs. In general, it is questionable whether customary subtraction designs really succeed in isolating only the neuropsychological function of interest, even with a carefully matched baseline condition (e.g. for stimulus complexity and motor demands). Moreover, assessment of task related effects in patient groups, as well as group-by-task interaction effects, may be problematic due to aspecific factors, such as increased arousal or distress. Next to these issues that are to be resolved, future designs will need to optimize methodological aspects such as sufficiently large sample sizes, adequate matching between patients and controls (for age, gender, education level, among others), and homogeneous patient groups with regard to medication history, illness duration and severity, and comorbid diagnoses.

Even in the ideal situation of no methodological drawbacks, the *interpretation* of data still remains a challenge as well. We need to reconsider the functional significance of hypoperfusion or hyperactivation in a specific brain region and what it tells us about the underlying neuronal response. Altered state-related oxygenation levels might be secondary to structural and/or neurochemical abnormalities and the specificity of the findings for OCD is yet to be assessed. There is an obvious need for designs adopting an integrated approach, for example by comparing multiple tasks within the same subject sample and using multimodal imaging. Also, most imaging studies in OCD published to date have been cross-sectional. For that reason, longitudinal designs, combing structural, functional and

biochemical (PET or MRS) imaging techniques in sufficiently large, homogeneous cohorts are likely to hold the greatest promise for unraveling the pathogenesis of psychiatric disorders such as OCD.

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3

Neuroimaging in panic disorder

van den Heuvel OA, Groenewegen HJ, Witter MP, Veltman DJ

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Abstract

The neuroanatomical correlates of panic disorder (PD) can be investigated with the aid of several complementary neuroimaging study designs. These include structural studies detecting morphological abnormalities, resting state measurements of cerebral blood flow and metabolism, provocation designs using pharmacological challenge agents, functional imaging designs during the performance of cognitive and/or emotional tasks, and ligand studies measuring receptor distribution and affinity. Results of most of these studies support the involvement of structures in the temporal lobe, specifically the amygdala, the hippocampus and the parahippocampal gyrus, and several subregions of the prefrontal cortex in panic disorder symptomatology. In addition, a number of different neurotransmitter systems have been implicated in the hypersensitivity of the fear network. Although the spontaneous panic attack is the most prominent characteristic of the disorder, results of imaging studies seem to reflect mainly other manifestations in these patients, such as anticipatory and generalized anxiety, avoidance behavior and conditioned fear responses. Theoretical models in PD are based on the assumption that each component of the disorder is linked to a distinct neuroanatomical system in the brain. Since a clear description of the emotional state at the time of data acquisition is lacking in most studies, proper neuroanatomical differentiation between panic attacks, anticipatory anxiety and other related symptoms in PD is not yet possible. In addition, various anxious states, including panic attacks, are not specific for panic disorder, but rather characterize anxiety disorders in general as well as ‘physiologic’ anxiety and fear responses. This necessitates between-diagnosis comparisons. Moreover, to investigate whether the observed neuroanatomical and neurophysiological abnormalities predispose to panic disorder, or are secondary to chronic symptomatology or medication, longitudinal follow-up measurements should be performed. Considering the fast development and innovation of imaging technology, increasing the temporal and spatial resolution, future research in this field is promising.

Introduction

Panic disorder (PD) is characterized by recurrent, spontaneous panic attacks, i.e. acute episodes of intense fear, accompanied by physical as well as cognitive symptoms. Several theoretical models have been proposed to explain the pathophysiology of PD. In this context, a wide range of disparate issues have been addressed, including dysfunctional serotonergic and/or noradrenergic systems[1-5], aberrant responsivity to CO_2 at the level of the brainstem[6], hyperresponsivity of the amygdala and associated limbic structures[7;8], and abnormal functioning of the GABA-benzodiazepine receptor complex[9] or cholecystokinin-B (CCK_B) receptor[10]. However, the extensive literature addressing the neuroanatomical structures and neurotransmitter systems assumed to be implicated in PD, and the inconsistencies between findings, show the complexity of the topic.

An alternative approach to investigate the pathophysiology of PD is neuroanatomical differentiation between symptom dimensions[11;12]. Gorman and colleagues[11] brought together biological-pharmacological and cognitive-behavioral findings in PD and linked the three main components of the illness, anticipatory anxiety, panic attacks and phobic avoidance, to three distinct neuroanatomical systems in the brain: the brainstem, the limbic circuit and the prefrontal cortex, respectively. In a revised version of their hypothesis[7], the amygdala was introduced on the basis of its role in conditioned fear[13] and the suggested overlap between conditioned fear and PD. Daekin and Graeff[1;5], in contrast, suggested PD to be mainly associated with unconditioned fear. Their theory was based on the dual role of serotonin (5-HT), a neurotransmitter which, on the one hand, facilitates conditioned fear responses via activation of limbic structures, such as the temporal cortex, amygdala and hippocampus and, on the other hand, inhibits unconditioned fear responses via brainstem structures, specifically the dorsal periaqueductal grey (PAG). They suggested that conditioned fear relates to generalized and anticipatory anxiety, while unconditioned fear is akin to panic. Tests in healthy volunteers supported this dual role of 5-HT: administration of the 5-HT agonist fenfluramine attenuated (in a dose-dependent way) anxiety, induced by a simulated public speaking test (reflecting decreased unconditioned fear), whereas fenfluramine did not significantly change the amplitude of skin conductance during the conditioned fear test[14]. Considering that PD involves not only spontaneous panic attacks, but also anticipatory anxiety, avoidant behavior and even situation-based panic attacks, conciliation between the models of Gorman et al.[7;11] and Daekin and Graeff[1;5] is possible. Both theories assume neuroanatomical differentiation between different aspects of anxiety, irrespective of the diagnosis.

The development and sophistication of various structural and functional neuroimaging techniques have provided the opportunity for a further exploration of the neuroanatomical correlates of PD symptomatology, and future research using these techniques is promising. Thus, up till now several different imaging modalities and experimental paradigms have been employed in an effort to delineate the pathophysiology of PD and associated anxiety states. A brief overview of these structural and functional neuroimaging modalities will be provided in order to stipulate their possibilities and limitations.

Magnetic resonance imaging (MRI) can be used to detect morphological (or structural) as well as functional changes. Structural MRI involves segmentation of brain

structures of interest, enabling calculation of volumes or detection of focal abnormalities in feature or density. Computerized tomography (CT) represents another structural imaging technique, providing less contrast between tissue classes than MRI, however. Both structural imaging techniques enable visualization of morphological abnormalities independent of the actual state.

Functional imaging studies, in contrast, measuring blood flow or glucose metabolism, are dependent on state or trait anxiety levels at the time of tracer distribution or image acquisition. Positron emission tomography (PET), single photon emission computer tomography (SPECT) and functional MRI have been used to investigate resting state characteristics as well as provoked anxious conditions. In addition, cognitive paradigms enable specific activation of brain systems of interest. In contrast to the considerable temporal and spatial limitations of PET and SPECT techniques, functional MRI, measuring regional blood oxygenation level dependent (BOLD) signal changes, is suitable to investigate flow changes during experimental conditions in relatively smaller regions. However, the most frequently used 1.5 Tesla MRI scanners also cannot distinguish anatomical regions smaller than 10-15 mm, such as individual brainstem nuclei. Since functional MRI is a non-invasive tool and does not require radiolabeled tracers, it enables multiple measurements.

One other domain in neuroimaging research focuses on neurochemical characteristics. Using radiolabeled high-affinity ligands, PET and SPECT methods enable *in vivo* measurements of global and regional receptor density and affinity. An alternative approach includes magnetic resonance spectroscopy (MRS), measuring changes in regional relative concentrations of specific compounds, such as neurotransmitters or their metabolites. Similar to functional imaging studies that measure changes in perfusion and metabolism, these ligand and MRS studies are influenced by state variables.

On the basis of this brief overview, we may conclude that two major streams in neuroimaging research exist: one focusing on structural and functional abnormalities of specific neuroanatomical structures and functional brain circuits during different cognitive and/or emotional states, and another focusing on distinct and interacting neurotransmitter systems. What can they tell us in PD research? Results of resting state studies are supposed to reflect baseline differences between PD patients and healthy controls, consisting of anticipatory anxiety or phobic avoidance, reflected by altered activation patterns of temporal cortex and limbic structures. Challenge studies, in contrast, if these succeed in capturing the actual panic attack, are expected to visualize changes at the level of brainstem structures, thalamus and basal ganglia. Cognitive paradigms, with or without emotional manipulation, may further contribute to our understanding of specific brain regions involved in PD. Interestingly, ligand and MRS studies, as well as some challenge designs investigate altered neurotransmitter functioning and the interactions between different neurotransmitter systems. Neither the structural and functional approach of neuroanatomical circuitries nor the neurochemical approach can provide full insight in the pathophysiology of PD. Instead, integration of both approaches is needed.

In the present chapter, we critically discuss the results of various neuroimaging studies, keeping in mind their possibilities and limitations in probing the neuroanatomical and neurophysiological correlates of PD. To this end, we have categorized the

neuroimaging literature on PD according to the technique or paradigm used. First, we summarize the results of structural and resting state studies. Then, we describe studies using challenge agents, focusing on both neuroanatomical as well as neurochemical characteristics of PD. Next, abnormalities in neurotransmitter functioning, as addressed by ligand and MRS studies, are discussed. Since adrenergic ligand studies are lacking and there is only one serotonergic ligand study in PD, most results concern the benzodiazepine receptor complex and the associated gamma-aminobutyric (GABA)-ergic system. Finally, the results of some recent fMRI studies will be discussed. Although many fMRI paradigms contain a psychological challenge, we discuss these studies separately from those studies using pharmacological challenge.

Structural imaging studies

As mentioned before, structural abnormalities are assumed not to be dependent on the actual psychological or neurophysiological state of the subjects at the time of image acquisition, but rather seem to reflect static or slow-developing processes. Structural changes may be the result of vascular or neurochemical changes due to long-term symptomatology or chronic use of psychotropic medication. Alternatively, these morphological abnormalities may be due to genetically based, early developmental disturbances that predispose to PD. Since prospective follow-up studies are lacking, no conclusions can be drawn so far concerning the time of onset and mechanisms underlying the structural changes in PD.

Findings of structural imaging studies in PD are summarized in Table 3.1. Early MRI studies investigating neuroanatomical changes in lactate-sensitive PD patients reported increased focal abnormalities in the medial temporal cortices, mainly involving the right hemisphere[15;16]. These changes were correlated with illness duration and total number of lifetime panic attacks. Moreover, a trend was found between the incidence of structural abnormalities and the age of onset of the illness, suggesting a prognostic value[15]. A considerable methodological drawback of these studies was the inclusion of PD patients using benzodiazepines before and during the measurements.

Using qualitative CT and electroencephalography (EEG), Lepola et al.[17] investigated 45 unmedicated PD patients and found non-specific abnormalities with both techniques in some patients, while the vast majority exhibited normal EEG and CT findings. Since no control group was included, results are difficult to interpret. In contrast, another study combining EEG with structural imaging, found EEG-screening, similar to the lactate-provocation test, useful in identifying PD patients with a high probability of MRI brain abnormalities[18]. PD patients with non-epileptic EEG abnormalities compared with PD patients without EEG abnormalities and healthy control subjects showed increased morphologic brain abnormalities. These abnormalities mainly concerned the septo-hippocampal region.

Later studies, using quantitative volumetric MRI methods, confirmed the involvement of temporal regions in panic disorder, as suggested by the results of these early qualitative MRI studies. The first quantitative MRI measurements by Vythilingam et al.[19]

showed decreased volumes of left and right temporal lobes in PD patients compared with healthy control subjects. Also a trend to whole brain volume reduction was found, but this finding may be partly gender biased. Another methodological drawback of this study was the presence of comorbid psychiatric disorders. Contrary to their expectations, no significant difference in hippocampal volume was found between patients and controls.

Inconsistent with the results of Vythilingam et al.[19], later quantitative structural MRI studies have found volumetric changes of specific subregions of the temporal lobes, such as the amygdala[20;21], hippocampus[20] and parahippocampal gyrus[22]. Amygdaloid involvement in PD has been supported by preclinical evidence for its role in conditioned fear and the evaluation of emotionally relevant information[13]. Although one would expect the amygdala to be larger[23;24], not smaller, decreased bilateral amygdaloid volumes were found in PD compared with controls[20;21]. It has been suggested that the structural regional atrophy within the amygdala might be accompanied by functional increased sensitivity or hyperexcitability. Whether volume reduction of the amygdala predisposes to PD or rather reflects a neurodegenerative process secondary to the disorder is unclear. However, no significant correlation was found between volumetric changes of the amygdala on the one hand, and duration of the symptoms, number of panic attacks and demographic variables on the other hand. Although these correlational results, which have not yet been replicated, are inconsistent with those concerning temporal lobe measurements[15;16], they suggest a primary rather than a secondary process[21].

Uchida et al.[20] reported a trend for decreased mean volume of the left hippocampus in PD compared with healthy controls. They found a positive correlation between left hippocampal volume and illness duration, with recent cases showing smaller hippocampal regions than prolonged cases[20]. Left-to-right asymmetry of hippocampal absolute mean volumes (left < right) was found both in PD patients and in healthy control subjects[21]. However, when comparing normalized mean volumes, the hippocampal asymmetry remained in PD patients, but only reached near-significance in healthy controls. Voxel-based morphometry (VBM) analysis in PD revealed grey matter deficits in left parahippocampal gyrus[22]. This *structural* hippocampal and parahippocampal asymmetry is consistent with asymmetric left-to-right *functional* differences in these regions as found in resting state FDG- and $H_2{}^{15}O$ PET studies[25-28]. Massana et al.[22] suggested that the metabolic asymmetry in the (para)hippocampal region, attributed to increased right-sided metabolism, might reflect compensation for left-sided structural deficits in this region. Since a reduced left-sided hippocampal volume was also found in patients with posttraumatic stress disorder related to childhood physical and sexual abuse[29], this abnormality seems not to be restricted to PD but may constitute a general characteristic of anxiety disorders.

Considering the results of studies addressing structural differences in PD patients compared with control subjects, abnormalities and volume reductions mainly concern the temporal cortex or specific structures in the temporal lobe, such as hippocampus, parahippocampal gyrus and amygdala. Correlational analyses with clinical characteristics were found to be inconclusive. Inconsistencies in results may be explained by methodological issues: the use of non- or semi-quantitative MRI techniques[15;16;18], non-uniformity in the definition of regions of interest (ROI), gender bias[19], limited sample sizes[20;21], the presence of comorbid diagnoses[19], and the use of psychotropic medication[16]. Nevertheless, structural

Table 3.1. Structural imaging studies

Authors (public. date)	*Technique*	*Subjects*	*Main findings*	*Methodological comments*
Ontiveros et al. (1989)	Qualitative MRI	30 PD vs 20 co	43% of PD (compared to 10% of controls) showed abnormalities in temporal cortices, mainly right MTL. Correlation with age at onset, duration of illness and amount of panic attacks.	Retrospective clinical evaluation
Fontaine et al. (1990)	Qualitative MRI	30 PD vs 20 co	40% of PD (compared to 10% of controls) showed abnormalities in temporal cortices, mainly right-sided.	Benzodiazepines before and during measurements in PD
Lepola et al. (1990)	Qualitative CT and EEG	45 PD	Non-specific EEG changes in 24% incidental CT abnormalities in 20% of PD patients.	No control group
Dantendorfer et al. (1996)	Qualitative MRI and EEG	28 PD(+EEG-abn) vs 28 PD(-EEG-abn) vs 28 co	61% of PD(+EEG-abn) (compared to 18% of PD(-EEG-abn) and 4% of controls) showed MRI abnormalities, mainly in septohippocampal region.	
Vythilingam et al. (2000)	Quantitative MRI	13 PD vs 14 co	Decreased volumes bilateral temporal cortices. Trend to smaller whole brain volumes in PD. No hippocampal volume differences.	Comorbid diagnoses, unequal female/male-ratio (F>M in PD)
Uchida et al. (2003)	Quantitative MRI	11 PD vs 11 co	Decreased volume of left temporal cortex. Trend to smaller volumes of right temporal lobe, bilateral amygdala and left hippocampus. Left hippocampal volume correlated with duration of illness.	Limited group size
Massana et al. (2003a)	Quantitative MRI	12 PD vs 12 co	Decreased volume of bilateral amygdala. Hippocampal asymmetry (L<R) in both patients and controls. No volumetric differences in temporal and hippocampal cortices. No correlation with clinical variables.	Limited group size
Massana et al. (2003b)	Quantitative MRI (VBM)	18 PD vs 18 co	Decreased gray matter density in left parahippocampal gyrus.	

PD = PD patients, co = controls, PD(+EEG-abn) = PD patients with non-epileptic EEG abnormalities, PD(-EEG-abn) = PD patients without EEG abnormalities.

abnormalities in temporal structures are consistent with the results of metabolic changes in this region as reported by resting state functional imaging studies. Since no prospective longitudinal measurements have been performed, it is unclear whether structural changes predispose to the development of panic disorder or rather occur secondary to panic symptomatology or chronic use of psychotropic medication. Moreover, future research will need to investigate disorder-specificity of the findings, by comparing PD patients to subjects with other anxiety disorders.

Resting state imaging

Resting state studies assume PD patients to be at a baseline state at the time of data acquisition. Although study paradigms indeed try to create an atmosphere of 'rest' or neutral state (e.g. by a continuous performance task), the possibility exists that the scanning procedure has more emotional impact on PD patients than on healthy volunteers, inducing considerable anxiety or distress. Nazemi and Dager[30] investigated the psychological reactions and coping strategies, used in response to confinement in a MRI scanner, in 13 PD patients and 11 control subjects. Whereas the coping strategies of PD patients and healthy volunteers to general stress were dissimilar, they tended to converge in response to the stress induced by the experimental procedure. Prolonged MRI confinement resulted in a significant reduction in the fear of restriction, but not in the fear of suffocation.

The results of studies revealing baseline changes in PD are shown in Table 3.2. Using radiolabeled water ($H_2{}^{15}O$) PET, Reiman et al.[25;26] demonstrated abnormally low ratios of left-to-right parahippocampal blood flow and increased whole brain metabolism in PD patients who were vulnerable to lactate-induced panic attacks, compared with PD patients with a negative response on lactate challenge and healthy controls. They suggested, although could not prove yet, that the described asymmetry reflected increased right-sided metabolism. Although PET emission scans were acquired during resting state, preceding lactate-induced anxiety states may have influenced the results. For instance, Drevets et al.[31] showed that part of the results could be explained by vascular or muscular changes due to teeth clenching. In addition, vasoconstrictive effects of hyperventilation-induced hypocapnia following lactate-provocation may complicate the interpretation of the cerebral blood flow changes[7]. Another methodological drawback was the use of psychotropic medication during the experiment in some PD patients.

In subsequent studies, Nordahl and colleagues[27;28] examined cerebral glucose metabolism using ^{18}F-fluorodeoxyglucose (^{18}FDG) PET in unmedicated PD patients, irrespective of lactate-sensitiveness, during an auditory discrimination task. Although their results did not confirm whole brain hypermetabolism as reported by Reiman et al.[26], metabolic asymmetry in the (para)hippocampal region was replicated. This left-to-right asymmetry remained after imipramine treatment[28]. In addition, PD patients showed decreased metabolism in left inferior parietal and anterior cingulate cortices and increased metabolism in the medial orbitofrontal cortex compared with healthy controls[27]. Post-treatment metabolic measurements showed a decrease in the posterior orbitofrontal cortex in treated PD patients compared with untreated patients[28]. FDG PET was also used by

Table 3.2. Resting state studies

Authors (public. date)	*Technique*	*Subjects*	*Main findings*	*Methodological comments*
Reiman et al. (1984)	$H_2{}^{15}O$ PET	7 PD(+lactate-sens) vs 3 PD(-lactate-sens) vs 6 co	Decreased L/R-ratio in parahippocampal gyrus in PD(+lactate-sens) vs. PD(-lactate-sens) and co; no differences in whole brain metabolism.	Lactate provocation prior to PET measurement, limited sample size.
Reiman et al. (1986)	$H_2{}^{15}O$ PET	8 PD(+lactate-sens) vs 8 PD(-lactate-sens) vs 25 co	Decreased L/R-ratio in parahippocampal gyrus and increased whole brain metabolism in PD(+lactate-sens) vs. PD(-lactate-sens) and co.	Lactate provocation prior to PET measurement, 4 patients used psychotropic medication.
Nordahl et al. (1990)	^{18}FDG PET	12 PD vs 30 co	Decreased L/R-ratio in (para)hippocampus, decreased metabolic rate in left inferior parietal and anterior cingulate cortices, increased metabolism in medial orbitofrontal cortex, no global differences.	
Nordahl et al. (1998)	^{18}FDG PET	9 imipramine-treated PD vs 12 untreated PD vs 43 co	Decreased L/R-ratio in (para)hippocampus remained after imipramine treatment, decreased metabolism in posterior orbitofrontal cortex in treated vs unmedicated PD patients.	
Bisaga et al. (1998)	^{18}FDG PET	6 female PD vs 6 female co	Increased L/R-ratio in (para)hippocampal gyrus, decreased metabolism in R inf parietal and sup temporal cortices. No correlations with clinical variables.	Limited sample size.
De Cristofaro et al. (1993)	HMPAO SPECT	7 lacate-sens. PD vs 5 co	Decreased perfusion in bilateral hippocampus, decreased L/R-ratio in inferior prefrontal cortex and increased perfusion in L occipital lobe.	Lactate provocation prior to SPECT measurement, limited sample size.
Eren et al. (2003)	HMPAO SPECT	22 PD vs 19 co	Hypoperfusion in bilateral inferior frontal cortex.	

PD(+lactate-sens) = PD patients with positive response on lactate provocation, PD(-lactate-sens) = PD patients without positive response on lactate provocation.

Bisaga et al.[32] in six female lactate-sensitive medication-free PD patients and 6 female healthy control subjects. Instead of a decreased left-to-right ratio, they reported increased left-sided hippocampal and parahippocampal metabolic rates in the PD patients compared with the normal subjects. In addition, relatively decreased metabolic flow was reported for right inferior parietal and superior temporal cortices. No correlations were found between clinical variables and the PET findings.

An alternative technique to measure metabolic changes is Tc99m-hexamethylpropyleneamine oxime (^{Tc99m}HMPAO) SPECT. Comparing 7 lactate-sensitive drug-naive PD patients with 5 normal subjects, De Cristofaro et al.[33] found significant left-to-right asymmetry in the inferior prefrontal cortices, considered to be due to increased right-sided cerebral blood flow. In contrast to repeatedly reported right-sided hypermetabolism in the (para)hippocampal regions, *hypoperfusion* was found bilaterally in the hippocampal regions of these PD patients. Recently, Eren et al.[34] used the same technique, although without lactate provocation prior to SPECT measurement. They found a significant decrease in perfusion bilaterally in the inferior frontal cortex. Correlation analyses with disease severity scores did not reach significance after correction for multiple comparisons. In contrast to other studies, Lucey et al.[35;36] did not find any difference between PD patients and controls, but instead reported reduced metabolism in the caudate nucleus and the prefrontal cortex in OCD and PTSD patients compared with controls and PD patients.

Summarizing, the main findings of resting state measurements in PD show involvement of (para)hippocampal and prefrontal regions, presumably reflecting chronic anticipatory anxiety and cognitive modulation processes. Inconsistencies exist concerning lateralization and direction of effect, e.g. decreased or increased cerebral flow and metabolism compared with controls. Resting state differences may be confounded by underlying structural changes, prolonged effects of pre-test lactate challenge, vascular and muscular artifacts, as well as differences in scanning-related anxiety levels.

Challenge designs

Several methodological and physiological issues have to be considered when imaging the neurophysiological correlates of panic attacks. Since natural panic attacks occur at random intervals, challenge agents are necessary to induce and capture an attack. Many of these panicogenic agents have been used, such as sodium lactate[37], CO_2[38;39], the cholecystokinin B (CCK-B) receptor agonist cholecystokinin tetrapeptide (CCK_4) and its synthetic analogue pentagastrin[40;41], the noradrenergic alpha2-antagonist yohimbine[42;43] and the 5-HT-agonist fenfluramine[44]. Challenge agents should meet several criteria[45]. First, the agent should mimic closely naturally occurring anxiety, rather than induce a ‘cold’ or ‘as-if’ anxious emotion. This implies both physical and psychological, and peripheral as well as central characteristics of anxiety. Second, it should be replicable in order to be a useful research tool. Third, the induced symptoms should be short-lived or readily reversible. Finally, the agent should be able to reflect both trait and state responses, i.e. differentiate between normal and pathological subjects or diagnostic entities and between groups with various

responses to treatment, respectively.

Since panic attacks, occurring naturally as well as agent-induced, are brief, radiotracers with a temporal resolution compatible with this short time frame, such as $H_2{}^{15}O$, are used. However, the $H_2{}^{15}O$ PET technique visualizes changes in regional CBF, not metabolism. Although it is assumed that increased neuronal activity in a given region is accompanied by increased blood flow in that region, the possibility exists that the uncoupling of CBF and metabolism may occur under circumstances of heightened stress, such as a panic attack[7].

Another issue of consideration are the hemodynamic consequences of hyperventilation during the panic attacks. Hyperventilation-induced hypocapnia causes vasoconstriction. Subsequent diminished CBF then counteracts the expected increases in perfusion, as found in the study of Stewart et al.[46]. To correct for the vasoconstrictive effects of hyperventilation, pCO_2-adjusted CBF values can be used[47]. However, even after correction for differences in pCO_2, PD patients were found to be more sensitive to the vasoconstrictive effects of hyperventilation than comparison subjects[48]. The implied hypersensitivity was confirmed by the results of Dager et al.[49], using magnetic resonance spectroscopy (MRS) to measure brain-lactate levels during voluntary hyperventilation. Despite a comparable stable level of pCO_2, PD patients exhibited significantly greater rises in brain lactate than comparison subjects. Additional mechanisms have been suggested to underlie this hypersensitivity in PD, such as fear-associated stimulation of the locus coeruleus causing noradrenergic-mediated vasoconstriction[4] or of the parabrachial nucleus leading to global cerebral vasoconstriction[50].

Based on theoretical models of neuroanatomical differentiation between anticipatory anxiety and panic attacks, results of challenge studies, in case they succeed in inducing a panic attack at the time of data acquisition, are presumed to reflect the neuroanatomical correlates of the attack. Since the brainstem and ventral hypothalamus are assumed to play a crucial role in panic, it is important to realize that small brainstem nuclei, such as locus coeruleus, PAG and raphe nuclei, and hypothalamic nuclei cannot be distinguished using current imaging techniques. Table 3.3 summarizes the results of different challenge paradigms performed in PD.

CO_2-challenge

During an explorative study addressing the role of hyperventilation in panic, the panicogenic effect of 20 minutes continuous inhalation of a 5%/95% CO_2/O_2 mixture was detected accidentally[39]. At the same time, another group reported panic attacks in anxious subjects following a single vital-capacity inhalation of a mixture of 35%/65% CO_2/O_2[38]. Both procedures are still frequently used in panic research (see for a review Rassovsky & Kushner[51]). However, as mentioned before, an increase in pCO_2 levels alters CBF, thereby potentially confounding and/or masking CBF changes produced by the panic attack. Therefore, in neuroimaging studies using CO_2 challenge, residual pCO_2 increases or decreases are used to mathematically correct the global CBF values[26;47]. In normal subjects, increased pCO_2 levels result in vasodilatation and enhancement of global CBF. Levels of pCO_2 and global CBF have been found to return to normal within 50 seconds post-initiation of CO_2 inhalation[52].

Compared with healthy volunteers, PD patients showed a decrease in global CBF,

and stable pCO_2-adjusted CBF, in response to hypocapnia and vasoconstriction induced by hyperventilation. Whereas normal controls returned to baseline levels soon after CO_2-challenge, PD patients exhibited a prolonged abnormal pattern of CBF response to provocation[52]. In contrast to their hypothesis, Mathew and Wilson[53] found no differences in perfusion between normal controls and anxious patients after CO_2 challenge. However, subjects, both controls and anxious patients, who experienced challenge-induced anxiety showed a more modest increase in CBF than subjects who did not experience anxiety. The finding that the panicogenic effects are not exclusively found in PD patients, but also in other anxiety disorders, such as specific phobias[54], as well as in healthy controls subjects[54;55], limits the use of CO_2 challenge for diagnostic purposes in PD.

Lactate-challenge

Since the classic experiment of Pitts & McClure[37], lactate-challenge is considered to be of diagnostic significance for panic disorder. Based on earlier studies reporting higher physical exercise-induced serum lactate levels in subjects with anxiety disorders compared with controls[56;57], these authors hypothesized lactate to be anxiogenic. To test this hypothesis, they infused 14 anxious patients and 10 healthy control subjects with sodium lactate and placebo. All anxious patients, except for one, and only two healthy control subjects, experienced severe anxiety following lactate infusion. These results have been replicated in several studies[58-63]. Successful treatment of the panic with antidepressants or benzodiazepines has been shown to block this lactate-induced anxiety[58;59;61;63]. Lactate comes close to fulfilling the requirements of an ideal challenge agent, as defined by Guttmacher et al.[45]. Although the exact mechanism underlying the anxiogenic properties of lactate remains unclear, hypersensitivity of central chemoreceptors has been suggested[11;64].

Stewart et al.[46] used dynamic SPECT with Xenon-133 to measure regional CBF changes in 10 drug-free PD patients and 5 healthy controls subjects before and during lactate-challenge. Six of the 10 PD patients and none of the control subjects experienced lactate-induced panic attacks. Whereas lactate infusion markedly raised whole brain perfusion in controls and those PD patients who did not panic, a minimal increase or even decrease in CBF was found in PD patients experiencing an attack. Although pCO_2 was not monitored during the experiment, this effect has been attributed to a large extent to lowered pCO_2 and vasoconstriction secondary to hyperventilation. During the panic attack, increased perfusion was found in the right occipital cortex.

In their $H_2{}^{15}O$ PET study, Reiman et al.[65] found that lactate-induced panic attacks correlated with increased perfusion in several regions of the brain, mainly in the bilateral temporal lobe. Although the involvement of these temporal regions was not found in lactate-challenge in non-panicking PD patients and controls, activation of the same temporal regions was also found to be involved during normal anticipatory anxiety in healthy volunteers[66].

Cholecystokinin (CCK_4) and pentagastrin challenge

CCK_4, as well as its analogue pentagastrin, has a high affinity for CCK_B-receptors. This subtype of CCK receptors is especially abundant in several brain regions implicated in fear responses, such as the rostral brainstem, hippocampus, amygdala and several parts of the cerebral cortex[67-69]. A close functional relationship between the GABA-ergic system and

CCK has been found in rodents; benzodiazepines have been shown to antagonize CCK-induced activation of hippocampal neurons[70] and CCK significantly enhanced GABA release in striatum, frontal cortex and hippocampus[71]. CCK_B-receptor agonists induced panic attacks and anxiety, in a dose-dependent manner, in healthy controls as well as in PD patients[40;72], although the sensitivity for this challenge is enhanced in PD [41]. The CCK_B-antagonist L-365,260 antagonized CCK_4-induced anxiety[73]. In addition, pretreatment with the serotonin-noradrenalin reuptake inhibitor imipramine or the selective serotonin reuptake inhibitor fluvoxamine decreased the vulnerability for CCK_B-agonists in PD patients, suggesting an interaction between CCK and other neurotransmitter systems[74;75].

In search of neuroanatomical correlates of anxiety in healthy volunteers, using CCK_4 as a panicogenic agent, Benkelfat et al.[76] reported increased regional CBF in the claustrum-insular-amygdalar region, the anterior cingulate cortex and the cerebellar vermis. Javanmard et al.[77] extended these findings by showing specific regional CBF changes as a function of time. Whereas early effects of CCK were accompanied by increases in regional CBF in the hypothalamic region, subsequent measurements showed increased regional CBF in the claustrum-insular region. In addition, decreased regional CBF was found in the medial frontal cortex.

So far, only one imaging study in PD has been performed using pentagastrin as panicogenic agent. Boshuisen et al.[78] compared brain perfusion before pentagastrin challenge ("anticipatory anxiety") and after subsiding the challenge-induced panic symptoms ("rest"), in 17 PD patients and 21 healthy volunteers. Both before and after pentagastrin challenge, differences between PD patients and healthy control subjects were found in virtually the same fear-related brain regions, although these activation patterns clearly differed in intensity. Thus, increased activation was found in the parahippocampal gyrus, superior temporal cortex, hypothalamus, anterior cingulate cortex and midbrain; decreased activation was reported for the precentral gyrus, inferior frontal cortex, right amygdala and anterior insular cortex. Unpublished data of this group showed a high panic incidence in PD patients during pentagastrin challenge, although control subjects experienced anxious arousal as well. Panic in PD patients as well as anxious arousal in controls was correlated with increased regional CBF in the parahippocampal gyrus, basal ganglia and parts of the prefrontal cortex, and decreased perfusion in the precentral gyrus, thalamus and other parts of the prefrontal cortex. Interestingly, in controls but not in PD patients, activation of the hypothalamus and bilateral amygdala was found during pentagastrin-induced anxiety. In addition, in PD partial normalization of the regional CBF patterns was found after successful pharmacological treatment[79]. Thus, the results of Boshuisen et al.[78;79] failed to demonstrate clear-cut neuroanatomical differentiation between different anxious states in PD as proposed by theoretical models.

Yohimbine challenge

Yohimbine, an α_2-adrenergic receptor antagonist, enhances synaptic availability of noradrenalin (NA) and increases sympathetic activity by blocking pre-synaptic α_2-autoreceptors. The locus coeruleus (LC) plays a central role in noradrenergic mediation of stress and anxiety. A comprehensive review addressing the LC-NA system in pathological anxiety has been published by Sullivan et al.[4]. Yohimbine, as a challenge agent, has some disadvantages. First, noradrenergic agents appear to induce 'as-if' anxious symptoms,

without the full emotional impact of the naturally occurring panic attacks[45]. Second, the specificity of yohimbine-challenge is limited, since also patients with PTSD were found to experience yohimbine-induced anxiety[80]. Also, adrenalin itself has been used as pharmacological agent in PD[81]. Since adrenalin is known to produce peripheral symptoms of arousal without penetrating the blood-brain barrier, anxiety during adrenalin provocation is therefore unlikely to result from a central neurochemical mechanism, as has been proposed for lactate and CO_2[11].

Compared with control subjects, PD patients show more yohimbine-induced subjective anxiety and increased cardiovascular biochemical responses, despite similar increases in NA levels[42;43;82]. Also, levels of 3-methoxy-4-hydroxyphenylglycol (MHPG), the peripheral marker of central NA turnover, are increased in PD patients following administration of yohimbine[42]. Drugs that reduce the locus coeruleus firing rate, such as the α_2-adrenergic agonist clonidine, have been shown to possess antipanic properties[83]. Acute clonidine administration also produced a greater decrease in anxiety and plasma MHPG levels of PD patients compared with normal volunteers, supporting the hypothesis of noradrenergic dysregulation in PD[84;85]. Moreover, treatment with the serotonin reuptake inhibitor fluvoxamine reduced yohimbine-induced anxiety in PD patients[86], illustrating the interaction between the noradrenergic and serotonergic system.

Neuroanatomical correlates of yohimbine challenge were investigated in healthy volunteers by Cameron et al.[87], using $H_2{}^{15}O$ PET. Compared with placebo, yohimbine produced a significant decrease in global perfusion and increased regional CBF in the medial frontal and insular cortices, as well as the thalamus and cerebellum. Since all subjects hyperventilated after yohimbine challenge, part of the decreased global perfusion may be explained by hyperventilation-induced hypocapnia. One subject, experiencing a yohimbine-induced panic attack, showed increased instead of decreased whole brain CBF. Increased activation of medial frontal cortex was correlated with increases in anxiety ratings.

An early, and so far the only, imaging study with yohimbine-challenge in PD patients has been done by Woods et al.[88]. Using ^{99mTc}HMPAO SPECT, they measured regional CBF changes after bolus injection of yohimbine and saline in 6 medication-free PD patients and 6 healthy control subjects. All PD patients and only one control subject experienced higher anxiety levels in response to yohimbine. Yohimbine decreased frontal cortical flow in PD patients, but not in controls.

Fenfluramine challenge

The serotonin (5-HT) agonist fenfluramine facilitates 5-HT release and inhibits 5-HT reuptake, in this way increasing serotonergic function. As mentioned before, serotonin is considered to play a dual role in anxiety. Animal experiments as well as clinical studies have given evidence for anxiogenic as well as anxiolytic characteristics of this neurotransmitter. Serotonergic projections to the brainstem and forebrain arise from the dorsal raphe nucleus (DRN) and the medial raphe nucleus (MRN), both modulating parallel functioning systems[1-3;5]. The MRN mainly projects to the lateral hypothalamus, temporal cortex, amygdala and hippocampus. The DRN, on the other hand, projects largely to brainstem structures such as the PAG and LC, as well as to the ventral hypothalamus, thalamus and basal ganglia. Reciprocal relationships exist between the raphe nuclei and the

Table 3.3. Challenge studies

Authors (public. date)	*Challenge/Technique*	*Subjects*	*Main findings*	*Methodological comments*
Ponto et al. (2002)	CO_2/ $H_2{}^{15}O$ PET	14 PD vs 12 co	Decreased global CBF and stable pCO_2-adjusted CBF during challenge in PD, compared to stable CBF and increased pCO_2-adjusted CBF in controls	
Stewart et al. (1988)	Lactate/ ^{133}Xe SPECT	6 PD (+lactate-sens) vs 4 PD (-lactate-sens) vs 5 co	Less increased (or decreased) whole brain CBF in PD (+lactate-sens) during lactate challenge compared to PD (-lactate-sens) and controls and increased regional CBF in right occipital region during panic attack.	Limited sample size
Reiman et al. (1989)	Lactate/ $H_2{}^{15}O$ PET	8 PD (+lactate-sens) vs 9 PD (-lactate-sens) vs 15 co	During panic attack in PD (+lactate-sens) increased CBF mainly in bilateral temporal cortex.	
Drager et al. (1994)	Lactate/ MRS	8 PD vs 8 co	Higher brain lactate levels before, during and after lactate infusion in unmed. lactate-sens. PD, vs med. PD and controls. After infusion, dissociation between decreasing blood lactate and increased brain lactate.	Limited sample size, medication in 5 out of 8 PD patients.
Boshuisen et al. (2002)	Pentagastrin/ $H_2{}^{15}O$ PET	17 PD vs 21 co	Anticipatory anxiety > rest in PD vs controls: increased activation in parahippocampal, superior temporal, anterior cingulate cortex, midbrain and hypothalamus.	
Boshuisen et al. (2003)	Pentagastrin/ $H_2{}^{15}O$ PET	17 PD vs 21 co	During panic in PD and arousal in controls: increased activation in parahippocampal gyrus, basal ganglia and prefrontal cortex. Hypothalamic and amygdalar activation in controls, not in PD.	
Meyer et al. (2000)	Fenfluramin/ $H_2{}^{15}O$ PET	9 PD vs 18 co	Decreased activation left posterior parietal-superior temporal cortex in PD compared to controls at baseline; greater increase in activation in same region after fenfluramine challenge in PD.	No placebo control groups, post-challenge scans after subsiding panic symptoms.
Woods et al. (1988)	Yohimbine/ HMPAO SPECT	6 PD vs 6 co	Decreased regional CBF in frontal cortex after yohimbine injection, compared to placebo, in PD, not in controls.	Limited sample size

locus coeruleus. Serotonergic projections from the DRN inhibit firing of LC neurons; noradrenergic innervation from the LC excites DRN neurons. In contrast, afferent noradrenergic projections from the LC inhibit the MRN. Serotonergic inhibition of the PAG is assumed to be crucial in preventing unconditioned fear responses such as panic attacks[1;5]. This inhibitory effect is strengthened by noradrenergic inhibitory innervation of the PAG from the LC and the presence of GABA-ergic receptors at the level of the PAG. In contrast to the inhibiting effect of 5-HT in unconditioned fear responses, 5-HT facilitates conditioned fear responses (e.g. generalized and anticipatory anxiety) via activation of the limbic structures such as the amygdala and hippocampus.

The differentiation between the anxiolytic and anxiogenic properties of 5-HT is important for interpreting the results of fenfluramine challenge in panic disorder patients. Increased fenfluramine-induced anxiety was found in PD patients[44;89]. Since described anxiety symptoms were different from the classic characteristics of panic, these results suggest that fenfluramine enhanced anticipatory anxiety, rather than triggering a panic attack. This idea is supported by the results of Mortimore & Anderson[90], who investigated the effect of fenfluramine in resting state anxiety and CO_2-induced panic attacks in 13 medication-free PD patients during a double-blind placebo-controlled crossover design. Compared with placebo, fenfluramine increased anxiety prior to CO_2-challenge but attenuated CO_2-induced panic.

So far, only one imaging study using fenfluramine-challenge in PD has been published[91]. To compare regional CBF changes 20 and 5 minutes before and 20 and 35 minutes after fenfluramine challenge, $H_2{}^{15}O$ PET was used in 9 PD patients and 18 healthy volunteers. The study design lacked a placebo control condition. Compared with controls, PD patients showed a decreased regional CBF in the left posterior parietal-superior temporal cortex. Following fenfluramine challenge a greater increase in perfusion in the same region was found in PD patients compared with control subjects. No correlation was found between the presence of a fenfluramine-induced panic attack and peak voxel activity. Moreover, since fenfluramine-induced panic attacks, when present, subsided within 5 minutes of fenfluramine administration and the post-fenfluramine PET scans were acquired at 20 and 35 minutes after challenge, fenfluramine-induced activation of the left posterior parietal-superior temporal cortex in PD patients is unlikely to reflect the panic attack. More research on the relationship between serotonin and neuroanatomical correlates of baseline anxiety and panic attacks in PD patients is required.

Combining the results of challenge studies in PD, no clear conclusion concerning the neuroanatomical correlates of panic attacks can be drawn. Although many studies support the involvement of temporal and limbic structures (e.g. the amygdala, hippocampus and parahippocampal gyrus) in non-panic anxious states of PD, results with regard to the neuroanatomical correlates of panic attacks are inconsistent. Several explanations may be put forward for this. The first problem is capturing the attack in an experimental setting. The second problem is that the results of many challenge studies lack proper differentiation of the anxious states during data acquisition. Third, rapid habituation of the amygdala response may explain the inconsistencies concerning amygdaloid involvement in challenge-induced anxiety in PD. Finally, since the amygdala is only 15 mm of size and brainstem nuclei such as PAG and LC are even smaller, visualization of these regions is problematic

due to the limited spatial resolution of PET and SPECT, favoring the use of functional MRI techniques in future research.

Concerning the role of the monoaminergic systems in PD, challenge studies have shown that both NA and 5-HT are involved in the modulation of anxious states. Moreover, complex reciprocal interactions between the neurotransmitters exist. In addition to these monoamines, the GABA-ergic system has been investigated indirectly by the use of CCK and pentagastrin. As will be discussed below, more insight in the role of GABA has been provided by ligand and MRS studies.

Ligand studies

The GABA-benzodiazepine receptor complex

Based on the anxiolytic effects of benzodiazepines, most ligand studies in PD have addressed the benzodiazepine receptor function and its influence on $GABA_a$ transmission. Modulation of the GABA-benzodiazepine receptor complex is assumed to underlie the pharmacological actions of benzodiazepines as well as to mediate the anxious emotion itself. Conventional benzodiazepines act as benzodiazepine receptor agonists and facilitate the inhibitory effects of GABA, resulting in their sedative, anxiolytic and anticonvulsant effects. Inverse agonists, such as FG7142, in contrast, were found to produce opposite effects, i.e. increased anxiety and arousal[92]. Flumazenil and its synthetic analogue iomazenil are antagonists. They block the effects of both agonists and inverse agonists, but have few intrinsic effects themselves[93]. Although this spectrum of agonist activities at the benzodiazepines receptor may explain pharmacological effects in human anxiety, aberrant patterns have been found in PD. Panic disorder patients, compared with healthy controls, were found to be less sensitive to the conventional benzodiazepine diazepam using saccadic eye movement velocity as a dependent measure, suggesting a functional subsensitivity of the GABA-benzodiazepine complex in brainstem regions controlling saccadic eye movements[94]. Whereas conventional benzodiazepines are able to reduce anticipatory anxiety in PD patients, high-potency benzodiazepines are necessary to attenuate anxiety levels during a panic attack.

Abnormal benzodiazepine receptor functioning in PD may be explained by different mechanisms[95]. First, PD patients may have an overproduction of an endogenous inverse agonist. Second, it is possible that PD patients have a deficiency in a naturally occurring benzodiazepine full agonist. Very little is known about endogenous benzodiazepines ligands. The third hypothesis implies a shift in benzodiazepines receptor 'set-point' towards the inverse agonist direction. This theoretical hypothesis predicts reduced efficacy of benzodiazepines agonists, increased efficacy of inverse agonists and the emergence of partial inverse agonist actions for antagonists, such as flumazenil. Administration of flumazenil to PD patients resulted in an increase of panic attacks and subjective anxiety in comparison to controls[95;96]. This anxiogenic effect of flumazenil argues against the presence of endogenous anxiogenic (inverse agonist) ligands.

Several studies have investigated the benzodiazepine receptor function in PD using ^{123}I-iomazenil SPECT[97-101] or ^{11}C-flumazenil PET[102;103], as summarized in Table 3.4. Early studies were limited by the use of non-quantitative methods for the estimation of

receptor binding[97-99]. Other methodological caveats, limiting comparison of results, were the inclusion of diseased control subjects[97-99], the existence of comorbid diagnoses[99], the use of antidepressants[99;100] and limited group size[102]. In addition, timing of the paradigm seems to be of importance: whereas most studies reported baseline differences, Bremner et al.[101] were able to compare PD patients who panicked during the scan with PD patients who did not. The panickers, compared with the non-panickers, showed a decreased benzodiazepine receptor binding in the prefrontal cortex. In contrast to the results of Bremner et al. [101], who reported decreased binding in frontal cortex to be correlated with panic severity, Abadie et al.[103] did not find an effect of symptom severity.

Baseline differences in benzodiazepine receptor binding have been reported for several brain regions, mainly the prefrontal[97;98;100;102] and temporal cortices[97-102]. In contrast, no differences have been found by Abadie et al.[103], which may be explained by the inclusion of patients with different anxiety disorders instead of only PD. Whereas Malizia et al.[102] reported a global reduction in benzodiazepine receptor binding, with the greatest significance in the right orbitofrontal cortex and insula, no whole brain differences were found by Bremner et al.[101]. Concerning frontal involvement, some studies reported increased[98;100] and some decreased[97;102] benzodiazepine receptor binding in this region. In the temporal cortex, benzodiazepine receptor binding has been found to be reduced[97;99;101;102], specifically in the hippocampus[101], although increased binding in the temporal lobe was reported by Brandt et al.[100]. Altered benzodiazepine receptor binding has also been found in the occipital cortex[97], precuneus[101] and caudate nucleus[101].

To summarize, SPECT and PET studies investigating benzodiazepine receptor binding in PD have generated inconsistent results. However, decreased binding (global as well as regional) compared with control subjects, seems to be the main finding at the baseline state in PD. Decreased global and/or regional benzodiazepine receptor binding at baseline may be explained by different mechanisms[102]. First, modification of $GABA_A$-subunit composition, for instance by increased expression of α_6 subunits to which flumazenil does not bind, may be the result of genetic polymorphisms or environmentally induced modification of receptor configuration. Second, increased concentration of brain GABA may induce down-regulation of the GABA-benzodiazepine receptor complex[104]. However, instead of increased GABA concentrations, Goddard et al.[105], using MRS, found a 20% decrease in occipital cortex GABA levels in PD patients compared with controls. Also, serotonergic and noradrenergic changes influence benzodiazepine receptor binding[9]. Finally, structural brain abnormalities, such as temporal lobe atrophy, may explain decreased flumazenil binding.

Other ligand studies

So far, most ligand studies in PD focused on the GABA-ergic system. However, the use of other radiolabeled ligands, e.g. to measure changes in the adrenergic and serotonergic systems, is desirous and promising. Very recently, Neumeister et al.[106] reported the results of the first serotonergic ligand study in PD. Binding of the very selective 5-HT_{1A} receptor radioligands ^{18}F-trans-4-fluoro-N-2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]-N-(2-pyridyl)cyclohexanecarboxamide (FCWAY) was determined in 16 unmedicated PD patients and 15 healthy volunteers using quantitative PET measurements. PD patients, compared with controls, showed lower 5-HT_{1A}-receptor binding in the anterior and posterior cingulate

Table 3.4 Ligand studies in benzodiazepine receptor binding

Authors (public. date)	*Ligand/Technique*	*Subjects*	*Main findings*	*Methodological comments*
Schlegel et al. (1994)	^{123}I iomazenil SPECT	10 PD vs 10 co (+epilepsy)	Decreased benzodiazepines receptor binding in prefrontal, occipital and temporal cortex in PD patients compared to controls.	Qualitative measurements, use of carbamazepine in diseased control subjects.
Kuikka et al. (1995)	^{123}I iomazenil SPECT	17 PD vs 17 co	Increased R/L-ratio (R>L) in prefrontal cortex in PD patients compared to controls.	Qualitative measurements.
Kaschka et al. (1995)	^{123}I iomazenil SPECT	9 PD (+MDD) vs 9 co (+dysthemia)	Decreased benzodiazepines receptor binding in left lateral temporal cortex in PD patients compared to controls.	Qualitative measurements, use of medication in both groups, comorbidity, diseased control subjects.
Brandt et al. (1998)	^{123}I iomazenil SPECT	12 PD vs 9 co	Increased benzodizepine receptor binding in right supraorbital cortex and right temporal cortex in PD patients compared to controls.	Use of antidepressants (although naïve to benzodiazepines).
Malizia et al. (1998)	^{11}C flumazenil PET	7 PD vs 8 co	Global reduction in benzodiazepines receptor binding in PD patients compared to controls with largest reduction in right orbitofrontal cortex and insula.	Limited sample size.
Abadie et al. (1999)	^{11}C flumazenil PET	10 'anxiety' vs 10 co	No differences in benzodiazepines receptor binding between the groups; no correlation between binding and state- and trait anxiety scores.	Anxious subjects instead of PD patients.
Bremner et al. (2000)	^{123}I iomazenil SPECT	13 PD vs 16 co	Decreased benzodiazepine receptor binding in left hippocampus and precuneus and increased binding in right caudate nucleus and cuneus in PD patients compared to controls; panicking PD patients (N=7) showed decreased binding in prefrontal cortex compared to non-panicking PD patients (N=6).	

co (+epilepsy) = control group with epilepsy, PD (+MDD) = PD patients with depression, co (+dysthemia) = control group with dysthemia

cortices and in the raphe nuclei. No significant differences were found between PD patients with or without a comorbid depressive disorder.

Functional MRI experiments

Notwithstanding the advantage of increased spatial and temporal resolution and non-invasive visualization of cerebral blood flow changes, functional MRI studies are sparse in PD. The first functional MRI study in PD was done by Bystritsky et al.[107], using imagery exposure. Six PD patients and 6 healthy control subjects performed directed imagery of neutral, moderate and high anxiety-related situations based on an individually determined behavioral hierarchy. Increased activation during high anxiety blocks compared with neutral blocks was found in the right inferior frontal cortex, the right hippocampus, bilateral anterior cingulate cortex extending into the anterior prefrontal gyrus, and the posterior cingulate cortex extending into the precuneus. Although anxiety ratings were significantly higher in PD patients compared with controls, no panic attack occurred during the experiment. Increased activation in the amygdala could not be visualized, due to signal drop out in that region of the MRI images. Moreover, since the small sample size did not permit between-group statistical comparisons, replication of the findings in a more extended sample is needed.

Maddock et al.[108] compared 6 PD patients with 8 control subjects while making valence judgments of threat-related and neutral words using functional MRI. Compared with the healthy volunteers, the PD patients showed increased activation of the left posterior cingulate and dorsolateral prefrontal cortices in response to threat-related words. In addition, PD patients had significantly more right greater than left asymmetry in the parahippocampal region. Although PD patients reported more anxiety during the test than the controls, no amygdaloid response was found.

In a recent study, we investigated the disease-specificity and neuroanatomical correlates of cognitive and emotional Stroop interference effects across different anxiety disorders[109]. In contrast to the results of Maddock et al. [108], increased right-sided amygdaloid and hippocampal activation was found in PD patients, correlating with increased interference in response to panic related threat words[109]. Activation patterns in PD patients were compared with those in OCD and hypochondriasis patients and healthy volunteers. Whereas in OCD patients emotional interference was restricted to disease-specific (OCD related) words, in PD patients frontal-striatal activation was found during color naming of both OCD and panic related words. However, color naming of panic related words was significantly slowed and was correlated with additional activation of the right amygdala and hippocampus. Although PD patients did not panic during the experiment, they showed increased activation of the rostral brainstem during color naming of panic related threat words. Hypochondriasis patients showed a similar activation pattern as PD patients, but without the amygdaloid response. In contrast to the robust emotional interference effects, PD patients showed minimal interference on color naming of incongruent color words, i.e. the cognitive variant of the task.

Results from the few fMRI experiments published so far do not support the clear

neuroanatomical differentiation between panic and non-panic anxiety as supposed by Gorman et al.[11] and Deakin & Graeff[1]. Although these fMRI paradigms have shown to provoke considerable non-panic anxiety and distress in PD patients, no panic attacks during MRI image acquisition have been reported. However, increased activation during these non-panic anxious states has been found in brain regions assumed to be related to both anticipatory anxiety and panic. Provocation experiments using fMRI, enabling visualization of brain activation changes during panic attacks with good temporal and spatial resolution, are needed to further investigate this issue.

Another domain of fMRI research, desirable but not yet addressed in PD so far, consists of cognitive paradigms with or without emotional manipulation, e.g. memory tasks. PD patients show an explicit memory bias for threat stimuli, compared with neutral stimuli[110]. Although the hippocampus has been implicated in PD, no imaging studies have been performed to investigate if altered hippocampal functioning is responsible for the impaired memory function. Moreover, to differentiate between global anxiety traits and disorder specific abnormalities, comparison with other anxiety disorders is needed.

Conclusions

Structural and resting state studies have been performed to investigate the neuroanatomical and neurophysiological characteristics of PD patients at rest. Focal abnormalities and volume reductions have been found mainly in the temporal lobe, specifically in the amygdala, hippocampus and parahippocampal gyrus. Resting state results mainly support involvement of the (para)hippocampal regions and the prefrontal cortex. Since prospective follow-up designs are lacking, it is not clear whether these abnormalities predispose to panic disorder or rather reflect secondary processes due to long-term symptomatology or chronic use of medication. Moreover, the specificity of these findings for PD still has to be established by comparing different anxiety disorders or different symptom dimensions. Based on the assumption of the limbic system to be involved in anticipatory anxiety, it might be argued that these baseline studies have visualized anticipatory anxiety or experiment-induced distress rather than a baseline state characteristic of PD. The scanning environment may induce claustrophobia more easily in PD patients than in healthy volunteers, confounding the results. Probably the most important and most difficult question in imaging PD seems to be: at what (anxious) state is the subject at the time of image acquisition?

The same issue is relevant with respect to challenge studies. Challenge studies do not always succeed in capturing the panic attack. Therefore, challenge-induced anxiety in PD varies from anxious baseline states to near-panic anxiety or real panic attacks. Other important methodological issues are differences in time frames of the experimental paradigms, limited temporal resolution of PET and SPECT techniques (missing visualization of rapidly habituating structures), limited spatial resolution (missing activations of small regions of interest, e.g. nuclei in brainstem and hypothalamus) and hemodynamic changes due to hyperventilation-induced hypocapnia.

Ligand studies in PD have mainly investigated the GABA-benzodiazepine receptor complex. Most studies have reported altered baseline benzodiazepine receptor

binding, both increased and decreased, in temporal and frontal brain regions. Panic symptomatology has been correlated with decreased benzodiazepine receptor binding in prefrontal regions, although this finding still needs to be replicated. So far, only one study has used a serotonergic radioligand, showing decreased 5-HT_{1A}-receptor binding in cingulate cortex and raphe nucleus. No ligand studies have been performed in PD to investigate the pharmacokinetics of (nor)adrenergic receptors. Considering the complexity of interacting neurotransmitter systems and their differential roles in the mediation of normal as well as pathological anxiety, future research should incorporate paradigms suitable to compare well-described anxious states before and after pharmacological and cognitive-behavioral treatment.

Although functional MRI paradigms have been used to investigate cognitive functions in many psychiatric disorders, these studies are limited in PD research. Results from fMRI studies using cognitive paradigms, e.g. memory tasks, may contribute to a better understanding of the brain regions involved in PD and the mechanism behind the increased attentional bias and explicit memory bias to threat stimuli in these patients.

Theoretical models in PD are based on the assumption that each component of the disorder is linked to a distinct neuroanatomical system in the brain. However, since a clear description of the emotional state at the time of data acquisition is lacking in most imaging studies, proper neuroanatomical differentiation between panic attacks, anticipatory anxiety and other related symptoms in PD is not yet possible. To better establish the neuroanatomical correlates of different anxious states, in PD patients as well as in patients with other anxiety disorders and healthy controls, different neuroimaging modalities should be combined. For instance, a promising design would be a combination of an adrenergic or serotonergic ligand study and a cognitive fMRI paradigm during a controlled emotional state, with subsequent volumetric measurements of those brain regions identified during functional imaging. In addition, longitudinal follow-up measurements are needed to investigate the effects of medication, psychotherapy, long-term symptomatology and comorbidity. Moreover, comparison between PD patients, their first-degree relatives and non-related control subjects may elucidate underlying genetic predisposition. Also, neuroanatomical correlates as revealed by imaging studies, can be used as phenotypes in further genetic determination.

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Attenuation correction of PET activation studies in the presence of task related motion.

van den Heuvel OA, Boellaard R, Veltman DJ, Mesina CT, Lammertsma AA

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Abstract

Motion induced misalignment between transmission and emission scans can result in erroneous estimation of regional tissue activity concentrations. If this motion is of a random nature, mismatch between transmission and emission scans is likely to result in diminished signal-to-noise ratios. In case of task related motion, however, corresponding systematic reconstruction artefacts may lead to false-positive or false-negative results.

The purpose of the present study was to investigate whether implementation of an image registration (IR) method, which allows for motion-corrected attenuation correction, would improve accuracy of $H_2{}^{15}O$ PET activation studies. To evaluate the performance of this method, phantom studies as well as studies in human subjects were performed. Results were compared with three alternative methods: standard attenuation correction without motion correction, calculated attenuation correction, and no attenuation correction.

The phantom measurements showed that, for quantitative assessment of regional activity concentrations, the IR method was superior to the other attenuation correction methods. In a single-subject study with intentional task related motion during a visual stimulation paradigm, false-positive results, obtained with the standard attenuation correction method, disappeared after attenuation correction using the IR method. Finally, a group analysis of 11 patients indicated that an increase in signal-to-noise ratio was obtained with the IR method. Therefore, in our view, the IR method should be considered as a first choice for attenuation correction in PET activation studies.

Introduction

Correction for tissue attenuation is a vital step in obtaining quantitative PET data. The most commonly used method to correct for tissue attenuation is by direct measurement using a separately acquired transmission scan. Alternatively, tissue attenuation can also be calculated. In this case, not only assumptions have to be made about density of brain tissue, but also about density and thickness of the skull. Relative advantages and shortcomings of both methods have been described in detail by Huang et al.[1]. As measured attenuation correction requires fewer assumptions, it has become the accepted standard. An important problem with measured attenuation correction is the possibility of patient motion between transmission and subsequent emission scans. This motion can lead to misalignment between transmission and emission scans, resulting in inaccurate attenuation correction. This, in turn, may introduce erroneous estimations of regional activity concentrations. In case of $H_2{}^{15}O$ PET activation studies, motion can have two effects. If motion is random, transmission-emission mismatch is likely to result in a general reduction of signal-to-noise-ratios. On the other hand, if motion co-occurs with an experimental condition (i.e. is task related), systematic reconstruction artefacts will occur, which may lead to both false-positive and false-negative results. A PET activation protocol with multiple injections usually takes one to two hours, during which time the subject is supposed not to move. Whilst the use of head fixation devices restricts larger movements, it will not abolish them completely[2;3]. Although realignment of reconstructed images is an essential and standard step in data processing, it will only realign emission scans. The procedure cannot correct for possible mismatches between emission and transmission scans and, consequently, also not for associated attenuation correction errors. Therefore, correct alignment of emission and transmission scan data is important, in particular in the case of task related motion.

For activation studies, where only changes in activity in repeat scans on the same subject are of interest, several options exist for dealing with motion between transmission and emission scans. In theory, no attenuation correction should introduce the same error in repeat scans and, therefore, not affect the detection of changes. Secondly, calculated attenuation correction can be considered. Finally, a motion corrected attenuation correction can be performed using a suitable image registration (IR) method[4]. The latter method has the advantage that it is also applicable to ligand studies, as described elsewhere[5]. However, application of this method for ligand studies still needs validation and the application of the method may not be feasible in case of poor statistical quality of dynamic PET data. In addition, the IR method has only been evaluated on a region of interest basis in a limited number of patients[6].

The purpose of the present study was to evaluate the IR method on a voxel by voxel basis and to assess which attenuation correction method performs best for activation studies. To this end, both phantom studies and studies in human subjects (single subject validation study and clinical group analysis) were used to compare the IR based attenuation correction with the following alternatives: standard attenuation correction using the transmission scan without motion correction, contour based calculated attenuation correction, and no attenuation correction at all.

Methods

All scans were acquired on an ECAT EXACT HR+ PET scanner (CTI/Siemens, Knoxville, TN), the characteristics of which are well documented [7;8]. In the present study transmission scans of 10 min were acquired in 2D mode for all studies. Transmission scans are based on three rotating ^{68}Ge rod sources, each about 150MBq for the studies described in this paper. All emission scans were acquired in 3D mode and data were reconstructed using FORE[9] and 2D ordered subset expectation maximisation (OSEM)[10;11] applying 4 iterations with 16 subsets[12]. Images were post-smoothed using a Hanning filter, resulting in an average resolution of about 7mm full width at half maximum (FWHM) in all directions. During reconstruction, a zoom of two was applied resulting in voxel sizes of 2.526 x 2.526 x 2.425 mm^3.

1. Implementation of image registration method

The IR method was first described and tested by Andersson et al.[4] and subsequently used by Smith et al.[6]. We have implemented this method for this study on SUN Ultra 10 workstations running under Solaris 2.7. As a detailed description of the method is presented in these papers, it will only be summarized here. The present implementation of the IR method consisted of the following steps[13]: (1) reconstruction of emission data without attenuation correction (AC); (2) calculation of transformation matrices between non-AC emission images and the first non-AC emission image; for this purpose the registration package AIR3.0.8, developed by Woods et al.[14], was used; (3) application of the inverse transformation matrices to the reconstructed transmission scan image; (4) forward projecting the transformed, and thus aligned, transmission scan images to yield motion corrected attenuation correction factors (ACF's) and; (5) finally, use of these motion corrected ACF's to reconstruct the emission scan data with attenuation correction. Note that this alignment will provide motion corrected attenuation factors. An additional alignment of the reconstructed emission images is also performed within the SPM99 software, as explained later. Although in theory we could reuse the calculated transformation matrices by the IR method within the SPM program, this has not been implemented.

The IR method is based on a number of assumptions. Firstly, it is assumed that the transmission scan and first emission scan, which is acquired immediately after the transmission scan, are well aligned. Secondly, it is assumed that transformation matrices can be derived accurately from non-AC emission scans. Thirdly, it is assumed that forward projected transmission images yield the same attenuation correction factors as those derived directly from transmission scans when patient motion is absent, i.e. when no transformation matrices need to be applied. Reconstruction and forward projection of transmission data might, however, reduce noise in the ACF's. Therefore, in the present study, this step was also applied to the first emission scan.

2. Phantom studies

Two phantom studies were performed to determine the accuracy of several steps involved in the IR method: one with a solid homogeneous 20 cm diameter cylindrical phantom, the other with a 3D brain Hoffman phantom (Data Spectrum, Hillsborough, NC, USA).

For the homogeneous phantom, which was filled with 30 MBq ^{68}Ge, a transmission scan of 10 minutes and emission scans of 20 million true counts were acquired for 3 positions. A count based emission scan was applied to obtain emission data with similar statistics as those obtained during patient studies (20-25 million true counts). The second position was 10 mm shifted vertically with respect to the first position and, subsequently, the third position was moved 10 mm laterally with respect to the second position, as indicated in Table 4.1. These shifts are a non-integer number of the voxel size (2.526x2.526x2.425mm^3) and are therefore unlikely to result in truncated results within the calculated transformation matrices. Movements were performed including the scanner bed and using the scanner bed motion controller. For the first position emission data were reconstructed using attenuation correction factors, which were derived directly from the transmission scan data, and using attenuation correction factors obtained after reconstruction and forward projection of the transmission data, but without any transformations. The latter experiment was performed to evaluate the accuracy of the algorithms used for reconstruction and forward projection of transmission scan images. Next, for the other positions, emission scans were reconstructed with attenuation correction based on: (1) the transmission scan acquired at the same position as the emission scans and (2) the transmission scan acquired at the first position, but corrected for motion using the IR method as described above. Reconstructed emission images based on both attenuation correction factors were compared using subtraction analysis. In addition, agreement between transformations calculated by the IR method and the actual position offsets were evaluated. Since a ^{68}Ge phantom was used for this experiment, emission spillover within the transmission scans is present. However, effects of emission spillover within the transmission scans may be ignored, since transmission scans at all positions must suffer from equal emission spillover due to the negligible radioactive decay of ^{68}Ge during the course of the experiment. In addition, the total amount of activity of 30MBq within the phantom will only result in a minor spillover effects within the transmission scan, as shown elsewhere[15].

Table 4.1. Accuracies of the IR method for a homogeneous phantom

Position Index	*Translation x, y, z (in mm)*	*Detected translation x, y, z (in mm)*	*Difference of pixel values after IR*
1	0, 0, 0		
2	0, 10, 0	0.22, 10.23, 0.41	<3%
3	10, 10, 0	10.40, 9.62, 0.35	<3%

Direction x= lateral, y= upwards, z= along scanner's axis.
IR= image registration based attenuation correction

The study with the 3D brain phantom, filled with 37 MBq ^{18}F, was performed in a similar way. In this case, however, four positions were used (Table 4.2). The second position was moved 10 mm upward from the first position, the third position was moved 10 mm in the axial direction with respect to the second position, and the fourth position was rotated 5 degrees clockwise along the axial axis with respect to the third position. With the latter

position also the accuracy of the method in the presence of a rotation could be determined. Again the scanner bed motion controller was used to carry out the movements, Rotation was determined using a calliper. During the evaluation of the method, manual segmentation was performed to exclude the bed from the transformations.

For all positions, transmission scans and emission scans of 20 million true counts were acquired. Data were analysed in the same way as for the homogeneous phantom experiment, i.e. with subtraction analysis. To evaluate the accuracy of the IR method in more detail, it was used to calculate the transformations amongst non-AC emission images between position 1 and 2, 2 and 3, 3 and 4 and 4 and 1, respectively. Multiplying all these transformation matrices should result in the identity matrix, a procedure described as the full circle theory[16]. Differences of the latter matrix with the identity matrix are a measure of the overall accuracy of the registration performance. The main advantage of the full circle theory is that it is also independent of inaccuracies in the applied phantom positions and rotations.

Table 4.2. Results obtained with the IR method for a 3D brain phantom

Position Index	*Translation x, y, z (in mm)*	*Detected translation x, y, z (in mm)*	*Difference of pixel values after IR*
1	0, 0, 0		
2	0, 10, 0	0.18, 9.72, 0.36	<2.1%
3	0, 10, 10	0.29, 9.67, 10.86	<2.0%
4	0, 10, 10 and 5° rotation along z-axis	0.13, 9.81, 10.62 and 4.3° rotation along z-axis	<3.0%

Direction x= lateral, y= upwards, z= along scanner's axis.
IR= image registration based attenuation correction

3. Validation study in human brain

This validation study comprised a simple visual cortex stimulation paradigm in a healthy volunteer. The transmission scan was followed by 12 emission scans (90 seconds each) using 445 MBq $H_2{}^{15}O$ injections. After the first 6 emission scans a controlled z-axis transformation of 7 mm was performed. The visual cortex stimulation paradigm consisted of a block design of two conditions; a baseline blind condition and a condition with visual cortex stimulation using high frequency binocular light flashes. Conditions were balanced over the scanning session. In this way 3 baseline and 3 active conditions were scanned in random order preceding and following the controlled transformation. Random motion of the subject was restricted using a head fixation strap, but as mentioned before, no complete immobilisation can be reached in this way.

Image reconstruction was performed in 4 ways: (1) without attenuation correction (NAC), (2) using the transmission scan to reconstruct all 12 emission scans neglecting the 7 mm z-axis transformation (Standard AC), (3) using a contour-based (CB) calculated attenuation correction, and (4) using the IR method. For patient studies, a radio-opaque head holder, with an average attenuation of only 2%, is used. It is therefore assumed that

the effects of including the head holder within the IR method on the accuracy of the calculated attenuation factors may be ignored. Reconstructed emission images were realigned, normalised and re-binned to voxel sizes of 3x3x3 mm, and smoothed by 10 mm, using SPM99 (Wellcome Department of Cognitive Neurology, http://www.fil.ion.ucl.ac.uk/spm). Subtraction analysis was performed comparing visual cortex stimulation to the baseline blind condition. Moreover, difference maps were created to compare the main effect of visual cortex stimulation before and after z-axis transformation.

4. Case-report of task related motion during $H_2{}^{15}O$ PET symptom provocation

To illustrate the impact of inaccurate attenuation correction due to subject motion, one case with task related motion during a $H_2{}^{15}O$ PET symptom provocation experiment is presented. This case concerns a female patient with obsessive compulsive disorder during a $H_2{}^{15}O$ PET activation study in which contamination fear was provoked using a block design consisting of two conditions: baseline condition (neutral pictures) and symptom condition (dirty pictures). Following the transmission scan 8 emission (4 baseline, 4 symptom) scans were performed, following 445 MBq $H_2{}^{15}O$ injections. The order of the conditions was fully randomised. Ninety seconds scans were acquired whilst the subject watched the pictures on a screen mounted in front of her. Motion of the head was restricted by the use of a head fixation strap.

Image reconstruction was performed using NAC, standard AC, CB and IR methods. Reconstructed images were realigned, normalised and smoothed and contrast images were calculated to investigate the effect of the symptom provocation.

To simulate the effect of motion, the first emission scan was copied 8 times to be sure that the regional distribution of activity concentrations equal for all scans. Attenuation correction was applied to these 8 identical emission scans using transmission scans, which were transformed in the opposite direction to observed patients motion. In this way differences in reconstructed data could only be the result of the induced mismatch between emission and transmission data. Subtraction analysis was performed to measure the effect of patient motion independent of difference in brain activation.

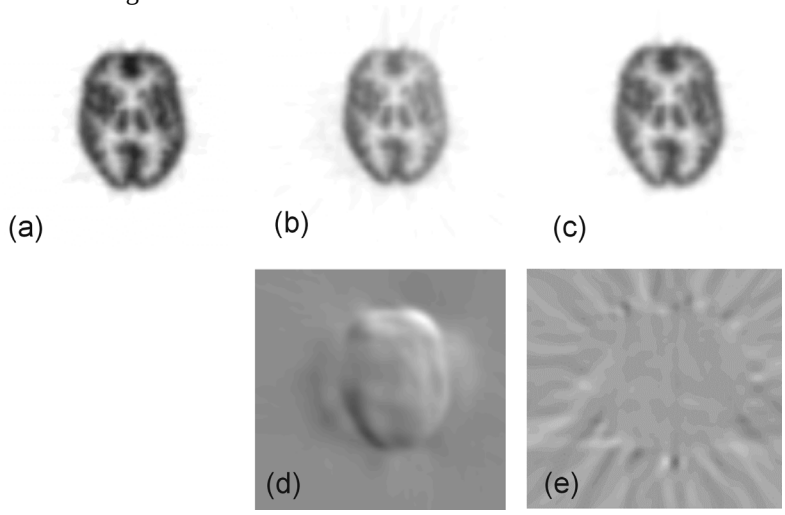
5. OCD-group analysis

A group analysis based on 11 patients with obsessive-compulsive disorder was performed to evaluate the effects of different attenuation correction methods on the overall result when data of more patients are combined. During the $H_2{}^{15}O$ PET activation study contamination fear was provoked using a block design consisting of two conditions: baseline condition (neutral pictures) and symptom condition (dirty pictures). Image reconstruction was performed using NAC, standard AC, CB and IR methods. Reconstructed images were realigned, normalised and smoothed and contrast images were calculated to investigate the effect of the symptom provocation. Results obtained with the different attenuation correction methods were compared. A detailed treatise of paradigm and clinical results is beyond the scope of this paper and will be reported elsewhere.

Results

1. Phantom studies
Results obtained for the homogeneous cylindrical phantom are shown in Table 4.1. ROI values of the emission images for position 1 reconstructed with attenuation correction derived from the transmission scan directly and with attenuation correction based on forward projection of a reconstructed transmission scan image agreed within 3% and with an average difference less than 1%, illustrating the accuracy of the algorithms used for reconstruction and forward projection of transmission scan data. The latter data were derived using several 1cm diameter ROI's placed within the phantom, Note also the excellent agreement between calculated and true transformations. Differences of ROI values larger than 20% were observed when transmission-emission misalignments were neglected, while these differences reduced to within 3% when the IR method was applied.

Figure 4.1. Reconstructions of emission scan of 3D brain phantom at position 3 (see Table 4.2) using (a): transmission scan of position 3, (b): transmission scan at position 1, (c): motion corrected transmission scan of position 1, (d): difference image between image a and b, (e): difference image between image a and c.



For the 3D brain phantom data are summarized in Table 4.2 and Figures 4.1 and 4.2. Use of the IR method resulted in emission pixel values which agreed within 3% as compared with the motion free attenuation correction, while differences larger than 15% were observed when the misaligned transmission scan was used without any motion correction. Figure 4.1 illustrates the observed difference for emission data reconstructed using attenuation correction with and without motion correction. Table 4.2 also shows the accuracy of the calculated transformations, i.e. actual offset was calculated within 0.9 mm and rotation along the axial axis within 1 degree. Figure 4.2 shows the transformation matrix obtained

according to the full circle theory, i.e. the product of all transformation matrices amongst all positions. It can be seen that residual errors after four transformations are very small, i.e. total translation errors smaller than 3 mm and a rotation error less than 1 degree.

Figure 4.2. Residual transformation matrix after application of full circle method

$$\begin{pmatrix} 1 & -7.2^{-4} & 9.2^{-4} & 0.07 \\ 7.2^{-4} & 1 & 1.9^{-4} & -0.24 \\ -9.2^{-4} & -1.9^{-4} & 1 & 0.11 \\ 0 & 0 & 0 & 1 \end{pmatrix}$$

2. Validation study in human brain

Effects of visual cortex stimulation are shown in Figure 4.3 en Table 4.3. Massive activation of the primary visual cortex was found, independent of the image reconstruction method used. Since the two conditions were equally balanced over the two sub-sessions of the experiment, effects of misalignment between transmission and emission scans were equal for both baseline condition and active condition with visual cortex stimulation. Therefore, in this case of task-unrelated motion, subtraction analysis obscured the problem of misalignment.

Figure 4.3. Single-subject validation experiment: main effects visual cortex stimulation. (a): image registration attenuation correction, (b): no attenuation correction, (c): contour based method, (d): standard attenuation correction.

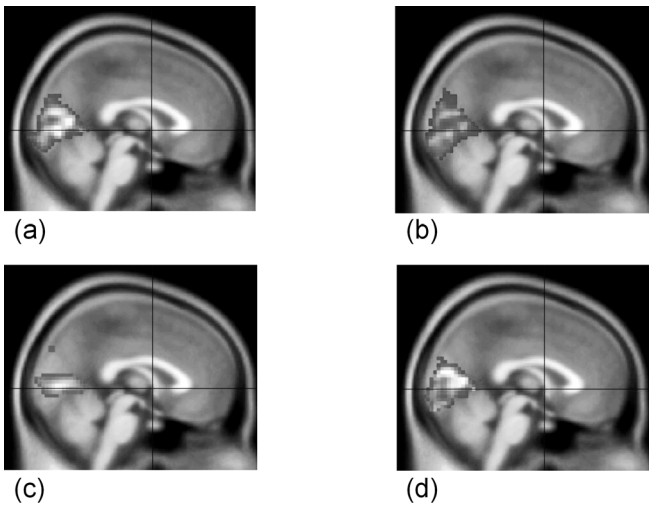


Table 4.3. Single-subject validation experiment: main effects visual cortex stimulation

	Talairach coordinates x, y, z (in mm)	*Cluster size (in voxels)*	*z-value*	*p-value (corrected)*
IR	15, -87, -6	2624	5.83	<0.0001
NAC	15, -87, -6	2770	6.17	<0.0001
CB	6, -78, 3	1023	5.38	0.003
standard	0, -69, 9	2120	5.68	<0.0001

IR= image registration, NAC= no attenuation correction, CB= contour based method

To compare the main effects of visual cortex stimulation before and after 7 mm z-axis transformation, difference maps were calculated (Table 4.4 and Figure 4.4). Using IR attenuation correction no effects of the 7 mm z-axis transformation were observed (i.e. complete correction). When no attenuation correction was used, border artefacts appeared bilaterally. Inferior parietal and prefrontal artefacts appeared after calculated and standard attenuation correction, respectively.

Figure 4.4. Single-subject validation experiment: difference maps before and after transformation. (a): image registration attenuation correction, (b): no attenuation correction, (c): contour based method, (d): standard attenuation correction.

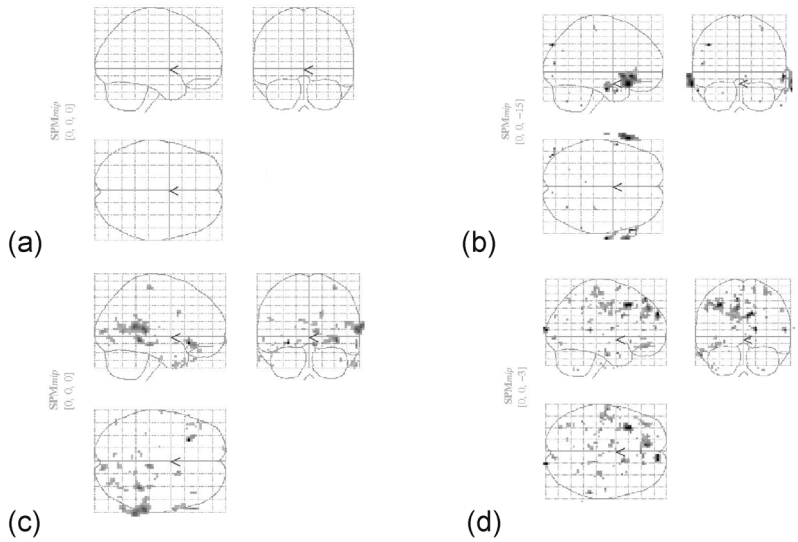


Table 4.4. Single-subject validation experiment: difference maps before vs. after 7 mm z-axis transformation

	Talairach coordinates x, y, z (in mm)	*Cluster size (in voxels)*	*z-value*	*p-value (corrected)*
IR	-	-	-	-
NAC	-69, 21, -18	180	4.54	0.037
	75, -9, -24	195	4.52	0.037
CB	-27, 24, -9	23	5.15	0.025
	63, -36, 9	421	4.64	0.025
standard	-33, 18, 45	114	4.98	0.007
	12, 60, 33	46	4.97	0.007
	18, -99, 9	13	4.89	0.007

IR= image registration, NAC= no attenuation correction, CB= contour based method

3. Case-report of task related motion during $H_2{}^{15}O$ PET symptom provocation

Randomisation of the conditions resulted is an unequal distribution of the conditions over the total study period in this patient, starting with 4 symptom conditions followed by 4 baseline conditions. Halfway the experiment, between the fourth and fifth emission scan, patient motion of about 4 mm in the z-axis occurred. Before and after this z-axis transformation no other significant patient motion occurred. Combination of poor randomisation and task related motion therefore resulted in systematic misalignment between the initial transmission scan and the fifth to eighth emission scans, which all corresponded to the baseline condition. In contrast, alignment of the transmission scan to the first four emission scans, corresponding to the symptom condition, was much better.

Table 4.5. Case report: main effects during provocation of contamination fear. Symptom condition > baseline condition

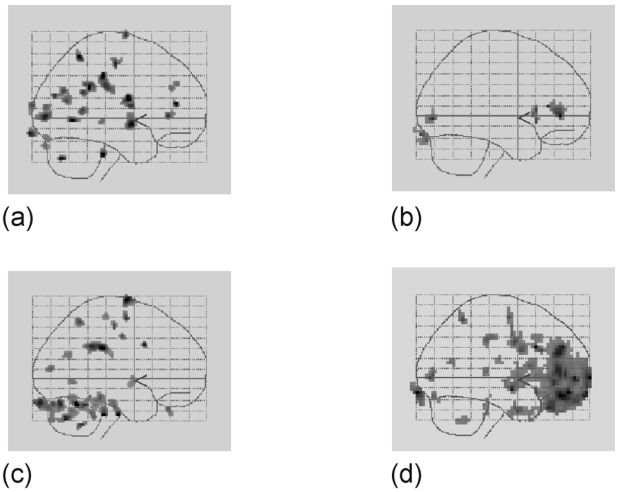
	Talairach coordinates x, y, z (in mm)	*Cluster size (in voxels)*	*z-value*	*p-value (corrected)*
IR	-	-	-	-
NAC	-	-	-	-
CB	-	-	-	-
standard	-9, 18, 30	29	5.40	0.002
	51, 51, -15	833	5.27	0.005
	-3, -99, -15	58	4.98	0.022
	0, 42, -18	1127	4.89	0.036

IR= image registration, NAC= no attenuation correction, CB= contour based method

Figure 4.5d shows the results of the subtraction analysis, comparing symptom with baseline conditions in case of standard attenuation correction. Large ventromedial prefrontal cortex

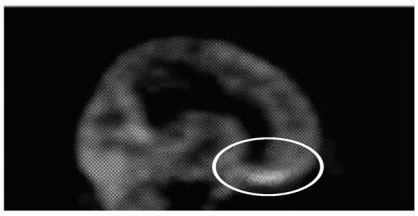
activation was found, which could have been associated with provocation of contamination fear. Different results, however, were found with alternative attenuation correction methods. Figures 4.5a, 4.5b and 4.5c illustrate results of subtraction analyses when IR, NAC, and CB methods were used, respectively (see also Table 4.5). The ventromedial prefrontal regions disappeared completely.

Figure 4.5. Case report: main effects during provocation of contamination fear. Symptom state > baseline. (a): image registration attenuation correction, (b): no attenuation correction, (c): contour based method, (d): standard attenuation correction.



To simulate the effect of motion, independent of difference in brain activation, the first emission scan was corrected for attenuation using 8 differently transformed transmission scans. The result of this simulation is shown in Figure 6, where the difference map of the SPM analysis is projected onto a reconstructed emission scan. The highlighted ventromedial prefrontal cortex indicates a false-positive area, which was detected as significantly activated in the SPM analysis. Note that this region corresponds with the results shown in Figure 4.5d.

Figure 4.6. Case report: simulation experiment



4. OCD-group analysis

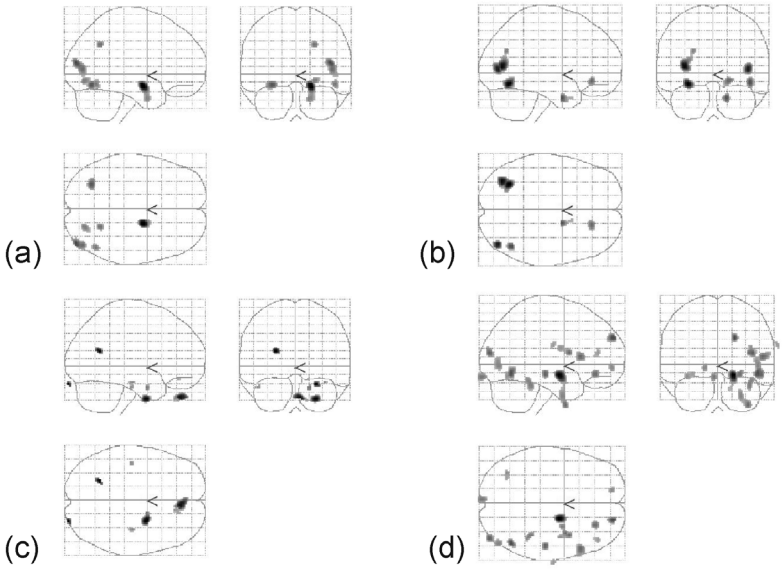
Table 4.6 and Figure 4.7 show the results of provoked contamination fear in 11 OCD patients compared with their baseline condition. Activation of the left amygdala was found to be more significant when using the IR method compared with standard attenuation correction. Moreover, when no or calculated attenuation correction was used, no significant difference between the conditions was found.

Table 4.6. Group analysis (N=11 OCD patients): main effects during provocation of contamination fear. Symptom condition > baseline condition

	Talairach coordinates x, y, z (in mm)	*Cluster size (in voxels)*	*z-value*	*p-value (corrected)*
IR	18, -6, -14	183	4.76	0.039
NAC	-	-	-	-
CB	-	-	-	-
standard	18, -6, -12	137	4.75	0.049

IR= image registration, NAC= no attenuation correction, CB= contour based method

Figure 4.7. Group analysis (N=11 OCD patients): main effects during provocation of contamination fear. Symptom state > baseline (a): image registration attenuation correction, (b): no attenuation correction, (c): contour based method, (d): standard attenuation correction.



Discussion

As illustrated in this study, subject motion during PET examination may lead to misalignment between transmission and emission scans. When standard attenuation correction is used this transmission-emission mismatch can result in both underestimations and overestimations of regional activity concentrations. If random motion occurs, transmission-emission mismatch is likely to result in diminished signal-to-noise ratios. This effect is shown both in the single subject validation study and the clinical group analysis. In the case of task related motion, as clearly shown in the clinical case-report, systematic reconstruction artefacts occur, leading to false-positive or false-negative results. Therefore, possible remedies to reduce motion induced reconstruction artefacts were compared.

Subtraction analysis is independent of subject motion when no attenuation correction is used. Results of the present study confirm that motion induced false-positive results, found when standard attenuation correction is used, disappear in the case of subtraction analysis without attenuation correction. Table 4.3 shows highest Z-values for NAC compared with IR, standard and CB methods. In case of no attenuation correction, however, border artefacts and extra-cranial activations appear, possibly due to the relatively higher pixel values near the border of the object. Moreover, this method is not suitable in the case of quantitative analyses, such as those used for ligand studies.

The contour-based attenuation correction methods involve image reconstruction without attenuation correction followed by manual geometric fitting of contours[17]. The present results show less significant results and artefacts when the CB method is used. This can be explained by increased noise in emission data resulting in irregular drawn contours, which in turn lead to increased noise and artefacts in reconstructed data. The CB methods neglect or only approximate density inhomogeneities within the head, thereby introducing inaccuracy. This drawback is of less importance in the case of $H_2{}^{15}O$ PET activation studies, but makes its use in ligand studies less attractive. Secondly, the method requires user intervention and selection of thresholds, which can easily introduce errors. To reduce user intervention, more automated contour-finding algorithms have been described in which object contours are estimated through back projection of emission sinogram borders, using the positions of maximum slopes in projection data, rather then from reconstructed images[17;18]. The advantage of the latter method is that no extra reconstruction is needed. However, the algorithm still assumes uniform skull and extra-cranial tissue thickness. Weinzapfel et al.[19] tackled the problem of natural variations in scalp and skull thickness, by using the reciprocal of each emission sinogram to generate the ACF's. The implementation of the CB method (CTI PET systems, ECAT software version 7.1), used in this study, however, might be less applicable for PET studies due to its poor performance in case of low count statistics in PET emission data.

A third approach is the realignment of repeated brief transmission scans with an initial high statistics transmission scan. Repeated brief transmission scans of less than 2 minutes, prior or immediately after each of the subsequent emission scans, can be performed to align the initial high quality transmission scan to the emission scans[20-22]. Since such low-counts transmission scans are not suitable for attenuation correction, the initial high quality

transmission scan is used to obtain accurate ACF's. However, even with this procedure, correct alignment between the short transmission scans and emission scans is not assured. The latter may be especially important in case of PET activation studies, where subject motion might be related with a specific condition or stimulus. In this case transmission scans need to be acquired while presenting the same condition to the subject as during each emission scan. The latter procedure, however, may confound subjects' task related activity, for example by inducing habituation.

A fourth alternative to prevent misalignment of emission and transmission data is simultaneous transmission and emission scan acquisition[23-26]. Simultaneous acquisition of transmission and emission scans reduces the efficiency of the technique significantly. The increase in noise must be weighted against the advantages of reduction in scanning time and the absence of misalignment between the scans. In addition, spill over from emission data into transmission data and vice versa might also result in less accurate attenuation correction and reduced quantification of PET scans.

Finally, optimal alignment between transmission and emission data, thereby improving the accuracy of attenuation correction, can be obtained by the image registration method, as proposed by Andersson et al.[4;5]. In the present study, this method gave the best reduction of noise and false-positive results. In addition, the group analysis showed that this method provided optimal statistics. Andersson tested the method on phantoms and a very few patient studies. Later, Smith et al.[6] tested this method on a limited number of patients. In the latter study, however, the effects of subject motion on attenuation correction and the performance of this image registration method was only evaluated for 2 regions of interest placed laterally in the brain. Therefore, the present study gives a more elaborate evaluation of the method, using phantom, single subject and group studies applying a whole-brain voxel based statistical analysis.

The phantom data showed excellent accuracy of the algorithms for image registration, reconstruction and forward projection of transmission scan data. The average difference was less than 1% for a homogeneous cylindrical phantom and less than 3% for a 3D-brain phantom. In contrast, differences larger than 15% were observed when misaligned transmission scans were used without any motion correction.

The human studies showed that the IR method reduced noise for a group analysis and removed false positive results in the case of task related motion, while improving the signal from expected activated areas. This was in contrast with NAC and CB methods. By using no attenuation correction, reduction of noise can be achieved at the cost of extra-cranial artefacts and loss of statistical power. The CB methods showed similar results, which might be due to poorly drawn (automatic) contours as a result of limited statistics in emission data. Despite the limitations of NAC and CB methods, they have advantages over standard AC in case of large patient motion.

Conclusion

For quantitative and parametric analyses of PET activations studies, the image registration method, which corrects for motion between transmission and emission scans, is superior to

reconstructions using standard (i.e. no motion correction) measured attenuation correction, contour based calculated attenuation correction and no attenuation correction. Therefore, in our view, the IR method should be considered as a first choice for attenuation correction in PET activation studies.

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5

Frontal-striatal system in planning complexity: A parametric functional MR version of Tower of London Task

van den Heuvel OA, Groenewegen HJ, Barkhof F, Lazeron RHC,
van Dyck R, Veltman DJ

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Abstract

In the present study, we sought to investigate which brain structures are recruited in planning tasks of increasing complexity. For this purpose, a parametric self-paced pseudo-randomised event-related functional MRI version of the Tower of London task was designed. We tested twenty-two healthy subjects, enabling assessment of imaging results at a second (random effects) level of analysis. Compared with baseline, planning activity was correlated with increased blood oxygenation level dependent (BOLD) signal in the dorsolateral prefrontal cortex, striatum, premotor cortex, supplementary motor area and the visuospatial system (precuneus and inferior parietal cortex). Task load was associated with increased activity in these same regions. In addition, increasing task complexity was correlated with activity in the left anterior prefrontal cortex, a region supposed to be specifically involved in third order higher cognitive functioning.

Introduction

Planning, i.e. the ability to achieve a goal through a series of intermediate steps, is an essential component of higher-order cognitive processes, such as problem solving. Central in the concept of the neuronal substrate for cognitive planning is the position of the dorsolateral prefrontal cortex, but other brain regions, like the premotor, cingulate, and insular cortices and the striatum have also been implicated in cognitive planning[1;2]. Whereas until recently insight in the role of brain structures in planning processes was primarily based on lesion studies in patients, contemporary studies are able to employ human brain imaging techniques. The results of such functional neuroimaging studies have suggested that a distributed cortico-cortical and cortico-subcortical network is being activated during complex planning tasks, although there is yet no complete agreement about the contribution of the various components of such neuronal networks.

A direct and very frequently used test to probe planning processes is the Tower of London task, originally developed by Shallice[3]. The Tower of London task has been modified in various ways to make it suitable for testing different components of cognitive planning in clinical and experimental psychological settings. The complexity of the task can range from trivial, requiring only one obvious move, to highly complex. The more complex versions of the task require the planning of intermediate, in many instances counter-intuitive steps to successfully solve a problem. Individuals with decreased prefrontal functioning, such as those with neurosurgical lesions[3], neurodegenerative diseases like Huntington's or Parkinson's disease[4-6] or psychiatric disorders like obsessive-compulsive disorder[7] and schizophrenia[8] are impaired on the task, in particular when complexity increases.

The Tower of London task is also very suitable to study in healthy subjects the patterns of brain activation in the context of planning tasks using Positron Emission Tomography (PET)[4;9-11] or Single-Photon Emission Computer Tomography (SPECT)[12;13]. The results of these imaging studies agree on the involvement of the dorsolateral prefrontal cortex and parieto-occipital regions (visuospatial system) during planning. However, activation of other brain regions, such as the cingulate and insular cortices, and the striatum, has not been found across all mentioned studies. Furthermore, the results of a functional MRI study using the Tower of London task of Lazeron et al.[14], confirmed activation of the dorsolateral prefrontal, parietal, cingulate and insular cortices but failed to show striatal activation. Several methodological differences between these studies may explain the inconsistencies in the results. First, different baseline conditions have been used, ranging from low-level conditions (watching a blank screen) to conditions involving motor activity (moving beads without a goal) or cognitive functioning (counting beads). Second, differences in the execution of the task exist: whereas in some studies subjects used a touch screen to perform the task[4;9;11], in other designs subjects were requested to execute their moves mentally[10;14]. A third likely source of inconsistency concerns the differences in task load. Because in subtraction designs the specificity of active vs. baseline differences for the (cognitive) function under study is often questionable[15;16], several researchers have used designs in which task load during performance of the Tower of London task was manipulated. For example, Baker et al.[10], in comparing 'difficult' (4-5 moves) and 'easy' trials (2-3 moves), found task load to be correlated with increased regional cerebral blood

flow (rCBF) in rostrolateral and dorsolateral prefrontal cortex, and the parietal and cingulate cortices. Owen et al.[9] reported increased activation in striatum and thalamus, but not in cortical areas. Lazeron et al.[14] did not find any difference between their 'easy' and 'difficult' versions, but they used a more difficult design comparing 2-4 moves to 5-7 moves. The only study employing a parametric approach in studying task load[4], used five levels of planning and showed a correlation between planning complexity and increased rCBF in the right caudate nucleus, and prefrontal and cingulate areas. The involvement of visuospatial areas in the parietal lobe, however, seemed not to be related to the task load. A general methodological drawback in the studies performed so far, is the lack of control over performance differences, due to the use of blocked designs. Since performance is likely to deteriorate at higher complexity, this is especially relevant for parametric tasks.

It must be assumed that the various cortical and subcortical brain structures that have been demonstrated to be involved in planning processes, execute these functions as "nodes" in distributed neuronal networks[1]. Employing a neuronal network model for planning processes, Dehaene and Changeux[17] proposed multiple hierarchic levels with ascending and descending streams for execution of planning and evaluation of this behaviour, respectively. This model also stresses that planning behaviour cannot be related to a single region, but rather relies on multiple neuronal circuits coding for specialised subprocesses such as working memory, plan generation and internal reward. These subprocesses may differentially contribute to different levels of planning behaviour; some sub-processes may be relatively independent of task load, while other sub-processes are involved mainly at higher levels of planning behaviour. The main aspect in increasing planning complexity is the increasing amount of sub-goals and counterintuitive moves, while holding in mind the overall main goal. Koechlin et al.[18] introduced the term "branching" for this process of integrating working memory with attentional resource allocation, a third order higher cognitive function. They found, using a multitask experiment, branching to rely on activation of the rostral part of the prefrontal cortex. To be able to investigate these specific sub processes and to differentiate between different brain regions in their contribution to different levels of planning behaviour, parameterisation of the task is essential. Therefore, the main aim of the present study was to determine the effects of task load in planning execution.

The present study employs event-related functional MRI, as a method to study activation patterns on a trial-by-trial basis. This enabled us (i) to randomise items with regard to task complexity, and (ii) to include correct responses only. A large number of subjects (n=22) was included, with the aim of performing second-level or random effects analyses, allowing for generalisation of our results. On the basis of the considerations above, we hypothesised that increased planning complexity would be associated with increased activity in dorsolateral prefrontal, visuospatial and striatal regions. In addition, we expected rostral areas of the prefrontal cortex to be specially involved in the higher levels of planning execution.

Materials and methods

Subjects

Twenty-two healthy right-handed subjects (11 men and 11 women; mean age 29.9 years, range 21-49 years) performed the Tower of London task, while functional MRI data were collected. Subjects were recruited among university students and staff. The ethical review board of the VU Medical Centre approved of the study and all participants provided written informed consent.

Figure 5.1. Display screen of the Tower of London task as used in the present study.
A: planning condition. B: baseline condition.

Task paradigm

To ensure that participants were familiar with the procedure, the test was explained and practised outside the scanner before MR imaging was performed. The present version of the Tower of London task consisted of six conditions: a baseline condition and five planning conditions ranging from 1 to 5 moves. In the planning condition, subjects were presented, on a single screen, a starting configuration and a target configuration with the instruction "count the amount of steps" displayed at the top and two answers at the bottom (Figure 5.1A). In both configurations, three coloured beads are placed on three vertical rods, which can accommodate one, two and three beads, respectively. One bead can be moved at a time and only when there is no other bead on top. Subjects were requested to determine the minimum number of moves necessary to reach the target configuration and to press the button corresponding to the side of the screen (left or right) where the correct answer was presented. In the baseline condition, subjects simply had to count the total of yellow and blue beads, a task which does not require any planning activity, the display being similar to the planning condition, but the instruction now being "count the total amount of yellow and blue beads" (Figure 5.1B). Moreover, the number of beads of each color in the two configurations, used for the baseline condition, was unequal, with the aim of preventing planning activity. We used a pseudo-randomised, self-paced design with a maximum response duration of 30 seconds for each trial. We adopted a pseudo-randomised design to control for any overflow effects (i.e. persevering of task related cognitive processes after a difficult trial). Therefore, each trial of 3 or more moves was followed by a baseline trial. No feedback regarding the answers was provided during the task.

Data Acquisition

Imaging was performed on a 1.5 T Sonata MR system (Siemens, Erlangen, Germany) with a standard circularly polarised head coil. Stimuli were generated by a Pentium PC and projected on a screen at the end of the scanner table, which was seen through a mirror mounted above the subject's head. Two magnet-compatible four-key response boxes were used to record subject's performance and reaction times (RT). To reduce motion artefacts, the subject's head was immobilised using foam pads; in addition, we used automatic online motion correction.

Anatomic imaging included a coronal 3D gradient-echo T1-weighted sequence (matrix 256x160, voxel size 1x1x1.5 mm, 160 sections). For functional MR imaging, a echo planar imaging sequence (TR 3.045 sec., TE 45 msec., matrix 64x64, field of view 192x192 mm, flip angle 90^{o}) was used, creating transversal whole-brain acquisitions (35 slices, 3x3 mm in-plane resolution, 2.5 mm slice thickness, 0.5 mm interslice gap). In total, 433 EPI volumes per subject were scanned. The distribution frequency of event types was based on RT data of a pilot study, so that a similar amount of scans (around 70 EPI volumes) was acquired per subject for each of the six conditions.

Data Analysis

Psychometric data were analysed using a standard statistical package. Imaging data were analysed using SPM99 (Wellcome Department of Cognitive Neurology, http://www.fil.ion.ucl.ac.uk/spm). After discarding the first 4 volumes, time-series were corrected for differences in slice acquisition times and realigned. Spatial normalisation into approximate Talairach and Tournoux space was performed using a standard SPM EPI template. Data were resliced to 2x2x2 mm voxels and spatially smoothed using a Gaussian kernel of 6 mm.

Next, data were analysed in the context of the General Linear Model, using delta functions convolved with a canonical hemodynamic response function to model responses of varying length to each type of stimulus. In addition, error trials were modelled separately as a condition of no interest. For each subject, weighted contrasts were computed for simple main effects (all active conditions vs. baseline) and for task load. Contrast images containing parameter estimates for main effects and task load were entered into a second level (random effects) analysis. All results are reported at $p < 0.05$ (z-score > 5.00) corrected for multiple comparisons.

Results

Task performance

Mean performance scores were 94.2 % (SD = 1.68) for baseline (mean RT = 3.7 sec, SD = 0.88), 97.6 % (SD = 2.02) for 1 move (mean RT = 4.4 sec, SD = 1.11), 95.4% (SD = 3.99) for two moves (mean RT = 5.8 sec, SD = 1.27), 96.7 % (SD = 3.58) for three moves (mean RT = 7.4 sec, SD = 1.90), 89.9 % (SD = 7.17) for four moves (mean RT = 10.1 sec, SD = 2.51) and 82.2 % (SD = 12.59) for five moves (mean RT = 15.0 sec, SD = 4.02). Both the decrease in performance ($F(4,18)=12.4$, $p<0.0001$) and the increase in RT ($F(4,18)=64.3$, $p<0.0001$) were statistically significant.

Imaging data

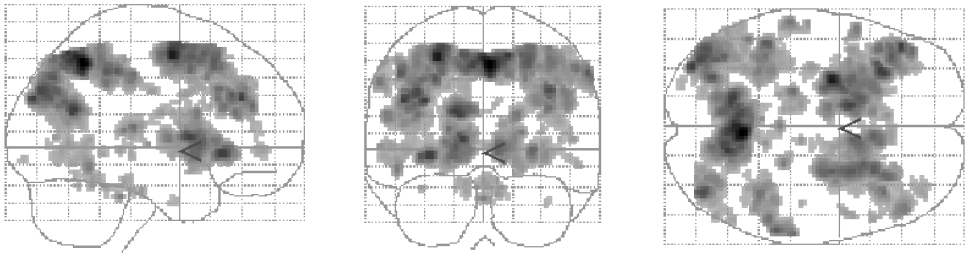
1. Planning vs. baseline

Regions showing increased BOLD signal during planning compared with baseline (Table 5.1 and Figure 5.2) were bilateral precuneus (Brodmann's area (BA) 7), bilateral inferior parietal cortex (BA 40), bilateral premotor cortex (BA 6), right dorsolateral prefrontal cortex (BA 9 and 46), left supplementary motor area, left caudate nucleus, right globus pallidum and right insular cortex.

Table 5.1. Maxima of regions showing significant ($p<0.05$ ($z>5.00$), corrected) BOLD signal increase during planning compared with baseline.

Region of activation	*Left/Right*	*Brodmann's area*	*Talairach coordinates*			*Cluster size (in voxels)*	*z-value*
			x	y	z		
Dorsolateral PFC	R	46	46	36	28	5	5.57
	R	9	40	30	30	7	5.17
Premotor cortex	L	6	-24	20	50	12	5.17
	L	6	-26	6	52	31	5.17
	L	6	-26	-4	54	7	5.08
	R	6	26	-4	54	90	6.05
SMA	L	32	-6	20	46	3	5.02
Precuneus	L	7	-4	-56	48	277	6.42
	R	7	6	-58	50		5.47
Inferior parietal	L	40	-38	-78	34	12	5.34
cortex	L	40	-60	-36	44	2	5.14
	R	40	34	-84	28	60	5.61
	R	40	28	-72	40	7	5.20
	R	40	48	-42	44	7	5.18
	R	40	40	-66	26	1	5.03
Caudate nucleus	L		-8	8	0	8	5.01
Globus pallidus	R		12	6	6	41	5.53
Insular cortex	R		32	24	-4	23	5.59

Figure 5.2. Glass brain renderings showing BOLD signal increase correlating with planning versus baseline.



2. Task load

Increased task load (Table 5.2) was correlated with increased BOLD signal in those areas that already showed an increased signal compared with the baseline condition, i.e. bilateral precuneus (BA 7), bilateral inferior parietal cortex (BA 40), bilateral premotor cortex (BA 6 and 8), right dorsolateral prefrontal cortex (BA 9 and 46) and left supplementary motor area. In addition, a number of areas showed increased BOLD signal specifically related to increased task load (Figures 5.3 and 5.4), i.e. left dorsolateral prefrontal cortex, left insular cortex, right pallidum and right caudate nucleus. The left rostral prefrontal cortex (BA 10) was also involved in the highest levels of planning complexity only. The reverse contrast (decreased BOLD signal associated with increased task load) did not show significant activations except for bilateral primary visual cortex.

Table 5.2. Maxima of regions showing significant ($p<0.05$ ($z>5.00$), corrected) BOLD signal increase correlating with increased task load.

Region of activation	*Left/Right*	*Brodmann's area*	*Talairach coordinates*			*Cluster size (in voxels)*	*z-value*
			x	y	z		
Anterior PFC	L	10	-36	60	8	2	5.02
Dorsolateral PFC	L	46	-40	50	16	15	5.52
	L	46	-44	32	24	23	5.27
	L	9	-44	36	38	33	5.37
	R	46	40	36	24	134	5.72
	R	9	44	32	32		5.13
Premotor cortex	L	6	-24	16	56	197	5.98
	L	6	-18	-12	58	3	5.19
	L	6	-32	-4	58	23	5.29
	R	6	26	10	58	212	5.69
	R	8	28	34	42	33	5.60
SMA	L	32	-6	16	50	94	5.97
Precuneus	L	7	-12	-56	52	323	6.86
	R	7	6	-60	48		5.50
Inferior parietal	L	40	-42	-40	36	51	5.89
cortex	L	40	-48	-42	46	87	5.73
	L	40	-40	-78	34	7	5.47
	L	40	-40	-50	16	15	5.52
	L	40	-24	-76	50	5	5.37
	R	40	38	-72	34	52	5.83
	R	40	50	-42	42	20	5.64
	R	40	48	-42	50	3	5.00
	R	40	34	-64	44	3	5.06
Caudate nucleus, caput	R		18	6	18	23	5.43
Globus pallidus	R		12	4	4	23	5.58
Insular cortex	L		-32	20	0	53	5.88

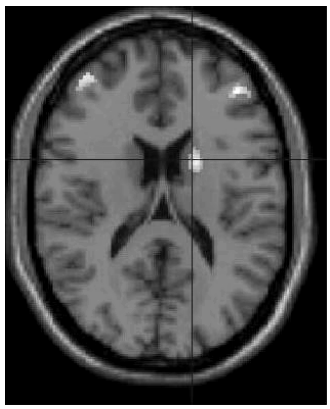


Figure 5.3.
BOLD signal increase in bilateral dorsolateral prefrontal cortex and right caudate nucleus, correlating with increased task load.

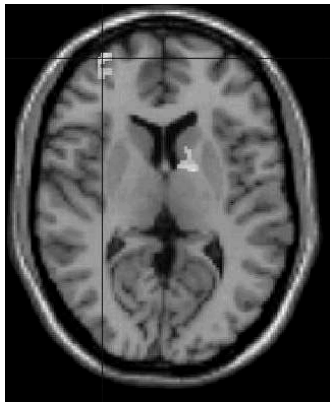


Figure 5.4.
BOLD signal increase in left Brodmann's area 10 and right pallidum, correlating with increased task load.

Discussion

In this paper we present a parametric event-related functional MRI version of the Tower of London task, suitable to investigate the effects of increasing complexity in planning processes. In agreement with the results of earlier imaging studies, the present fMRI data show that the process of planning, compared with baseline, was strongly correlated with activation of the right dorsolateral prefrontal cortex (BA 9 and 46), bilateral premotor cortex (BA 6 and 8), bilateral precuneus (BA 7) and inferior parietal cortex (BA 40), left supplementary motor area (BA 32), right insular cortex, and bilateral striatum[4;9-14;19]. Our parametric approach also shows that with increasing planning complexity a number of additional areas comes into play, i.e. the left dorsolateral (areas 9 and 46) and rostral prefrontal cortices (BA 10) as well as the right caudate nucleus. These are, at least in part, novel observations.

Involvement of the dorsolateral and rostral prefrontal areas is in agreement with the findings of Baker et al.[10]. It has been postulated that manipulation processes, operating on

information already stored in memory, engage dorsolateral prefrontal cortex[20]. Rostral prefrontal cortex activation, on the other hand, may reflect a more complex third level of executive control, holding temporarily in mind an ongoing goal while first completing intermediate or sub-goals, although we cannot rule out the alternative explanation, i.e. that rostral prefrontal activation merely reflects increasing task difficulty. Burgess et al.[21] investigated the phenomenological correlates of prospective memory and found activation of the rostral prefrontal areas correlated with maintenance of intention. This higher-order supervisory cognition, specific for humans, functions beyond the manipulation in dorsolateral prefrontal cortex and maintenance in ventrolateral prefrontal regions[20;22].

We found dorsolateral prefrontal activation to be right lateralised in the comparison planning vs. baseline, but was bilaterally occurring as a function of task load. Lateralisation probably exists, with spatial predominance at the right hemisphere and non-spatial predominance at the left side, but there is no consensus about the exact nature of this lateralisation. While Baker et al.[10] found right rostral prefrontal cortex activity to be strongly correlated with planning complexity, in present study left lateralisation was found for this rostral prefrontal area (Brodmann's area 10).

In the present study, right striatal activation (head of the caudate nucleus) was correlated with task load. Striatal involvement in planning complexity has been described before[4;9]. In conjunction with the increased activity of dorsolateral and rostral prefrontal areas, this suggests a role for the frontal-striatal system in complex planning tasks. In terms of forebrain circuits, this indicates an involvement of particular basal ganglia-thalamocortical circuits[23;24] which are directed at various parts of the prefrontal cortex. Interactions between the prefrontal cortex and the basal ganglia are thought to play a role in the selection and switching of behavioural components[25], which are crucially important for the execution of a (complex) planning process. In the context of the neuronal network model of Dehaene and Changeux[17], our finding of involvement of various cortical and subcortical regions related to increased planning complexity, strengthen the idea of distributed cortico-cortical and cortico-subcortical networks as a neuronal basis for such higher order cognitive processes. The involvement of the striatum might represent, at least in part, the predicted role of a reward system in the cognitive processes[26].

As mentioned before, most previous studies used a block design with an easy and a difficult planning condition, thereby restricting the investigation of task load effects to a subtraction analysis of both conditions. That no task load effects were found in the functional MRI study of Lazeron et al.[14] may be explained by this operationalisation of "easy" and "difficult" task conditions. The main aspect of planning complexity is presumably the amount of sub-goals to be attained before reaching the main goal. The "easy" condition of Lazeron et al. consisted of 2 to 4 moves items. Part of the 3 moves items and all 4 moves items need the counterintuitive intermediate steps characteristic for planning complexity. In the "difficult" condition subjects had to solve 5 to 7 moves items, which are quite hard to perform. Therefore, the difference between "easy" and "difficult" may have been obscured by the presence of complex items in the "easy" condition and the occurrence of ceiling effects in the "difficult" condition.

Our results are also at variance with Dagher et al.[4], who conducted a parametric analysis and divided areas involved in planning behaviour into those that did and those that did not

correlate with task complexity. Brain regions where rCBF correlated with planning complexity included bilateral dorsolateral prefrontal, premotor and anterior cingulate cortex and right caudate nucleus. In contrast with our results, Dagher et al.[4] found parietal activation not to be correlated with task complexity. Since this PET study consisted of only 6 subjects with 12 measurements per subject, all data were analysed using a fixed effects analysis, and results were presented uncorrected for multiple comparisons. Another important confounding factor may have been that subjects had to perform the planning task by touching the screen to move the beads. Motor execution is not essential for planning and may produce motion artefacts, confounding experimental effects. For this reason, we asked our subjects to execute their plan by making the moves mentally. Even without motor execution, massive activation of motor and visuospatial areas was found. Parietal activation caused by motor execution, independent of specific planning behaviour, is not correlated with task complexity, while visuospatial activity necessary for planning is indeed correlated to task complexity.

Compared with previous studies, the present version of the Tower of London task has two advantages. First, a self-paced, parametric design allows flexible responding as well as comparisons between subjects/groups at each task level, providing us with the opportunity to investigate subjects and groups with varying levels of performance. The increased comparability across groups, using the same task, is important for clinical studies, for instance in patients with decreased functioning of the frontal-striatal system. Second, in addition to variation in reaction time, performance may also vary in hit percentage. Event-related analysis enables a selective analysis of correct responses, by rejecting the false responses. Moreover, adequate randomisation of trials is possible in event-related designs, in contrast to block designs.

In summary, frontal-striatal and visuospatial systems are strongly involved in planning behaviour and their level of activity is related to task complexity as demonstrated by the described self-paced, parametric event-related version of the Tower of London task. By correcting for task performance differences, it will be possible to investigate planning behaviour in subjects with decreased frontal-striatal function.

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6

Amygdala activity in OCD patients with contamination fear: a study with oxygen-15 water positron emission tomography

van den Heuvel OA, Veltman DJ, Groenewegen HJ, Dolan RJ, Cath DC, Boellaard R, Mesina CT, van Balkom AJLM, van Oppen P, Witter MP, Lammertsma AA, van Dyck R

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Abstract

Previous imaging studies of obsessive-compulsive symptom states have implicated frontal-striatal and limbic regions in the pathophysiology of obsessive-compulsive disorder (OCD). Functional imaging studies, however, have yielded inconsistent results, presumably due to methodological differences (patient inclusion criteria, stimulus paradigm, imaging technique, and absence of control groups). In the present study, randomized presentation of contamination-related and neutral visual stimuli was used to investigate the neurophysiological correlates of contamination fear in a group of medication-free OCD patients with washing behaviors and healthy controls.

Twenty-one subjects (11 OCD patients and 10 healthy controls) were scanned using $H_2{}^{15}O$ PET. Subjects were presented with pictures of clean and dirty surroundings and were requested to make indoor/outdoor decisions to control for attention differences. State anxiety and obsessionality were rated after each scan using visual analogue scales.

Main effects of stimulus type (contamination vs. neutral) were found in bilateral occipital cortex in both groups. A significant group interaction effect was observed in the left amygdala reflecting enhanced activity in response to contamination stimuli in OCD patients. Sensitization effects were observed in the right amygdala in the OCD group; these paralleled an increase in levels of distress and obsessionality as well as a decrease in dorsolateral prefrontal activity.

The findings of the present study are consistent with the hypothesis of decreased frontal-striatal control of limbic structures, specifically the amygdala, resulting in an inadequate fear response in OCD patients with contamination fear.

Introduction

Obsessive-compulsive disorder (OCD) is a common psychiatric disorder, affecting more than 1% of the population[1]. This chronic disorder generally manifests itself first during adolescence and has a fluctuating course during adult life. OCD is characterized by recurrent intrusive thoughts (obsessions) and repetitive ritualistic behaviors (compulsions). These rituals can be highly time-consuming and result in considerable morbidity, whereas concomitant anxiety symptoms often are less prominent at clinical presentation.

Although the pathogenesis of OCD is incompletely understood, a number of neuroimaging studies have reported neuro-anatomical and functional abnormalities in OCD, particularly involving prefrontal-striatal and limbic regions. These studies, both volumetric and functional, have not yielded unequivocal results, however. Structural abnormalities in OCD have been described in the caudate nucleus in some studies[2-4], whilst in other studies no differences between OCD patients and normal controls were evident[5;6]. Jenike et al.[7], using magnetic resonance imaging (MRI), found significantly diminished total white matter and increased total cerebral cortex and opercular volumes in OCD patients compared with controls. These results were only partially confirmed by Kim et al.[8], who found increased gray matter (GM) density in frontal subcortical areas, but decreased GM density in other regions, in particular the cerebellum. In contrast, Szeszko et al.[9] reported reduced orbitofrontal and amygdala volumes (both GM and white matter), and lack of normal hemispheric asymmetry of the hippocampus-amygdala complex. Although these inconsistencies may reflect various methodological differences such as patient inclusion criteria, imaging modality, and data analysis techniques, they also suggest that volumetric abnormalities in OCD, if present, are at best subtle.

Early functional imaging studies in OCD have been performed using Positron Emission Tomography (PET), with ^{18}fluoro-deoxyglucose (FDG) as a tracer, assessing resting state glucose metabolism[10-13]. Baxter et al.[10] reported hypermetabolism of the left orbital gyrus and bilateral caudate nucleus. In addition, striatal hypermetabolism normalized after successful treatment, either pharmacotherapy[14] or behavioral therapy[15]. The normalizing effect of pharmacotherapy on caudate hyperactivity has been replicated by many groups[16-19]. In a similar vein, Rauch et al.[20] found that pre-treatment increased regional cerebral blood flow (rCBF) in orbitofrontal and posterior cingulate cortices predicted response to pharmacotherapy in OCD patients with prominent contamination fear.

To our knowledge, at present six imaging studies in OCD have been published in which symptom provocation was employed to differentiate between state and trait aspects. In a $H_2{}^{15}O$ PET study, McGuire et al.[21] used tactile stimuli (various contaminants sealed in glass tubes) in four medication-free male OCD patients. Symptom scores (and state anxiety) were correlated with increased activity in the right inferior frontal gyrus, basal ganglia, thalamus, left hippocampus and posterior cingulate cortex. Rauch et al.[22], using $C^{15}O_2$ PET, similarly compared responses to individually tailored provocative versus neutral stimuli in a sample of 8 male OCD patients and found increased relative regional cerebral blood flow during OCD symptom versus resting state in bilateral orbitofrontal cortex, right caudate nucleus and left anterior cingulate cortex. The same group performed a similar study in 10 OCD patients two years later, using functional MRI, also with idiosyncratic tactile stimuli[23]. Contrasting provoked vs. neutral state, increased activity was

found in the medial, right dorsolateral, and left ventrolateral prefrontal cortices (PFC), as well as in bilateral temporal cortex. At a lower statistical threshold, insular and anterior medial temporal regions were also found to be more active in the majority of patients. Since 9 out of 10 OCD subjects in the Breiter study[23] were taking medication at the time of scanning, Adler et al.[24] replicated the study in medication-free OCD patients and reported increased activity in the orbitofrontal, anterior cingulate, bilateral anterior and lateral temporal cortices, as well as the right medial temporal cortex, during symptom provocation compared with baseline.

In two studies, OCD subcategories were investigated. Cottraux et al.[25] used neutral vs. idiosyncratic obsession-related auditory scripts during $H_2{}^{15}O$ PET in 10 OCD patients with checking rituals and in 10 normal controls. In both groups, orbitofrontal perfusion was higher during provoked vs. neutral state. In addition, regional cerebral blood flow was higher in basal ganglia in controls. Finally, Phillips et al.[26] compared 'checkers' and 'washers' using standardized visual stimuli during functional MR imaging. Similar to the results of Cottraux et al., increased frontal-striatal activity in "checkers" and normal controls was found. In contrast, insular activity was observed in 'washers', although these effects did not survive in an interaction analysis. A methodological drawback of the Phillips et al. study was that the majority of patients were using psychotropic medication.

Taken together, these studies have implicated a number of regions in the pathophysiology of OCD. It has been hypothesized that frontal-striatal involvement reflects ritualistic behavior, even in normals[25], whereas increased medial temporal activity has been associated with (pathological) anxiety[21], and insular activity with disgust[26;27].

Similar to volumetric studies, inconsistent findings in functional imaging studies in OCD may result from a number of methodological issues, such as different imaging modalities, medication status, and small sample size. In addition, in all studies, with the exception of Phillips et al.[26], individually tailored and therefore heterogeneous stimuli were used, i.e. not only contaminants, but also stimuli related to aggression/danger or sexual obsessions. Therefore, it cannot be ruled out that different symptoms are associated with different neuronal substrates. In addition, the use of idiosyncratic stimuli prohibits assessment of group by task interactions. Moreover, most studies used tactile stimuli, which may necessitate ritual behavior (hand washing), interrupting the scanning session. Consequently, in these studies off-on paradigms had to be used, carrying the risk of order effects. For example, in a recent PET study it was shown that condition-related subject motion might produce considerable false-positives due to transmission-emission mismatches[28].

In the present study, we aimed at investigating neurophysiological correlates of contamination fear. Only OCD subjects with prominent contamination fear were included, and presented with 'clean' vs. 'dirty' stimuli. Similar to the Phillips et al. study [26], visual stimuli, matched for visual complexity were used, thereby obviating the need for cleaning rituals. Moreover, these stimuli, although ranging in severity, are universally aversive, so that the same paradigm could be used both in patients and control subjects. In contrast to the Phillips et al. study, we included only medication-free OCD patients. Finally, $H_2{}^{15}O$ PET rather than fMRI was used, as the advantage of high spatial resolution (fMRI) was offset by the advantages of PET, i.e. having scanning windows interspersed with rest to

allow return to baseline, a smaller risk of claustrophobic reactions, and the absence of susceptibility artifacts in regions of interest such as anterior medial temporal lobe, in particular in the amygdala, and orbitofrontal cortex. Each subject had 8 scans (4 active and 4 baseline), randomized across subjects (11 medication-free OCD patients and 10 normal controls), which allowed examination of condition by time interactions. The specific experimental prediction was that contamination fear would result in recruitment of those circuitries hypothesized to play a role in ritualistic behavior (frontal-striatal system), anxiety (amygdala) and disgust (insula).

Patients and Methods

Subjects

Eleven medication-free OCD patients (three women, eight men, age 40.5 ± 9.2 years) and ten normal control subjects (three women, seven men, age 36.5 ± 8.8 years) were included. All subjects were right-handed. Exclusion criteria were the presence of major medical illness, other major psychiatric disorders, and the use of psychotropic medication. Diagnosis was established using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I). The Yale Brown Obsessive-Compulsive Scale (Y-BOCS)[29] and the Padua Inventory-revised (Padua-IR)[30;31] were used to assess symptom characteristics and severity scores. Only subjects with prominent contamination fear as measured with the Y-BOCS symptom checklist were included. Scores on Y-BOCS and Padua-IR for OCD patients were 23.8 ± 6.1 and 73.3 ± 22.5, for controls 0 and 12.9 ± 11.9 respectively, which represented significant differences (Y-BOCS: $F(1,19)=179.4$, $p<0.001$; Padua-IR: $F(1,19)=50.0$, $p<0.001$). Subjects were recruited from the Outpatient Department of Psychiatry, VU University Medical Center and the Dutch Phobia Organization. All subjects gave written informed consent. The protocol was approved by the Medical Ethical Committee of the VU University Medical Center.

OCD symptom provocation paradigm

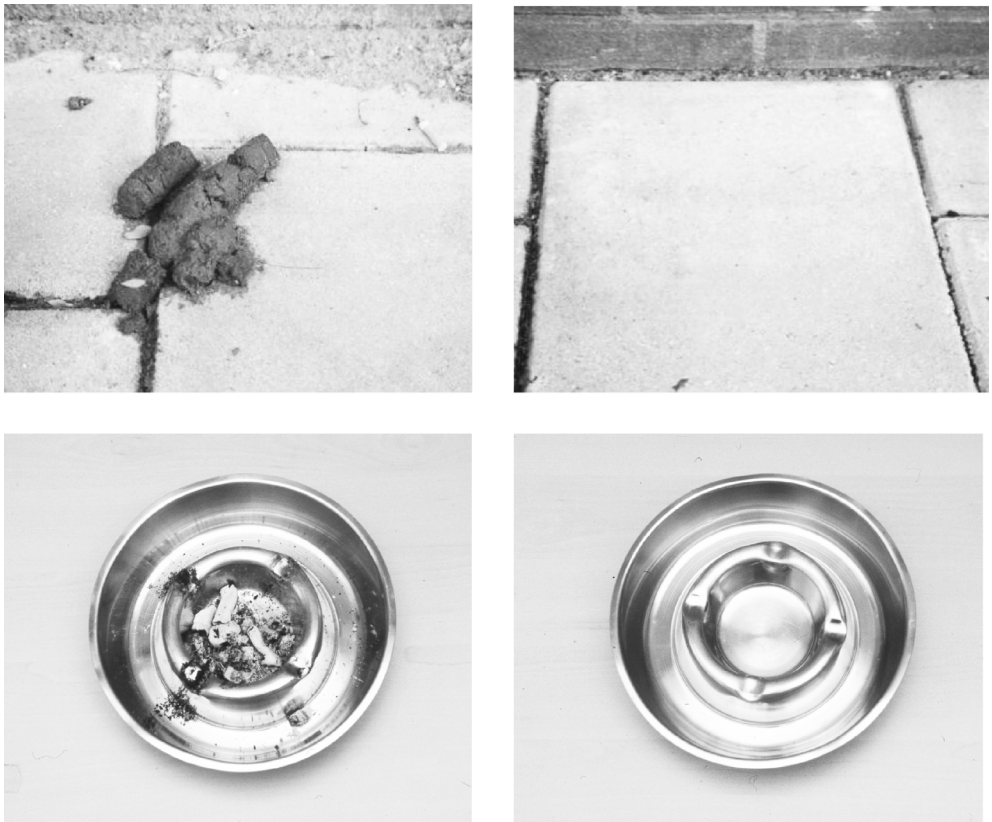
Visual stimuli were presented to each subject to provoke contamination fear while PET data were obtained. A scanning session consisted of 8 randomly ordered $H_2{}^{15}O$ PET scans: 4 in the control condition (clean pictures) and 4 in the symptom condition (dirty pictures). Twenty seconds after injection of $H_2{}^{15}O$, eleven pictures were presented sequentially on a high-resolution flat screen. Each picture was shown for three seconds, followed by a one second blank screen. To control for attention, subjects were asked to indicate for each picture (using a response box) whether it represented an indoor or outdoor situation. After each scan a resting period of eight minutes followed. During this resting phase, subjects were asked to rate subjective distress (ANX) and obsessionality (OBS) using 100-point analogue scales. In total 44 different dirty and 44 clean pictures were presented to each subject (examples are shown in Figure 6.1).

Image acquisition and reconstruction

PET scans were acquired on an ECAT EXACT HR+ scanner (CTI/Siemens, Knoxville, TN), the characteristics of which are well documented[32]. Prior to each scanning session, a

cannula was inserted into the left antecubital vein and normal saline was infused. A transmission scan was performed first for the purpose of attenuation correction of the subsequent emission scans. For each emission scan 10-15 ml normal saline containing 450 MBq of $H_2{}^{15}O$ was administered as a bolus injection using an infusion pump. Data acquisition lasted 90 seconds (20 seconds before, 44 seconds during and 26 seconds after stimulus presentation) and the interval between successive $H_2{}^{15}O$ injections was 8 minutes to allow for radioactive decay. In the present study, transmission scans of ten minutes were acquired in 2D mode for all studies. All emission scans were acquired in 3D mode. To rule out task related motion artifacts, data were reconstructed using an image registration method[28] with ordered subset expectation maximization (OSEM)[33;34] applying 4 iterations with 16 subsets[35]. For each subject, a T1-weighted structural MR-scan was also acquired using a 1.5 Tesla Sonata (Siemens, Erlangen) MR scanner (magnetization prepared-rapid acquisition gradient echo (MP-RAGE); inversion time = 300 ms, TR = 15 ms, TE = 7 ms, flip angle = 8^0, voxel size = 1x1x1.5 mm).

Figure 6.1. Examples of dirty and clean pictures in used symptom provocation design.



Statistical analysis

Behavioral data were analyzed using a standard statistical package. After reconstruction, PET-images were analyzed using SPM99 (Wellcome Department of Cognitive Neurology, http://www.fil.ion.ucl.ac.uk/spm). Spatial preprocessing consisted of realignment, transformation to approximate Talairach[36] anatomical standard space, as defined by the SPM99 template, and smoothing using a 10 mm Gaussian filter instead of the customary 16 mm for PET group studies, resulting in an overall FWHM of 12 mm. Since the human amygdala is about 15 mm, the present smoothing is considered to be optimal to detect activation changes, which are just over 12 mm in diameter[37]. Data were analyzed using a linear regression model. Weighted contrasts were computed for condition and time effects for each group, and for group by condition interactions. Analyses of covariance were also performed to assess correlations between imaging data and psychometric measurements. In view of the smoothness of the data after spatial preprocessing (ca. 12 mm FWHM), a combined height and extent threshold was used to report main effects of $p<0.001$ uncorrected with an extent threshold of 25 voxels. Interactions (group by task, and group by time) were masked with the appropriate main effect and are reported at $p<0.001$ uncorrected, with an extent threshold of 5 voxels.

Results

Psychometric data

Among patients, OBS scores reflected increased OCD symptomatology during the active condition vs. baseline (paired T-test: 30.3 ± 22.4 vs. 9.3 ± 13.0, $p=0.003$, one-tailed). An increase in OBS scores was also observed in normal controls (15.9 ± 17.9 vs. 0.6 ± 1.0, $p = 0.02$, two-tailed). As expected, in the OCD group, subjective distress as expressed by ANX scores was higher during the active condition than during baseline (32.1 ± 18.3 vs. 26.1 ± 21.4, $p=0.05$, one-tailed). For control subjects, we found no significant active vs. baseline increase in ANX scores (7.2 ± 5.4 vs. 9.3 ± 6.9, $p> 0.1$, two-tailed). Comparisons between the two groups revealed that OBS scores were higher in the OCD group at rest (ANOVA: $F(1,19)=4.4$, $p=0.05$) but not during the active condition ($F(1,19)=2.6$, $p>0.1$). ANX scores were higher in the OCD group at baseline ($F(1,19)=7.4$, $p=0.01$) as well as during the active condition ($F(1,19)=13.7$, $p=0.002$). Condition by time interactions were found only in the OCD group during the active condition, ANX and OBS scores showing an increase from the first (early) to the second (late) part of the session (ANX scores: 20.5 ± 33.9, $p=0.09$; OBS scores: 24.0 ± 28.6, $p=0.03$). Correlations between mean ANX and OBS ratings were only statistically significant during the active condition in the OCD group ($r=0.63$, $p=0.04$).

PET data

Main effects of symptom compared with baseline condition are listed in Tables 6.1 and 6.2. Provocation of contamination fear in OCD patients was correlated with increased regional cerebral blood flow (rCBF) in the left amygdala, extending into left anterior temporal cortex, left entorhinal cortex and hypothalamus, with additional activity in bilateral extrastriatal cortex (Figure 6.2). In control subjects, increased rCBF was found in left

dorsolateral prefrontal cortex, basal forebrain extending into orbitofrontal cortex, right caudate nucleus, left anterior temporal cortex and bilateral extrastriatal cortex (Figure 6.3).

Table 6.1. Main effects in 11 OCD patients: contamination stimuli > neutral stimuli

Region of activation	*Talairach coordinates*			*Cluster size (in voxels)*	*BA*	*Z-value*
	x	y	z			
Left amygdala region	-18	-4	-16	202		4.8**
Left occipital cortex	-44	-78	6	168	19	4.0*
Right occipital cortex	30	-68	-14	99	19	4.2*

BA= brodmann's area
*= p<0.001 uncorrected for multiple comparisons, **= p<0.05 corrected for multiple comparisons

Table 6.2. Main effects in 10 healthy control subjects: contamination stimuli > neutral stimuli

Region of activation	*Talairach coordinates*			*Cluster size (in voxels)*	*BA*	*Z-value*
	x	y	z			
Left dorsolateral prefrontal cortex	-18	44	32	96	9	3.7*
Basal forebrain/posterior orbitofrontal	0	0	-4	261	25	4.1*
cortex	4	12	-14			3.9*
Right caudate nucleus	18	10	14	37		3.7*
Left anterior temporal cortex	-42	28	-24	82	38	3.9*
Left occipital cortex	-44	-84	0	42	19	3.7*
Right occipital cortex	26	-80	-18	63	19	3.9*

BA= brodmann's area
*= p<0.001 uncorrected for multiple comparisons

Figure 6.2. PET subtraction images of provoked minus resting conditions for 11 OCD patients displayed on mean T1-weighted MRI show activation in left amygdala (Talairach coordinates x, y, z = -18, -4, -16).

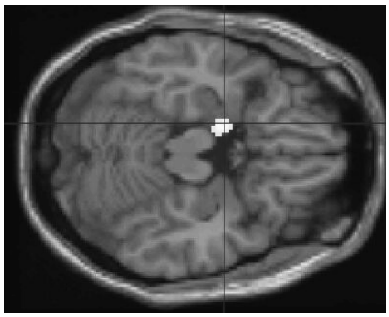
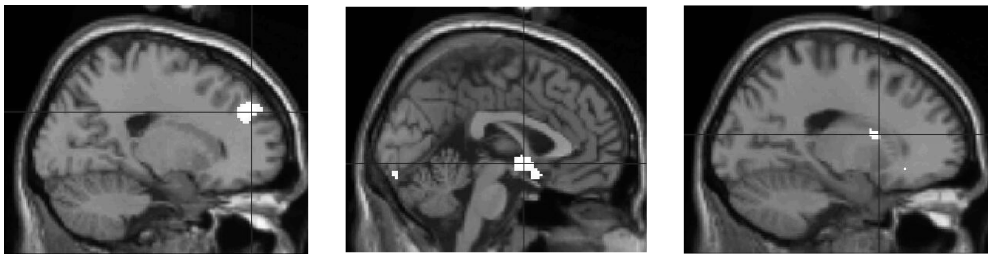


Figure 6.3. PET subtraction images of provoked minus resting conditions for 10 control subjects displayed on mean T1-weighted MRI show activation in left dorsolateral prefrontal cortex (Talairach coordinates x, y, z = -18, 44, 32), basal forebrain (Talairach coordinates x, y, z = 4, 12, -14) and right caudate nucleus (Talairach coordinates x, y, z = 18, 8, 16).



Group by condition interactions were found in left amygdala region, reflecting relative enhanced activity to contamination stimuli in the OCD group with relative decreases in left dorsolateral prefrontal cortex and right caudate nucleus (i.e. enhanced activity in normal controls) (Table 6.3 and Figure 6.4). Finally, significant time by condition interactions were observed in the right amygdala region in the OCD group, but not in controls (group by time by condition ANOVA, z-score = 2.75, p = 0.003 uncorrected, see Figures 6.5a and 6.5b), corresponding to a decrease in rCBF in this region during the successive resting state conditions and an increase in rCBF during the successive active conditions. These differential time courses for left and right amygdala in the provocation condition were confirmed by a region of interest (ROI) analysis, demonstrating a statistically significant negative correlation between left and right amygdala signal (r= -0.35, p= 0.022).

Figure 6.4.
Group by condition interaction analysis, showing increased rCBF in left amygdala (Talairach coordinates x, y, z = -18, -4, -16) during provoked versus resting conditions in OCD patients compared with control subjects and in right caudate nucleus (Talairach coordinates x, y, z = 18, 8, 16) in control subjects compared with OCD patients (statistical significance was thresholded at uncorr. p = 0.01 for display purposes).

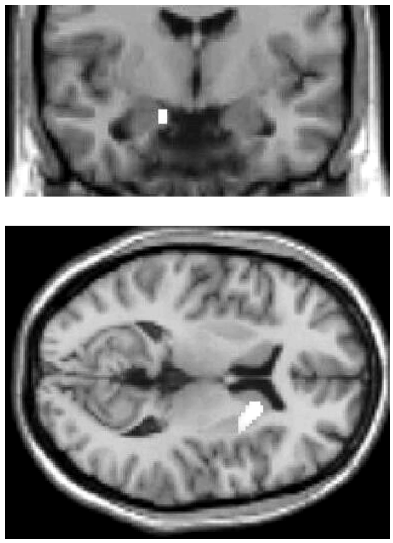


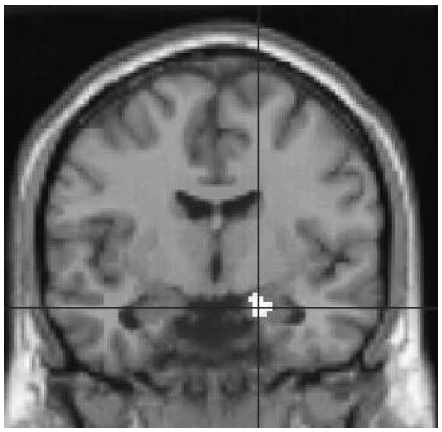
Table 6.3. Condition (contamination versus neutral stimuli) by group interactions

Region of activation	*Talairach coordinates*			*Cluster size (in voxels)*	*BA*	*Z-value*
	x	y	z			
OCD > controls						
Left amygdala region	-18	-6	-18	7		3.4*
Controls > OCD						
Left dorsolateral prefrontal cortex	-18	44	32	96	9	3.4*
Right caudate nucleus	18	10	14	37		3.3*

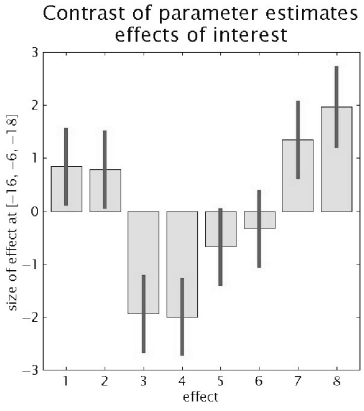
BA= brodmann's area, *= p<0.001 uncorrected for multiple comparisons

Figure 6.5.

A.
PET time by condition contrast images for 11 OCD patients displayed on mean T1-weighted MRI show activation in right amygdala (Talairach coordinates x, y, z = 16, -6, -18).



B.
Plot of size of effect (y-axis) in right amygdala for time by condition analysis in 11 OCD patients. Plot shows decreasing effect size over time during subsequent resting conditions (x-axis 1, 2, 3, 4) and increasing effect size over time during subsequent provoked conditions (x-axis 5, 6, 7, 8).



Results of correlational analyses are listed in Table 6.4. In the OCD group, OBS scores were correlated with increased activation of bilateral occipital cortex and decreased activation of right ventromedial prefrontal cortex, dorsolateral prefrontal cortex, and right

parietal cortex. No statistically significant correlations were found between ANX scores and regional cerebral blood flow changes.

Table 6.4. Covariation with OBS scores in 11 OCD patients

Region of activation	*Talairach coordinates*			*Cluster size (in voxels)*	*BA*	*Z-value*
	x	y	z			
Increase						
Right occipital cortex	40	-90	2	58	18	4.2*
	40	-74	8	127	19	3.9*
Left occipital cortex	-44	-78	8	148	19	4.2*
Decrease						
Right ventromedial prefrontal cortex	34	52	-14	87	11	3.7*
Right dorsolateral prefrontal cortex	46	34	32	30	9	3.7*
Right parietal cortex	44	-54	44	179	40	4.1*

BA= brodmann's area
*= $p<0.001$ uncorrected for multiple comparisons

Discussion

In the present study, $H_2{}^{15}O$ PET was used to investigate the neurophysiological correlates of contamination fear in a subgroup of obsessive-compulsive washers and healthy control subjects. To this end, rCBF was compared in both groups during presentation of neutral and contamination-related visual stimuli. Behavioral data showed that the present design was successful in inducing both increased subjective distress (ANX) and obsessionality (OBS). In the OCD group, ANX and OBS ratings were only moderately high, but showed an increase over time, implying that OCD patients became more sensitive, rather than habituated, to contamination stimuli. Nevertheless, all subjects were able to complete the scanning session.

Imaging data showed that symptom provocation in OCD patients was correlated with increased regional cerebral blood flow in left amygdala and bilateral extrastriatal cortex, while control subjects showed increased activation of the basal forebrain extending into posterior orbitofrontal cortex, left dorsolateral prefrontal cortex, right caudate nucleus and bilateral extrastriatal cortex. Group by condition interactions were found in the left amygdala in the OCD group and in left dorsolateral prefrontal cortex and right caudate in controls, whereas significant time by condition interactions were only observed in the right amygdala in the OCD group. The finding of amygdala involvement in OCD subjects during symptom vs. neutral condition is in agreement with a number of studies using fear paradigms in normal controls[27;38-44], supporting the role of the amygdala as a key structure in evaluating the behavioral significance of external stimuli[45]. Previous imaging studies in OCD, however, have observed amygdala involvement only in small subgroups and/or at

lower statistical thresholds[23;24], or not at all[21;22;25;26]. Possible explanations include statistical power (repeated measures vs. off-on paradigms) and stimulus type. Although the amygdala receives multimodal sensory information, projections from visual processing areas are particularly prominent[46]. Therefore, salient visual stimuli may give rise to amygdala activation more easily than tactile or auditory stimuli. In addition, the use of antidepressants is likely to suppress amygdala function (see Drevets[47] for a review).

In both groups, increased activity of bilateral extrastriatal cortex in the active condition compared with the baseline condition was observed. It has been postulated that these secondary visual areas play a critical role in perceptual processing of socially and emotionally relevant stimuli[39;48;49]. Morris and colleagues[39] demonstrated that activation of the amygdala resulted in context dependent modulatory effects in extrastriatal cortex. Lane et al.[50] investigated the interaction between emotion and attention in visual processing and found the extrastriatal visual cortex as well as the anterior temporal cortex activated by emotional valence, arousal and attention. In the present study, increased activity in the left anterior temporal pole during the active condition was found in the control group. The role of the anterior temporal cortex, as a hierarchically higher-order cortical area involved in visual processing and evaluation of the significance of current stimuli, might include retrieval of past emotional experiences. The anterior temporal cortex has reciprocal connections to frontal areas, in particular orbitomedial prefrontal areas, in this way enabling associated behavioral responses.

In the present study, differential effects in left and right amygdala were found. OCD patients showed increased activity in the left amygdala during the symptom condition, an effect that was stable over time. This left lateralized response is in accordance with results of earlier studies in normal volunteers[27;38-42]. However, in favor of the OCD group compared with healthy control subjects, a significant time by condition interaction was observed in the right amygdala, i.e. a signal decrease over time in the baseline condition compared with an increase during the symptom condition. This effect has not been described in previous OCD studies, but is likely to reflect the increase in ratings of subjective distress and obsessionality over time observed in the OCD group. In normal volunteers, several authors have reported habituation, not sensitization, of amygdala responses to alarming stimuli[38;41;42;48;51]. These effects have mostly been found in the right amygdala, but not in the left, from which it has been concluded that the right amygdala is involved in rapid emotional stimulus detection, whereas the left amygdala is associated with sustained stimulus evaluation. Alternatively, Morris et al.[40] proposed that the left amygdala has a role in conscious stimulus processing, whilst the right amygdala is associated with unconscious processing. Neither hypothesis, however, fits the data from the present study. A possible explanation is provided by the work of Schaefer et al.[52], who found increased amygdala activity when subjects maintained the negative emotional response during the delay following visual presentation of negative stimuli. This increase in amygdala response correlated with self-reported levels of negative affect. These findings suggest that maintenance of emotional responses by cognitive processes may operate, at least in part, by modulating neuronal activity within the amygdala. Similarly, Siegle et al.[53] reported that in subjects with major depression, processing of negative stimuli was associated with prolonged bilateral amygdala activity. Moreover, these responses were related to self-reported rumination. In the present study, however, due to the limited

temporal resolution of $H_2{}^{15}O$ PET, it was impossible to determine whether the sensitization effects observed in the OCD subjects were due to increases in amplitude or duration of responses to individual stimuli.

Contrary to expectations, when contrasting symptom vs. baseline, orbitofrontal activity was only observed in the control group, but failed to reach significance in the OCD group. Moreover, group by condition interaction effects in basal ganglia and dorsolateral prefrontal cortex were found in favor of normal controls compared with OCD patients. Involvement of the basal ganglia during symptom provocation in OCD subjects has been reported in most[21-23], but not all[24], previous studies, which was attributed by Adler et al.[24] to differences in medication status. Although in the present study only medication-free patients were included, this does not seem to be a plausible explanation since subjects in some of the above mentioned studies were off medication as well[21;22]. Whether paradigms employing tactile stimuli are generally more likely to produce basal ganglia activity is difficult to ascertain since most earlier studies lacked control groups. A possible explanation for the apparent lack of frontal-striatal involvement in OCD patients, is increased baseline regional perfusion, as suggested by the resting state FDG-PET studies by Baxter et al.[10;11]. To investigate this issue, we performed a post-hoc analysis comparing relative baseline activity between the two groups. OCD patients, compared with controls, showed increased activity in orbitofrontal and insular cortices; however, no differences were found in the basal ganglia (data not shown). These findings seem to be in agreement with those of Cottraux et al.[25], who found increased orbitofrontal perfusion during provoked vs. neutral state in both OCD patients and in healthy control subjects, presumably reflecting ritualistic behavior. In addition, increased activity in the basal ganglia was found in controls, but not in OCD patients. Increased frontal-striatal involvement in controls in response to contamination-related stimuli was also found by Phillips et al.[26]. While controls and "checkers" recruited the frontal-striatal system, contamination-feared OCD patients showed increased activity in the insular cortex, important in disgust perception.

Taken together, imaging findings from the present study indicate that presentation of contamination-related stimuli in normal subjects results in activation of regions assumed to be involved in higher order control. In contrast, in OCD subjects, condition effects were primarily observed in areas associated with processing of salient (i.e. threatening) stimuli. This interpretation receives some support from our finding that OBS ratings increased in both groups, but ANX ratings only in the OCD group. Moreover, ANX and OBS ratings showed significant correlations only during the symptom condition in the OCD group. However, we recognize that the specificity of visual analogue scales used in the present study as well as in others is debatable[22]; in particular, OBS ratings may reflect not only urge to ritualize, but also disgust.

With regard to these two emotional states, Phillips et al.[27] showed that separate regions were associated with fear and disgust. Whereas perception of fearful faces was correlated with activation of the left amygdala, perception of facial expressions of disgust was correlated with activation in right medial frontal and insular cortices. Very strong disgust also activated right putamen and thalamus, structures associated with the cortico-striatal-thalamic circuit[54]. Sprengelmeyer et al.[55] also reported right putamen and left insular cortex during presentation of disgusted, but not fearful, facial expressions, in support of the hypothesis of distinct neuronal networks involved in basic emotions like fear

and disgust. However, the issue whether the neuronal representation of emotion involves separate systems for individual emotions, or an integrated system able to code all emotions is still unresolved[56]. Although several studies support the category-based model, indicating that the neuronal mechanisms underlying fear and disgust might be at least separate in part[27;55], some studies also show amygdala involvement in perception of disgust, supporting a dimensional model[57]. Impaired recognition of disgust from facial expressions has been reported in patients with OCD[58], Huntington's disease[59] and brain injury in the area of basal ganglia and insular cortex[60]. In contrast to the results of Phillips et al.[26;27] and Sprengelmeyer et al.[55], in the present study no task related insular activity was found in the OCD group, although our post-hoc results indicate that this may have been due to increased activity in insular cortex at baseline in OCD patients compared with controls.

Finally, an explanation for the differential frontal-striatal involvement in the two groups in the present study is suggested by our finding that OBS ratings in OCD patients were inversely coupled to both ventromedial and dorsolateral prefrontal activity. An inverse correlation between OCD scores and prefrontal activity (albeit in the orbitofrontal cortex) was also reported by Rauch et al.[22] and Adler et al.[24]. This finding was attributed to a loss of inhibitory input from frontal structures. Similarly, negative correlations between amygdala and dorsolateral prefrontal activity were demonstrated in depressed subjects in the study of Siegle et al.[53]. Therefore, we suggest that the observed differences between OCD patients and controls reflect a failure of frontal-striatal circuitry in OCD patients to control the processing of negative (contamination-related) stimuli, resulting in an inadequate fear response, involving bilateral amygdala. The present data also indicate that repeated stimulation may lead to sensitization in OCD subjects. However, because we studied only OCD subjects with prominent contamination fear, these findings cannot be generalized to other OCD subtypes. Different OCD symptom dimensions might be associated with differential patterns of activation in ventral prefrontal, limbic and dorsal prefrontal regions[61]. Future research will need to investigate temporal characteristics of amygdala responses in OCD patients, to compare contamination and general threat stimuli in medication-free OCD patients and normal controls, and to compare OCD subgroups, by including subjects with prominent checking rituals. To investigate the specificity of the neurophysiological abnormalities found in OCD, comparison with results in other anxiety disorders, like hypochondriasis and panic disorder, or with mood disorders is important. Pre-post treatment studies, in case of pharmacotherapy as well as psychotherapy, may result in a more detailed understanding of the possibilities to influence the frontal-striatal system.

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7

Frontal-striatal dysfunction during planning in obsessive-compulsive disorder

van den Heuvel OA, Veltman DJ, Groenewegen HJ, Cath DC,
van Balkom AJLM, van Hartskamp J, Barkhof F, van Dyck R

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Abstract

Dysfunction of frontal-striatal, in particular orbitofrontal-striatal, circuitry has been implicated in the pathophysiology of obsessive-compulsive disorder (OCD), characterized by obsessions, ritualistic behavior, anxiety and specific cognitive impairments. In addition, neuropsychological studies in OCD have reported impairments in visuospatial tasks and executive functions, such as planning. The aim of the present study was to determine whether dorsal frontal-striatal dysfunction mediates planning impairment in OCD patients.

A parametric self-paced pseudo-randomized event-related functional MRI version of the Tower of London task was used in 22 medication-free patients with OCD and 22 healthy control subjects. This paradigm, allowing flexible responding and post hoc classification of correct responses, was developed to compare groups likely to differ in performance.

Behavioral results showed significant planning impairments in OCD patients when compared with control subjects. During planning, decreased frontal-striatal responsiveness was found in OCD patients, mainly in dorsolateral prefrontal cortex and caudate nucleus. In addition, OCD patients showed increased - presumably compensatory - involvement of brain areas such as the anterior cingulate, ventrolateral prefrontal and parahippocampal cortices, known to play a role in performance monitoring and short-term memory processing, respectively.

These findings support the hypothesis that decreased dorsal frontal-striatal responsiveness is associated with impaired planning capacity in OCD patients. Since described frontal-striatal dysfunction in OCD is independent of state anxiety and disease symptom severity, we conclude that executive impairment is a core feature in OCD.

Introduction

Clinical, neurosurgical and functional neuroimaging studies have provided evidence that dysfunctional prefrontal cortex-basal ganglia circuits are involved in the pathogenesis of obsessive-compulsive disorder (OCD). The high co-occurrence between obsessive-compulsive disorder (OCD) and other basal ganglia disorders strongly supports the so-called frontal-striatal hypothesis[1;2]. In addition, recent neuropsychological studies have shown cognitive impairments in OCD, particularly with regard to visuospatial processing, executive functioning, and motor speed[3;4]. Other cognitive domains appear to remain intact, indicating a specific, rather than a general, cognitive deficit.

Executive functioning implies different subdomains of higher order cognitive functioning. Planning, i.e. the ability to achieve a goal through a series of intermediate steps, is an essential component of higher-order cognitive processing, such as problem solving. Employing a neuronal network model, Dehaene and Changeux[5] proposed multiple hierarchic levels coding for specialized subprocesses of planning such as plan generation, working memory, and internal evaluation and reward. Some subprocesses seem to be relatively independent of task load, i.e. increasing planning complexity, while other subprocesses are mainly involved at higher levels of planning behavior.

A frequently used test to probe planning processes is the Tower of London task (ToL)[6], adapted by Shallice et al. from the Tower of Hanoi[7]. The ToL has been used to investigate planning in healthy control subjects using positron emission tomography (PET)[8-12], single-photon emission computed tomography (SPECT)[13;14] and functional magnetic resonance imaging (fMRI)[12;15-20]. The results of these imaging studies agree on the involvement of (dorsolateral) prefrontal cortex and parietal-occipital regions during planning. However, activation of other brain regions, such as cingulate[9;10;12;16;17;19] and insular cortices[9;10;17], striatum[8;10;12;16;17;20] and rostral prefrontal cortex[9;10;17], has not been found across all mentioned studies. These inconsistencies are likely to be explained by methodological differences between these studies, such as group size, scanning modality, analysis technique (regions of interest versus whole brain analysis), and details of task paradigm. For example, baseline conditions have not been uniform (low-level vs. matched for visual complexity and motor demands); also, some paradigms require mental execution, whereas in others a touch screen is used. Berg and Byrd[21] have emphasized the potential impact of such modifications, providing recommendations for constructing e.g. computerized versions of the ToL, to increase comparability across studies.

Neuropsychological studies of planning ability, as a measure of executive functioning, in OCD patients have not provided wholly consistent results. Veale et al.[22], using a computerized version of the ToL, found no difference in accuracy between OCD patients and healthy control subjects. However, when OCD patients made a mistake, they spent more time than controls in generating alternative solutions or in checking next responses. Normal accuracy in planning execution in OCD was also found by Schmidtke et al.[23], using the Tower of Hanoi; however, in this study no reaction time data were collected. Results of the study of Purcell et al.[24], comparing neuropsychological profiles of patients with OCD, panic disorder, and major depression, highlight the importance of task implementation. Although motor speed was decreased, OCD patients showed a normal ability to organize and execute a series of goal-directed moves on a planning task when

using a touch screen, providing external validation of ongoing performance. In contrast, when the task had to be executed mentally, OCD patients were significantly impaired. Whether impaired executive functioning is a trait feature of OCD, i.e. not secondary to present mood or anxiety symptoms, is not yet clear. In one study[25] impaired performance relative to controls was found in subclinical obsessive-compulsive subjects. However, in this study it was also found that performance was inversely correlated with symptom severity, in particular severity of checking behavior.

Although these neuropsychological studies, together with functional imaging studies in healthy subjects, suggest that the executive impairment found in OCD patients reflects dorsal prefrontal-striatal dysfunction, so far no imaging study has been published in which this hypothesis has been investigated. Functional imaging studies using executive tasks have been performed in schizophrenia[26] and major depression[27], whereas in OCD most studies have used symptom provocation paradigms. However, evidence of striatal dysfunction in OCD has been provided in a PET-study employing an implicit learning task[28;29]. In this study, OCD patients showed increased activity of posterior (temporal and parietal) cortical regions relative to controls, explained as compensatory mechanisms.

The aim of the present study was to investigate dorsal prefrontal-striatal function during performance of a planning task in OCD patients compared with healthy controls. To this end, we developed a parametric self-paced pseudo-randomized event-related version of the Tower of London task[17], suitable for fMRI. A self-paced parametric design allows flexible responding as well as comparisons between subjects or groups at each task level, resulting in increased comparability across groups with varying levels of performance. In addition, an event-related design enables *post hoc* classification of events based on subjects' responses, so that correct responses and errors can be analyzed separately. Based on previous studies, we expected task performance in OCD subjects to be impaired; in addition, we hypothesized this planning deficit to be reflected in decreased responsiveness of striatal and dorsal prefrontal regions as assessed using fMR imaging.

Methods

Subjects

Twenty-two OCD patients (7 males; age 34.4 (range 21-49) years) and 22 healthy control subjects (11 males; age 29.9 (range 23-51) years) performed the Tower of London task, while functional MRI data were collected. All subjects were right-handed, as assessed during a medical interview. Exclusion criteria were the presence of major medical illness, other major psychiatric disorders, and the use of psychotropic medication. Subjects had to be off medication for at least 4 weeks. Diagnoses were established using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I/P[30]). The Yale Brown Obsessive-Compulsive Scale (Y-BOCS)[31] and the Padua Inventory-revised (Padua-IR)[32;33] were used to assess symptom characteristics and severity scores (cf. Table 1). Patients were recruited from the outpatient clinic for anxiety disorders of GGZ Buitenamstel/VU University Medical Center (VUMC) and the Netherlands Anxiety, OCD and Phobia Foundation. The ethical review board of the VUMC approved the study and all participants provided written informed consent.

Task paradigm

A pseudo-randomized, self-paced version of the ToL was used, discussed in detail previously[17]. This version consisted of six conditions: a baseline condition and five planning conditions, ranging from one to five moves. In the planning conditions, subjects saw a starting configuration together with a target configuration with the instruction "count the number of steps". Two possible answers were shown, from which the correct one had to be selected (see Figure 5.1A). In both configurations, three colored beads were placed on three vertical rods, which could accommodate one, two and three beads, respectively. One bead could be moved at a time, and only when there was no other bead on top. Subjects were requested to determine the minimum number of moves necessary to reach the target configuration and to press the button corresponding to the side of the screen (left or right) where the correct answer was presented. In the baseline condition, subjects simply had to count the total amount of yellow and blue beads (see Figure 5.1B). A pseudo-randomized design was adopted to control for any overflow effects (i.e. perseverance of task related cognitive processes after a difficult trial). Therefore, each trial of three or more moves was followed by a baseline trial. No feedback regarding the answers was provided during the task. A maximum response duration of 30 seconds for each trial was applied. After performing the task, subjects were asked to rate subjective distress using 100-point analogue scales. To ensure that participants were familiar with the procedure, the test was explained and practiced outside the scanner before MR imaging was performed.

Data acquisition

Imaging was performed on a 1.5 T Sonata MR system (Siemens, Erlangen, Germany) with a standard circularly polarized head coil. Stimuli were generated by a Pentium PC and projected on a screen at the end of the scanner table, which was seen through a mirror mounted above the subject's head. Two magnet-compatible four-key response boxes were used to record subject's performance and response times (RT). To reduce motion artifacts, the subject's head was immobilized using foam pads.

Anatomic imaging included a coronal 3D gradient-echo T1-weighted sequence (matrix 256x160, voxel size 1x1x1.5mm, 160 sections). For functional MR imaging, an echo planar imaging sequence (TR 3.045s, TE 45 ms, matrix 64x64, field of view 192x192mm, flip angle 90^{o}) was used, creating transversal whole-brain acquisitions (35 slices, 3x3mm in-plane resolution, 2.5mm slice thickness, 0.5mm inter-slice gap). In total, 433 EPI volumes per subject were scanned. The distribution frequency of event types was based on RT data of a pilot study, so that a similar amount of scans (around 70 EPI volumes) was acquired per subject for each of the six conditions.

Data Analysis

Demographic and behavioral data were analyzed using SPSS software (version 11.0). Imaging data were analyzed using SPM99 (Wellcome Department of Cognitive Neurology, http://www.fil.ion.ucl.ac.uk/spm). After discarding the first 4 volumes, time-series were corrected for differences in slice acquisition times and realigned. Spatial normalization into approximate Talairach and Tournoux space was performed using a standard SPM EPI template. Data were resliced to 2x2x2mm voxels and spatially smoothed using a 6mm Gaussian kernel.

Next, data were analyzed in the context of the General Linear Model, using delta functions convolved with a canonical hemodynamic response function to model responses of varying length to each type of stimulus. In addition, error trials were modeled separately as a regressor of no interest. For each subject, weighted contrasts were computed for main effects, i.e. all active conditions vs. baseline. In addition, specific linear contrasts for task load were computed[34]. Contrast images containing parameter estimates for main effects and task load were entered into a second level (random effects) analysis. Main effects for each group are reported at $p<0.05$ significance level corrected for multiple comparisons; group interaction effects (masked with the appropriate main effect) are reported at an uncorrected threshold of $p<0.001$.

Results

Demographic and behavioral data

The groups did not differ significantly with respect to age and gender. Education level, however, was higher in control subjects. OCD symptom severity, as measured with the Y-BOCS and Padua questionnaires, was significantly higher in patients (Table 7.1). Analysis of behavioral data (ANOVA) showed significant differences in performance between OCD patients and controls at all task levels, whereas RTs were longer in OCD patients compared with controls only in the two easiest planning conditions (Table 7.2). Subjective distress scores were significantly higher in the OCD group compared with the control group (42 ± 25.5 vs. 21 ± 11.8, $F(1,42)=12.4$, $p<0.001$). Within groups, performance was not significantly correlated with trait- or state measures (all p's>0.1).

Table 7.1. Demographic characteristics

	22 healthy control subjects	*22 OCD patients*
	Mean (SD)	Mean (SD)
Age, yr	29.9 (7.4)	34.4 (8.6)
Sex (M/F)	11/11	7/15
Education (1-8)	7.36	5.67 *
Y-BOCS	0	22.2 (6.5) **
Padua-IR	9.9 (10.4)	61.3 (21.9) **

*= p<0.01, **= p<0.001

Table 7.2. Response times (RT, in seconds) and performance scores (PS, in %)

	22 healthy control subjects		*22 OCD patients*	
Condition	RT (SD)	PS (SD)	RT (SD)	PS (SD)
Baseline	3.71 (0.88)	94.2 (1.66)	4.23 (1.07)	88.3 (3.66)***
1 move	4.44 (1.10)	97.6 (2.04)	5.46 (1.39)**	78.5 (9.0)***
2 moves	5.79 (1.26)	95.4 (3.98)	6.66 (1.49)*	79.3 (15.99)***
3 moves	7.40 (1.89)	96.7 (3.58)	8.51 (2.08)	72.0 (15.7)***
4 moves	10.06 (2.48)	89.9 (7.11)	10.72 (3.41)	68.1 (14.3)***
5 moves	14.99 (3.99)	82.2 (12.6)	14.99 (5.54)	63.6 (21.3)***

* p<0.05 ** p<0.01 *** p<0.001

Imaging data

1. Main effects of task

In controls, regions showing increased BOLD signal during planning compared with baseline (Table 7.3) were found bilaterally in dorsolateral prefrontal (Brodmann area (BA) 9 and 46), motor and premotor (BA 4, 6 and 8), inferior parietal (BA 40), insular and superior occipital (BA 19) cortices, as well as bilateral precuneus (BA 7), right caudate nucleus and left globus pallidus. OCD patients showed increased BOLD signal in most of these regions as well. However, in contrast to control subjects, in OCD patients, no activation was found in the striatum during planning. In addition, activation was found in right cingulate cortex (BA 32). Group by task interaction analyses (Table 7.4) showed increased activation in controls compared with OCD patients in right dorsolateral prefrontal cortex (BA 9/46), right premotor cortex (BA 6), left cingulate cortex (BA 32), bilateral precuneus (BA 7), left inferior parietal cortex (BA 40), right caudate nucleus (Figure 7.1) and left putamen. No significant group by task interactions were found in OCD patients compared with controls.

Figure 7.1.
Increased BOLD signal in right caudate nucleus during planning compared with baseline in healthy control subjects compared with OCD patients.

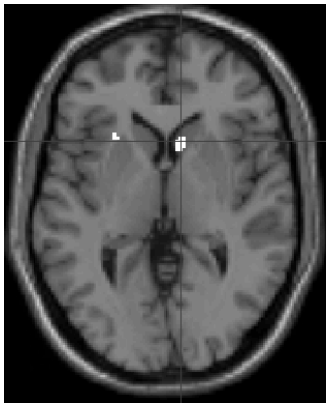


Table 7.3. Brain regions showing significant (p<0.05 corrected) BOLD signal increase during planning compared with baseline.

			22 healthy control subjects				*22 OCD patients*			
Region of activation	*L/R*	*BA*	*Talairach coordinates*			*z-value*	*Talairach coordinates*			*z-value*
			x	y	z		x	y	z	
Dorsolateral prefrontal cortex	L	9/46	-40	30	28	4.46				
	L	9					-44	38	30	3.78
	R	9	48	34	34	4.05				
	R	9/46					48	38	30	3.63
Cingulate cortex	R	32					10	24	42	3.78
(Pre)motor cortex	L	4/6	-20	-6	50	4.50				
	R	4	24	-12	52	4.31				
	L	6	-22	10	58	4.67	-24	12	52	5.69
	R	6	22	16	50	4.83	24	6	54	4.54
	L	8	-22	20	48	3.48				
	R	8	8	24	48	3.67				
Precuneus	L	7	-6	-54	48	5.05				
	L	7	-12	-62	22	4.90	-2	-56	54	4.84
	R	7	10	-58	48	5.59	8	-58	52	4.41
Inferior parietal cortex	L	40	-26	-44	48	4.52	-30	-48	46	4.03
	R	40	52	-40	46	3.82	38	-42	44	3.53
Insular cortex	L		-28	18	-2	4.91	-32	22	-4	3.65
	R		28	26	0	4.36				
Caudate nucleus	R		12	12	0	4.23				
Globus pallidus	L		-10	8	-2	4.04				
Superior occipital cortex	L	19	-42	-76	30	5.05	-38	-78	32	4.60
	R	19	38	-78	32	3.95				

Table 7.4. Brain regions showing significant (p<0.001 uncorrected) BOLD signal increase during planning compared with baseline

Region of activation	*L/R*	*BA*	*Talairach coordinates*			*z-value*
			x	y	z	
Healthy control subjects > OCD patients						
Dorsolateral prefrontal cortex	R	9/46	32	42	14	3.37
(Pre)motor cortex	R	4/6	24	-14	54	3.76
Cingulate cortex	L	32	-14	20	34	3.73
Precuneus	L	7	-10	-64	22	3.66
	R	7	10	-78	44	3.30
Inferior parietal cortex	L	40	-52	-28	32	3.21
Caudate nucleus	R		8	16	6	3.73
Putamen	L		-26	16	-6	3.74
OCD patients > healthy control subjects						
No significant regions						

2. Task load

In controls, increased task load (Table 7.5) was correlated with increased BOLD signal bilaterally in anterior prefrontal (BA 10), dorsolateral prefrontal (BA 9 and 46), cingulate (BA 32), premotor (BA 6 and 8) and insular cortices and the precuneus (BA 7). Furthermore, increases were observed in right inferior parietal cortex (BA 40), left superior occipital cortex (BA 19) and left caudate nucleus. OCD patients, in contrast, did not show increased activity in the caudate nucleus correlating with task load. Instead, task load was correlated with increased activation bilaterally in supplementary motor area, as well as left posterior globus pallidus, left parahippocampal gyrus, thalamus and dorsal brainstem, and right ventrolateral prefrontal cortex (BA 44).

Table 7.5. Brain regions showing significant (p<0.05 corrected) BOLD signal increase correlating with increased task load.

			22 healthy control subjects				***22 OCD patients***			
Regions	*L/R*	*BA*	*Talairach coordinates*			*z-value*	*Talairach coordinates*			*z-value*
			x	y	z		x	y	z	
Anterior prefrontal cortex	L	10	-24	52	2	3.29	-36	52	8	4.14
	R	10	38	50	-4	3.66				
	R	10	34	62	4	3.69				
	R	10/46	32	52	6	3.49	38	54	8	5.03

Table continues (next page)

Table 7.5 (continue)

			22 healthy control subjects				***22 OCD patients***			
Regions	*L/R*	*BA*	*Talairach coordinates*			*z-value*	*Talairach coordinates*			*z-value*
			x	y	z		x	y	z	
Dorsolateral prefrontal cortex	L	9					-44	32	34	4.62
	L	9/46	-44	38	24	4.86	-42	32	28	4.51
	L	46	-30	42	4	3.95				
	R	9	44	32	32	4.73	38	40	34	5.17
	L	8	-40	28	44	3.91	-30	36	46	5.30
	R	8	28	24	50	4.44	30	24	46	4.48
Ventrolateral prefrontal cortex	R	44					58	16	6	4.63
Cingulate cortex	L	32	-10	22	34	3.82	-6	34	28	4.50
	R	32	6	22	40	4.01	6	34	24	3.88
Supplementary motor area	L	6					-6	28	36	4.82
	R	6					6	20	44	5.55
(Pre)motor cortex	R	4/6	18	-10	58	4.46	26	-2	50	4.33
	L	6	-22	6	58	5.55	-34	16	56	4.48
	R	6	26	8	50	4.98	28	4	52	5.20
	L	6/8					-24	16	50	4.58
Precuneus	L	7	-10	-54	48	4.98	-2	-62	52	4.40
	R	7	8	-60	52	5.29	2	-54	52	4.52
Inferior parietal cortex	L	40					-50	-42	40	4.74
	R	40	50	-42	54	4.55	56	-40	40	4.66
Insular cortex	L		-32	20	-4	4.08	-32	20	-4	5.00
	R		34	22	-4	4.84	34	20	-6	4.33
Caudate nucleus	L		-18	4	16	3.78				
Globus pallidus posterior	L						-16	-6	4	4.36
Parahippocampal gyrus	L						-26	-24	-22	3.56
Thalamus	L						-14	-4	-4	3.42
Brainstem	L						-8	-22	-26	3.71
Occipital cortex	L	19	-42	-70	36	3.71	-40	-72	38	4.50

Group by task load interaction analyses (Table 7.6 and Figures 7.2 and 7.3a-c) showed increased activation in controls compared with OCD patients in left dorsolateral prefrontal

cortex (BA 46). In contrast, OCD patients, compared with controls, showed increased activation bilaterally in cingulate (BA 32), ventrolateral prefrontal (BA 47 and 45) and parahippocampal cortices, as well as left anterior temporal cortex and dorsal brainstem.

Table 7.6. Brain regions showing significant ($p<0.001$ uncorrected) BOLD signal increase correlating with increased task load

Region of activation	*L/R*	*BA*	*Talairach coordinates*			*z-value*
			x	y	z	
Healthy control subjects > OCD patients						
Dorsolateral prefrontal cortex	L	46	-32	40	4	3.38
OCD patients > healthy control subjects						
Cingulate cortex	L	32	-2	18	32	3.40
	R	32	9	26	26	3.49
Ventrolateral prefrontal cortex	L	47	-50	24	-10	3.68
	L	47	-46	24	0	3.51
	R	47	50	24	-2	3.84
	R	45	58	20	8	3.63
Anterior temporal cortex	L		-44	0	-32	3.54
Parahippocampal gyrus	L		-24	-24	-24	3.62
	R		24	-18	-26	3.54
Brainstem	L		-4	-26	-20	3.54

Figure 7.2.
Increased BOLD signal in left dorsolateral prefrontal cortex (BA 46) in healthy control subjects compared with OCD patients, correlating with task load.

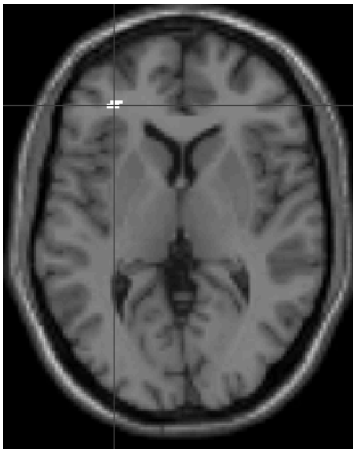
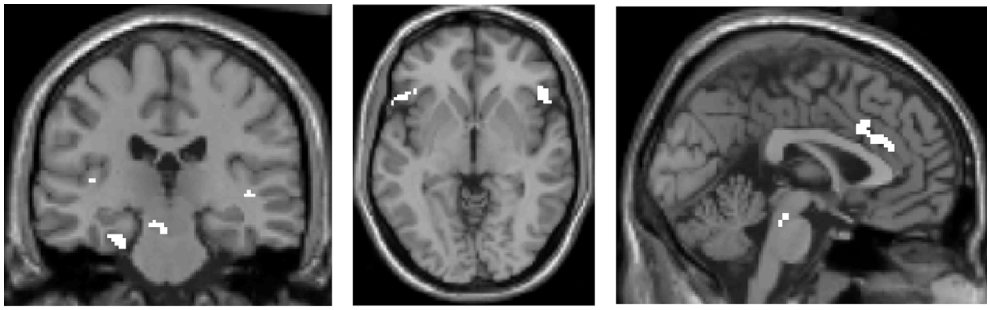


Figure 7.3. Increased BOLD signal correlating with task load in OCD patients compared with control subjects, in a): parahippocampal gyrus and brainstem, b): bilateral ventrolateral prefrontal cortex (BA 47 and 45), and c): cingulate cortex (BA 32).



Analyses of covariance

In OCD subjects, neither main effects for task nor task load effects were associated with symptom severity scores (Y-BOCS and Padua-IR), except for a small region in left ventrolateral prefrontal cortex (BA 44, z-value = 3.24). Additional analyses of covariance were performed to investigate whether the observed between-group differences could be explained by differences in demographic variables or levels of subjective distress. However, the above-described group by task interaction effects in favor of control subjects persisted after regressing out differences in age, education level, sex ratio, and state anxiety.

Discussion

In the present study, a parametric self-paced pseudo-randomized event-related fMRI version of the Tower of London task was used to investigate the neuronal substrate of planning in medication-free OCD patients compared with healthy control subjects. This paradigm allowed flexible responding as well as post hoc selection of correct trials, so that analyses of imaging data were not confounded by performance differences[35]. The present results not only confirm previous findings with regard to impaired planning capacity in OCD, but also clearly demonstrate decreased responsiveness of dorsal frontal-striatal circuits in OCD patients.

Behavioral data showed increased RTs in OCD patients only during the two easiest task levels, whereas performance scores were significantly lower compared with control subjects across all levels. These behavioral findings are in agreement with some, but not all, previous findings with regard to planning performance in OCD. While some studies have reported decreased response speed[24] or performance scores[25], other studies found unaffected planning capacity in OCD patients compared with healthy control subjects[22;23]. As discussed previously, these differences are likely to reflect methodological differences

in task implementation, such as mental performance vs. the use of a touch screen, or providing feedback vs. no feedback[21;24].

Imaging results showed increased task-associated activation in controls compared with OCD patients in several regions previously found to be involved in planning, in particular dorsolateral prefrontal cortex, basal ganglia, and parietal cortex. Task load correlated activity was found in left dorsolateral prefrontal cortex in control subjects compared with OCD patients. In contrast, OCD patients showed increased activation in bilateral cingulate, ventrolateral prefrontal and parahippocampal cortices, left anterior temporal cortex and dorsal brainstem, correlated with increased task load. Our finding of decreased responsiveness of dorsal frontal-striatal circuits during planning in OCD patients is in accordance with previous findings with regard to basal ganglia dysfunction in OCD in implicit learning[28;29]. However, in the Rauch et al. study[28], OCD subjects showed impaired performance during motor skill acquisition, suggesting basal ganglia-motor cortical rather than striatal-dorsal prefrontal dysfunction, as found in the present study. In addition, several functional imaging studies using symptom provocation designs have reported abnormalities in orbitofrontal-striatal function in OCD, thought to reflect its role in ritualistic behavior[36-40]. Most of these studies, however, lacked adequate control groups, or failed to find significant group by task interactions in subcortical areas[36;37;40]. Increased prefrontal-subcortical glucose uptake in OCD patients relative to controls, resolving after successful therapy, has also been reported in a number of resting-state imaging studies[41-45]. Although apparently in one investigation dorsal prefrontal abnormalities were found[43], the majority of these studies have reported increased orbitofrontal-striatal metabolism. Moreover, although dorsal and ventral PFC are known to project to dorsal and ventral parts of the striatum, spatial resolution of FDG-PET may have been insufficient to detect differences based on topographical organization within the basal ganglia. Therefore, the evidence for pathologically increased baseline activity in dorsal prefrontal-striatal, as opposed to ventral frontal-striatal, loops in OCD is still inconclusive. However, although the present functional MRI data do not allow baseline comparisons, our finding of decreased or absent prefrontal-striatal responsiveness is compatible with existing pathophysiological models of OCD hypothesizing basal ganglia disinhibition due to an altered balance between indirect, inhibitory and direct, excitatory cortico-striatal-thalamico-cortical circuits[46;47]. Whether the failure of OCD patients to recruit dorsal striatal-prefrontal regions in comparison with controls, as found in the present study, is specific for planning tasks is also not yet clear. In a recent fMRI study, van der Wee et al.[48] found decreased performance only at the highest task level in OCD patients compared with controls during performance of a spatial n-back task. Imaging results showed similar activity in bilateral dorsolateral prefrontal and parietal cortex in both groups, from which the authors concluded that (spatial) working memory in OCD was not abnormal. The findings of the present study provide support for this conclusion. Whereas it might be argued that in our paradigm not only planning complexity but also working memory load was (linearly) increased, our results reveal impaired planning at all levels, which is difficult to explain by differences in working memory capacity.

In the present study, OCD patients, compared with control subjects, showed increased activation of bilateral ventrolateral prefrontal, anterior cingulate and parahippocampal cortices, left temporal cortex and dorsal brainstem, correlated with task

load. These interaction effects may reflect both compensatory processes and increased arousal. Functional neuroanatomical subdivisions of the lateral prefrontal cortex (i.e. ventrolateral and dorsolateral PFC) have been proposed based on stimulus type (verbal vs. object/spatial[49]) [50] and process type (maintenance/manipulation), recent evidence favoring the latter hypothesis[51;52]. Thus, the VLPFC region (BA 44, 45 and 47) supports processes that transfer, maintain and match information in working memory. In contrast, the dorsolateral prefrontal cortex (BA 9 and 46) supports complex processes operating on information (spatial as well as non-spatial) that is currently maintained in working memory, such as monitoring, manipulation and higher-level planning. Beyond maintenance in VLPFC and manipulation in DLPFC, the anterior prefrontal cortex (BA 8 and 10) is associated with processes of a third level of executive control[53]. Increased VLPFC activity may reflect not only working memory load, i.e. the number of items kept 'on line', but also retrieval and maintenance of abstract rules used for problem solving[54]. In addition, it has been shown that VLPFC activity is selectively increased during arithmetic computations, in particular when subjects attempt to resolve a conflict between externally presented answers and internally computed solutions[55]. Such a mechanism may explain the increased activity of bilateral VLPFC associated with task load in OCD patients compared with control subjects observed in the present study.

Compared with control subjects, OCD patients also showed increased activity of anterior cingulate cortex (BA 32) correlated with task load. Anterior cingulate involvement in OCD has been observed at rest, during symptom provocation, and after having committed errors during cognitive tasks[37;40]. The ACC has been implicated in performance monitoring, error detection, conflict monitoring, response selection, and reward expectation[56]. ACC activity has been found prior to a response during correct trials and immediately following error trials, reflecting its role in error prevention and detection[57]. Ursu et al.[58] reported increased activation of the ACC in OCD patients, compared with control subjects, during both incorrect and correct trials, indicative of an overactive performance monitoring system in OCD, independent of the actual occurrence of errors. These results were replicated in a subclinical group of obsessive-compulsive undergraduates[59]. In addition to the ACC, the supplementary motor area (SMA) may also have a role in performance monitoring[60]. Increased activity in ACC and SMA was also observed during performance of a spatial n-back task in OCD patients, thought to reflect increased effort to develop an efficient strategy or increased error monitoring[48]. A functional neuroanatomical dissociation between these areas has been proposed, with error processing associated with the anterior cingulate region and response competition with SMA[61]. Increased performance monitoring during correct task performance, as well as during errors, may be characteristic of the critical self-evaluation of performance in OCD, leading to inappropriate need for correction and, consequently, repetitive behavior.

Increased activity correlated with task load was also observed in bilateral parahippocampal gyrus (PHG) in OCD patients relative to controls. Similar results were obtained by Rauch et al.[28] in OCD patients during a motor sequence learning paradigm. The authors hypothesized that dysfunction of corticostriatal systems in OCD resulted in compensatory recruitment of 'explicit networks' to perform the task. However, debriefing data from their study showed that OCD patients did not differ from controls in explicit knowledge with regard to the task. In the present study, PHG involvement may reflect

intermediate-term memory for spatial information, in addition to working memory, supported by parietal and lateral prefrontal cortex as discussed above, and long-term memory (more than about 2 minutes) provided by the hippocampal formation[62]. Increased activity of both PHG and VLPFC may therefore be secondary to OCD patients' failure to develop an adequate strategy, so that they need to rely on short- and intermediate term memory capacity to perform the task[3]. Connections exist between the rostral anterior cingulate cortex (BA 32) and parahippocampal gyrus[63]. Faw[64] described this system of ACC and adjacent dorsal medial prefrontal cortex, with extensions to the hippocampal stream, as a key player in spatiotemporal processing and attention, supporting executive functions located in dorsolateral prefrontal cortex.

Finally, we found an area of increased activation in left dorsal brain stem associated with task load in OCD patients compared with control subjects. This region was located within the reticular formation, extending rostrally into the periaquaeductal grey, and may therefore reflect increased arousal at higher task loads in our OCD group[65;66]. Arousal responses implicate activation of the hypothalamic-pituitary-adrenal (HPA) axis and the reticular activating system (RAS). The RAS is composed of cholinergic neurons from the pedunculopontine nucleus, stimulating the noradrenergic system of the locus coeruleus (LC), which in turn inhibits cholinergic output of the pedunculopontine nucleus[65]. Evidence from non-stress experiments suggests that phasic LC activity is associated with increased attention and task related performance[66-68], whereas tonically increased tonic activity of the LC results in attentional instability, decreased performance, and increased emotional reactivity[66;68]. Chronic stress, on the other hand, may lead to locus coeruleus damage, resulting in reduced output from the LC[69] and disinhibition of the pedunculopontine nucleus[70]. In the present study, our finding that increased dorsal brain stem activity in OCD was associated with task load suggests increased effort rather than increased subjective distress, although we cannot rule out the latter possibility.

To our knowledge, this study is the first to demonstrate impaired planning associated with decreased dorsal frontal-striatal responsiveness in OCD. Strengths of the study are the inclusion of medication-free subjects, a large group size permitting random effects analyses, and the use of a parametric self-paced event-related design. The present study is not without limitations, however. First, education level was higher in control subjects, although we controlled for performance differences by selecting correct responses only. In addition, post hoc analyses of covariance with regard to education level, sex ratio, and age were performed. Moreover, it has been shown that planning performance (both error rates and RTs) is not correlated with intelligence measures[71]. Second, although in the present study no clear correlations were found between symptom severity ratings and outcome measures, in accordance with previous studies[3;48], we did not specifically investigate OCD subgroups, e.g. with prominent washing or checking symptoms. Although little is known with regard to the generalisability of neuropsychological deficits across clinical subtypes, a dimensional model of OCD has been proposed, in which various symptom dimensions are associated with differential patterns of functional neuroanatomical abnormalities[72;73]. Recent data showed activation of VLPFC and caudate nucleus correlated with washing characteristics and dorsal regions (DLPFC, thalamus and putamen/globus pallidus) with checking symptoms[73]. Based on these subtype differences in provocation experiments, one might hypothesize that impaired planning and decreased responsiveness

of dorsal prefrontal-striatal circuits as found in present study, mainly concerns OCD patients with predominant checking symptoms. Another issue for future research is whether the dorsal frontal-striatal dysfunction observed in this study is specific for OCD or extends to other neuropsychiatric disorders. These include not only anxiety disorders, but also basal ganglia disorders such as Tourette's syndrome, and major depressive disorder (MDD). MDD is characterized by impairments in various executive functions, such as verbal fluency, and attentional set shifting[74]. However, in MDD, dorsal prefrontal baseline perfusion is decreased, rather than increased[75]. Moreover, MDD is not associated with striatal pathology[76]. Whereas depressive symptoms frequently co-occur in OCD, cognitive deficits in OCD are not associated with comorbid depression[3], suggesting different pathophysiological mechanisms in OCD compared with MDD. Future studies should also investigate whether the executive deficits in OCD are specific for the present paradigm, or can be replicated during other 'strategic' tasks, such as set-shifting tasks. Finally, to further elucidate the pathophysiology of OCD, the 'state-trait' issue needs to be addressed by using pre-post treatment designs.

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8

Disorder specific neuroanatomical correlates of attentional bias in obsessive-compulsive disorder, panic disorder and hypochondriasis

van den Heuvel OA, Veltman DJ, Groenewegen HJ, Witter MP, Merkelbach J, Cath DC, van Balkom AJLM, van Oppen P, van Dyck R

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Abstract

Attentional bias to disease relevant emotional cues is considered to be important in the causation and maintenance of affective symptoms in anxiety disorders. However, neuropsychological studies have reported conflicting results concerning the disease-specificity of attentional bias. Moreover, although frontal-striatal and limbic circuits have been implicated in attentional bias and emotion processing, neuroanatomical correlates of emotional interference across different anxiety disorders have not yet been investigated.

A functional MRI version of a cognitive and emotional Stroop task was used in 16 patients with obsessive-compulsive disorder (OCD), 15 patients with panic disorder (PD), 13 patients with hypochondriasis and 19 healthy control subjects. All subjects were medication-free. The paradigm consisted of congruent color words, incongruent color words, OCD related negative words, panic related negative words and neutral words.

During incongruent versus congruent color naming, all patient groups recruited additional posterior brain regions relative to controls, but only in OCD patients performance was impaired. In OCD, color naming of OCD related, but not panic related words, was associated with increased activation of frontal-striatal and temporal regions, although performance was not abnormal. In contrast, in PD patients, increased frontal-striatal involvement was found during color naming of both OCD and panic related words. In PD, color naming of panic related words was significantly slowed and was associated with increased activation of right amygdala and hippocampus. Hypochondriasis patients showed a similar activation pattern to PD patients.

The present results support the hypothesis of increased distractibility for irrelevant information in patients with OCD, PD and hypochondriasis compared with healthy control subjects, associated with frontal-striatal and limbic involvement. Clear differences between OCD patients on the one hand and PD and hypochondriasis patients on the other were found. Increased cognitive interference was observed only in OCD. Although OCD patients did not display an attentional bias in behavior relative to healthy controls, there was a clear and specific neuronal response during color naming of OCD related words, involving mainly ventral brain regions. In contrast, generalized emotional interference effects were found in PD and hypochondriasis, involving both ventral and widespread dorsal brain regions, reflecting not only unconscious emotional stimulus processing but also increased cognitive elaboration.

Introduction

Difficulty inhibiting irrelevant information, for instance obsessive thoughts and impulses, is a key feature of obsessive-compulsive disorder (OCD)[1;2]. Because most of their attentional resources are allocated to threat cues related to their concerns, OCD patients are limited in their ability to selectively attend to relevant information, while simultaneously ignoring irrelevant competing information[1]. Similar cognitive dysfunctions have been described for panic disorder (PD)[3]. Attentional bias in anxiety disorders is presumably not simply a by-product of emotional state, but may play a major role in symptom causation and maintenance[4-6]. The critical process of gating, i.e. inhibiting irrelevant information, has been linked to frontal-striatal function[7;8]. Impaired frontal-striatal function is considered to be of etiological importance in the affective, behavioral, as well as cognitive characteristics of OCD[9]. In contrast, the brainstem and limbic regions, such as the amygdala and (para)hippocampal region, are mainly implicated in the symptomatology of PD[10-13].

Cognitive interference occurs when processing one stimulus impedes simultaneous processing of another stimulus, as in Stroop‘s color-word task[14]. This task requires the ability to actively inhibit a fast automatic response (word reading) in favor of a slower voluntary response (color naming), resulting in increased response latencies and error rates during the interference condition (e.g. “red” printed in blue ink) compared with the facilitation (or baseline) condition (e.g. “red” printed in red (or black) ink).

Frontal involvement in interference processes was first demonstrated in lesion studies, showing impaired Stroop performance in patients with lesions in left[15] and right[16] lateral prefrontal cortex. More extensive investigation of the neuronal correlates of interference in healthy subjects was undertaken with the advent of positron emission tomography (PET)[17-21], functional magnetic resonance imaging (fMRI)[22-31] and magnetoencephalography[32]. In many[17-20;22;24;25;27;28;30;31], but not all[18;21;26;29;32] studies interference was associated with increased activation of anterior cingulate cortex (ACC). Prefrontal involvement in interference was reported in dorsolateral (DLPFC)[19;22;23;26-29;31;32], anterior (aPFC)[18;20;29;32] and ventrolateral (VLPFC)[21;25;26;28;29] prefrontal subregions. Recent imaging studies have shown that ACC is primarily involved in response-related processes, such as performance monitoring, response conflict and error detection[24;27;28;31;33;34]. In contrast, various prefrontal regions seem to be primarily responsible for the implementation of top-down attentional control (i.e. higher order regulation of hierarchically lower attentional processes) per se, although the relative contribution of specific prefrontal subregions is insufficiently clear[24;27;28;31]. Other regions associated with interference were (pre)motor[17;22;25;32], temporal[17;22;23;25;29;31], parietal[18-20;22;23;28;29;32], extrastriate[17;19-21;23;26;31] and insular[19;20;25] cortices, thalamus[19;20;23] and striatum[17;19;23;25].

In OCD patients, neuropsychological studies using the word-color Stroop task have provided mixed evidence for impaired selective attention compared with control subjects[1;35-40]. Some studies showed that OCD patients performed slower than control subjects[1;35] and made significantly more errors and had slower reaction times during the interference condition of the Stroop task[1;37]. This interference effect in OCD patients was augmented when situational anxiety was high[1]. Moreover, decreased Stroop performance in OCD patients was found to be correlated with decreased resting state regional cerebral

metabolism rates in lateral prefrontal cortex[35], in agreement with the notion of prefrontal involvement in the pathophysiology of OCD.

A potentially more powerful and specific way to investigate selective attention and response inhibition in anxiety disorders is based on an emotional analogue of the Stroop task[5]. When anxious patients are presented with colored words relevant to their concerns, automatic semantic processing will delay voluntary color naming. This interference effect, however, is not restricted to patients; control subjects, after priming to an anxious state, also show interference during color naming of threat words[41;42]. Whalen et al[43], using a counting version of the emotional Stroop task during functional MR imaging in healthy subjects, reported significant activation in the affective (rostral) subdivision of the ACC during emotional interference in the absence of a behavioral interference effect. In contrast, the traditional Stroop task was correlated with increased activation of the dorsal anterior cingulate cortex (cognitive subdivision of the cingulate cortex)[22]. The authors suggested that rostral ACC activation reflected a regulatory response during successful suppression of task-irrelevant emotional information.

Neuropsychological studies using the emotional Stroop task in various anxiety disorders, such as generalized anxiety disorder (GAD)[44-46], PD[47-53], OCD[53-56], posttraumatic stress disorder (PTSD)[57-59], social phobia[46;60-64] and spider phobia[65;66], all (but one[53]) showed robust interference effects in patients during color naming of threat related words. It is not yet clear, however, whether this effect is specific to disease related threat words or extends to general threat domains (or even positive emotional material), although some anxiety disorders seem to be more specific in their attentional bias than others. Whereas patients with OCD[54-56;67] and social phobia[46;63;64] showed a predominantly disease-specific attentional bias, threat word interference in GAD[46] and PD[49] was found to be more generalized[51;52]. A second issue of consideration in emotional interference is the explicit versus implicit nature of the attentional bias. Most studies using subliminal stimulus presentation support the assumption that interference is not dependent on conscious strategies[5;52;68;69].

To summarize, evidence from imaging data in healthy subjects as well as from neuropsychological studies in anxiety disorder subjects appear to be in agreement with the hypothesis of frontal involvement in emotional Stroop interference. However, direct evidence for the underlying neuronal substrate in anxiety disorders is lacking, with a single exception[70]. Shin and colleagues[70] found activation of the rostral ACC in non-PTSD Vietnam veterans during trauma-related emotional interference, similar to the results of Whalen et al[43] in healthy volunteers, but not in veterans with PTSD. Shin et al suggested that the absence of rostral ACC activation in PTSD patients reflected the inability of this region to inhibit amygdalar hyperresponsivity, although no amygdala activation was found during threat-related emotional processing in their PTSD patients. They did not include additional experimental groups, so that the specificity of their findings remains to be confirmed.

The aim of the present study was to investigate the neuronal substrate of both cognitive and emotional Stroop interference across anxiety disorders, by addressing the following questions. First, is increased cognitive interference, as found in OCD, syndrome-specific? Second, do anxiety disorder patients show an attentional bias only for stimuli

relevant to their concerns? Third, is the neuronal substrate of emotional interference different across anxiety disorders? To answer these questions, functional MRI data of patients with OCD, PD and hypochondriasis were compared with data of healthy control subjects, during performance of a Stroop task containing color-congruent color words, color-incongruent color words, OCD related negative words, panic related negative words and neutral words. Although emotional Stroop interference at a behavioral level can be demonstrated using either supra- or subliminal stimuli, we chose to present overt (supraliminal) stimuli based on observations that covert stimuli are not very effective in eliciting regional cerebral blood flow changes in the amygdalar region[71;72], unless in an aversive conditioning paradigm[73]. In agreement with the results of previous studies, we hypothesized that OCD patients would show increased interference effects for incongruent versus congruent color words. In addition, we expected significant interference effects in OCD for disease-specific threat words only, compared with generalized interference effects (i.e. for PD and OCD related words) in PD. To investigate whether any increased responsiveness to PD and OCD related words is specific to OCD and/or PD, we included a third experimental group of patients with hypochondriasis. In hypochondriasis, obsessions or compulsions are by definition restricted to illness concerns. However, compared with PD, in hypochondriasis patients these worries typically do not spiral into panic[74]. We expected to find frontal-striatal as well as limbic (including amygdala) regions correlated with emotional interference in all patient groups compared with healthy controls.

Methods

Subjects

Eighteen OCD patients (6 males, age 33.4±2.4 yrs (mean±SE)), 15 PD patients (8 males, age 33.7±2.5 yrs), 14 hypochondriasis patients (12 males, age 40.6±3.2 yrs) and 19 healthy control subjects (10 males, age 30.3±1.9 yrs) performed the Stroop task, while functional MRI data were collected. Mean age ($p=0.04$) and M/F ratio ($p=0.04$) were both higher in the hypochondriasis group compared with other groups. All subjects were right-handed. Patients were recruited from the outpatient clinic for anxiety disorders of GGZ Buitenamstel/VU University Medical Center (VUMC) and the Netherlands Anxiety, OCD and Phobia Foundation. The medical ethical review board of the VUMC approved of the study and all participants provided written informed consent.

Exclusion criteria were the presence of major internal and/or neurological illness, other psychiatric disorders and the use of psychotropic medication. Subjects had to be off medication for at least 4 weeks. One OCD patient had comorbid hypochondriasis, one hypochondriasis patient had comorbid PD and one OCD patient was found to have a vascular malformation near the ACC. These three patients were excluded from further analysis.

Diagnoses were established using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I/P)[75]. To assess symptom characteristics and severity scores, the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS)[76], the Padua Inventory-revised (Padua-IR)[77;78], the Yale-Brown Obsessive-Compulsive Scale for Hypochondriasis (Y-

BOCSH)[76] and the Whitley Index (WI)[79] were used. In addition, the Body Sensations Questionnaire (BSQ)[80] was administered to PD patients.

Task paradigm

The Stroop paradigm used in the present study consisted of 6 conditions: congruent color-words (e.g. "red" printed in red), incongruent color-words (e.g. "red" printed in blue), OCD related negative words (e.g. dirty, mess, uncertain), panic related negative words (e.g. heart attack, crowd, faint) and two baseline conditions with neutral words (e.g. percent, month, oval). OCD related words were selected based on studies of Lavy et al[55;66]; panic related words were derived from earlier research of attentional bias in PD[48].

Stimuli were presented in a block design, consisting of eighteen randomized blocks (three blocks of each condition), each containing sixteen words. Each word was presented during 2 seconds, followed by a 200ms blank screen. Subjects were asked to respond as fast as possible by pressing the button corresponding to the color of the ink (yellow, green, red, blue), irrespective of the meaning of the word. After performing the task, subjects were asked to rate subjective distress using a 100-point analogue scale.

Functional MRI data acquisition

Imaging was performed on a 1.5T Sonata MR system (Siemens, Erlangen, Germany) with a standard circularly polarized head coil. Stimuli were projected on a screen at the end of the scanner table, which was seen through a mirror mounted above the subject's head. Two magnet-compatible four-key response boxes were used to record subject's performance and response times (RT). To reduce motion artifacts, the subject's head was immobilized using foam pads.

For functional MR imaging, an axial echo planar sequence (TR 1.99sec., TE 45ms., matrix 64x64, field of view 192x192mm, flip angle 90°) was used (28 slices, 3x3mm in-plane resolution, 3.6mm between-plane resolution), which was selected based on its sensitivity to signal changes in (orbital) prefrontal cortex. In total 296 EPI volumes per subject were scanned, 48 EPI volumes in each condition. In addition, a coronal T1-weighted MR was acquired (matrix 256x160, voxel size 1x1x1.5 mm, 160 sections).

Imaging data analysis

Imaging data were analyzed using SPM99 (Wellcome Department of Cognitive Neurology, http:www.fil.ion.ucl.ac.uk/spm). After discarding the first 6 volumes, time-series were corrected for differences in slice acquisition times and realigned. Spatial normalization into approximate Talairach and Tournoux space was performed using a standard SPM EPI template. Data were resliced to 2x2x2mm voxels and spatially smoothed using a 6mm Gaussian kernel. Subsequently, data were band pass filtered, and analyzed in the context of the General Linear Model, using boxcar regressors convolved with the canonical hemodynamic response to model responses during each condition.

For each subject, weighted contrasts were computed for cognitive color-word interference (incongruent versus congruent color words), for OCD related emotional interference (OCD related negative words versus neutral words) and for panic related emotional interference (panic related negative words versus neutral words). These contrast images containing parameter estimates for main effects were entered into a second level

(random effects) analysis, using one-way ANOVA's for each contrast. Main effects and group by task interaction effects are reported at $p<0.05$ significance level corrected for multiple comparisons using the False Discovery Rate method[81].

Results

Clinical characteristics

OCD patients had significantly higher scores on the Y-BOCS (23.4±1.7) and Padua-IR (63.2±5.6) than subjects of other groups (ANOVA: $F(3,53)=144$, $p<0.001$ and $F(3,52)=22$, $p<0.001$), respectively). Hypochondriasis patients scored significantly higher on the Y-BOCSH (25.9±3.6) and the WI (24.4±2.4) than subjects of other groups (ANOVA: $F(3,55)=60$, $p<0.001$ and $F(3,55)=34$, $p<0.001$, respectively). Mean BSQ scores in PD patients were 38.1±3.3, in agreement with previous findings[82].

Task performance

Behavioral data of one hypochondriasis patient were lost due to technical problems. Mean response times (RTs) and performance rates for each group are listed in Tables 8.1a and 8.1b. Across groups, RTs showed a significant increase for incongruent versus congruent color words (ANOVA: $F(1,61)=129.8$, $p<0.001$) and panic related words versus neutral words ($F(1,61)=34.7$, $p<0.001$), but not for OCD words versus neutral words ($F(1,61)=0.7$, $p=0.40$). Between-group comparisons revealed that the increase for panic related words versus neutral words was greater in PD and hypochondriasis patients compared with both OCD patients (ANOVA: $F(1,30)=6.5$, $p=0.016$ and $F(1,27)=4.8$, $p=0.037$ respectively) and controls ($F(1,33)=9.1$, $p=0.007$ and $F(1,30)=10.4$, $p=0.003$ respectively). RTs for color naming of neutral words were also longer in PD patients ($F(1,33)=6.9$, $p=0.013$) and hypochondriasis patients ($F(1,30)=6.7$, $p=0.015$) compared with controls. The increase in RTs for incongruent versus congruent color words in OCD subjects was greater than in PD patients ($F(1,30)=4.2$, $p=0.049$) and marginally greater than in control subjects ($F(1,34)=3.4$, $p=0.076$). Correlation analyses showed significant associations between subjective distress scores and increased RTs for color naming of panic related in PD patients (Pearson's $r=0.56$, $p=0.047$) and for color naming of OCD words in OCD patients ($r=0.63$, $p=0.011$).

Table 8.1a. Response times, averaged within each condition for each subject (mean (± SE), in milliseconds)

	Controls	*PD*	*OCD*	*Hypochondriasis*
Congruent color	831 (± 31)	891 (± 33)	834 (± 32)	873 (± 34)
Incongruent color	985 (± 25)	1027 (± 28)	1062 (± 45)	1073 (± 34)
OCD related	874 (± 25)	949 (± 27)	943 (± 43)	953 (± 36)
Panic related	898 (± 20)	1057 (± 29)	986 (± 46)	1022 (± 34)
Neutral 1	870 (± 25)	967 (± 27)	934 (± 39)	984 (± 39)
Neutral 2	867 (± 28)	902 (± 26)	940 (± 41)	912 (± 33)

Table 8.1b. Performance scores, expressed as percentage of the total number of responses within each condition for each subject (mean (± SE), in %)

	Controls	*PD*	*OCD*	*Hypochondriasis*
Congruent color	83 (± 4.6)	87 (± 2.5)	85 (± 5.4)	79 (± 7.5)
Incongruent color	84 (± 4.5)	82 (± 5.2)	74 (± 7.4)	75 (± 7.3)
OCD related	86 (± 4.9)	93 (± 2.5)	89 (± 5.3)	86 (± 8.2)
Panic related	87 (± 4.9)	90 (± 3.2)	87 (± 6.2)	82 (± 8.0)
Neutral 1	84 (± 4.6)	88 (± 3.2)	85 (± 5.2)	80 (± 7.9)
Neutral 2	87 (± 4.9)	90 (± 3.8)	88 (± 6.4)	83 (± 7.9)

Analysis of performance scores across all groups (Table 1b) showed a significant increase in error rates during incongruent versus congruent color naming ($F(1,61)=5.6$, $p=0.021$), but not during color naming of panic related words versus neutral words ($F(1,61)=0.3$, $p=0.57$). The increase in error rates was greater in OCD subjects compared with controls ($F(1,34)=4.1$, $p=0.050$), whereas compared with controls performance was unimpaired in PD and hypochondriasis patients. Paradoxically, error rates *decreased* across groups during color naming of OCD related words versus neutral words ($F(1,61)=36.1$, $p<0.001$). Paired comparisons showed that this decrease was greater in hypochondriasis patients ($F(1,30)=5.8$, $p=0.022$) and marginally greater in PD patients ($F(1,33)=2.9$, $p=0.097$) compared with controls. Correlation analyses showed a significant negative association between subjective distress scores and performance during color naming of panic related words in the hypochondriasis group ($r = -0.72$, $p=0.008$).

Imaging data

1. Incongruent versus congruent

Across all groups, regions showing increased Blood Oxygenation Level Dependent (BOLD) signal during color naming of incongruent versus congruent color words (see Figure 8.1) were present in right aPFC, bilateral DLPFC, VLPFC, dorsal ACC, motor, premotor, supplementary motor, insular, inferior parietal, superior and middle temporal, temporal-occipital and peristriate cortices, striatum, thalamus, hypothalamus, rostral brainstem and cerebellar cortex, and in left fusiform gyrus. Group by task interaction effects are shown in Table 8.2. Compared with control subjects, increased activation was found in OCD patients in right precuneus, left parahippocampal gyrus and left brainstem, in PD patients in right fusiform gyrus and in hypochondriasis patients in bilateral inferior parietal cortex, precuneus, left insular cortex and left rostral brainstem. No regions of significantly increased activation were found in control subjects compared with patients.

2. OCD related versus neutral

Color naming of OCD related words compared with neutral words, across all groups (see Figure 8.1) was correlated with increased BOLD signal in bilateral VLPFC, left OFC, right middle temporal cortex, bilateral superior temporal cortex, right parietal-occipital cortex and right precuneus. Group by task interactions are shown in Table 8.3. Compared with

Figure 8.1. 'Glass brain' renderings showing main effects for the contrasts of interest (incongruent versus congruent color words, OCD related versus neutral words and panic related versus neutral words).

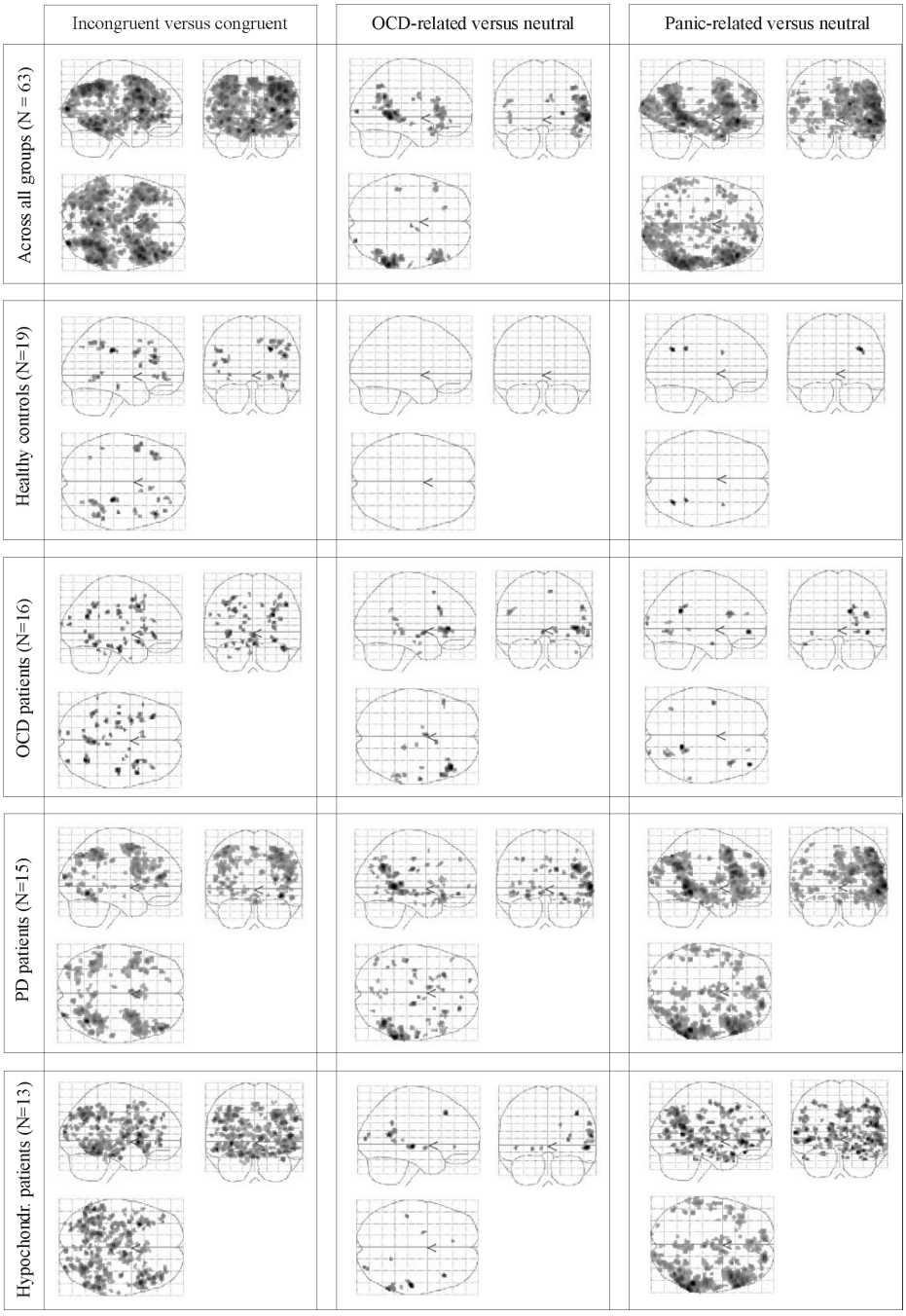


Table 8.2. Brain regions showing significant ($p<0.05$ corrected) BOLD signal increase correlating with cognitive interference (incongruent vs. congruent color words), group by task interactions (column > row).

	Healthy controls >	*OCD patients >*			*PD patients >*			*Hypochondriasis patients >*		
		Brain regions	*BA*	*z-value*	*Brain regions*	*BA*	*z-value*	*Brain regions*	*BA*	*z-value*
Healthy controls	-	R precuneus	7	3.46	R fusiform gyrus	19	4.91	R/L inf. parietal	39/40	4.04/4.61
		L parahippoc. g.	36	3.45				R/L precuneus	7/31	4.47/4.08
		L brain stem		3.35				L brain stem		4.57
OCD	No significant regions	-			R fusiform gyrus	19	4.96	No significant regions		
PD	No significant regions	No significant regions			-			No significant regions		
Hypoch.	No significant regions	No significant regions			No significant regions			-		

Table 8.3. Brain regions showing significant ($p<0.05$ corrected) BOLD signal increase correlating with OCD related emotional interference (OCD related vs. neutral words), group by task interactions (column > row).

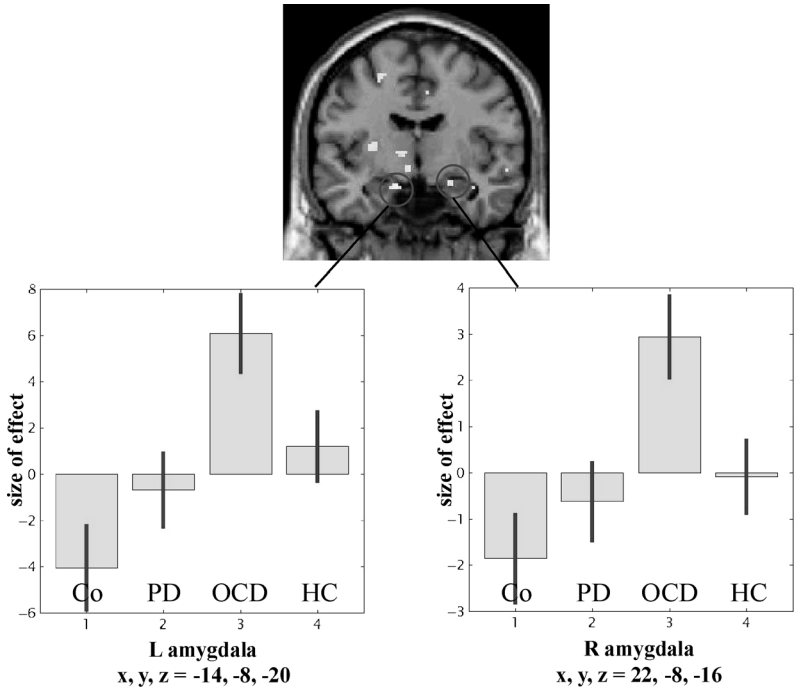
	Healthy controls >	*OCD patients >*			*PD patients >*			*Hypochondriasis patients >*		
		Brain regions	*BA*	*z-value*	*Brain regions*	*BA*	*z-value*	*Brain regions*	*BA*	*z-value*
Healthy controls	-	R VLPFC	45	3.86	L anterior PFC	10	4.18	R DLPFC	8	3.91
		L motor cortex	4	4.29	R medial PFC	8	3.67	R posterior ACC	31	3.78
		R SMA	6	4.01	R/L DLPFC	8/9	3.91/3.98	L rostral ACC	24	3.91
		R/L dorsal ACC	24	3.68/4.09	L OFC	47	5.03	L pallidum		3.77
		L putamen		3.74	L (pre)motor	4/6	4.93	R/L sup. temp.	22	3.56/3.63
		R/L amygdala		3.63/3.80	L dors. ACC	24/31	3.94	R/L mid. temp.	21	4.20/3.58
		L hypothalamus		3.80	R/L putamen		4.19/3.75			
		R/L sup. temp.	22	4.21/3.90	R sup. temp.	22	4.10			
					R mid. temp.	21	4.12			
					R/L parieto-occ.	39/19	4.28/3.83			
					R/L cuneus	18/19	4.67/4.11			
OCD	No significant regions	-			No significant regions			No significant regions		
PD	No significant regions	R amygdala		3.79	-			No significant regions		
Hypoch.	No significant regions	R VLPFC	45	4.39	L putamen		3.82	-		
		R mid. temp.	21	3.86	R mid. temp.	21	4.08			
		R SMA	6	3.84	L parieto-occ.	19/39	4.22			

Table 8.4. Brain regions showing significant (p<0.05 corrected) BOLD signal increase correlating with panic related emotional interference (panic related vs. neutral words), group by task interactions (column > row).

	Healthy controls >			*OCD patients >*			*PD patients >*			*Hypochondriasis patients >*		
	Brain regions	*BA*	*z-value*	*Brain regions*	*BA*	*z-value*	*Brain regions*	*BA*	*z-value*	*Brain regions*	*BA*	*z-value*
Healthy controls	-			No significant regions			R/L ant./rmed. PFC	10	4.21/4.19	R medial PFC	10	3.38
							L medial PFC	8	4.01	R DLPFC	8	3.67
							R DLPFC	9, 46	3.64	L VLPFC	47	3.64
							R VLPFC	45, 47	3.73	R OFC	11	3.94
							R OFC	11	3.97	L SMA	6	4.38
							L SMA	6	3.96	L caudate nucleus		3.62
							R dorsal ACC	24	3.70	L thalamus		3.84
							L rostral ACC	24	3.52	L mid. temp. cortex	21	4.05
							R thalamus		3.91	L sup. temp. cortex	22	3.96
							R mid. temp. cortex	21, 37	4.65	R hippocampus		3.89
							R amygdala		3.23			
							R hippocampus		3.57			
							R/L inf. parietal cortex	39	4.28/3.70			
OCD patients	No significant regions			-			L anterior PFC	10	4.46	R medial PFC		4.11
							R/L DLPFC	9, 46	3.99/3.67	R/L DLPFC	9	3.71/3.93
							R VLPFC	44, 45	4.49	L VLPFC	47	4.50
							R OFC	11, 47	4.76	R/L (pre)motor cortex	4, 6	4.41/4.46
							L medial PFC	8	4.30	L rostral ACC	32	3.82
							R/L (pre)motor cortex	6	4.62/4.81	L post. cingul. cortex	31	4.29
							R rostral ACC	24	3.57	R/L thalamus		4.37/4.17
							R/L thalamus		3.62/3.71	L hypothalamus		4.09
							R rostral brain stem		4.16	R/L mid. temp. cortex	21	4.17/3.77
							R/L mid. temp. cortex	21, 37	5.39/4.31	L sup. temp. cortex	22	3.76
							R hippocampus		3.24	R hippocampus		3.73
							R amygdala		3.21	R parahippoc. gyrus		4.20
							R/L pariet.-occ. cortex	19, 39	5.09/4.42	L pariet-occ cortex	39	3.92
							L precuneus	7	4.03	R/L cuneus	19	3.96/4.41
										R/L cerebellum		4.38/3.61
PD patients	No significant regions			No significant regions			-			No significant regions		
Hypoch. patients	No significant regions			No significant regions			No significant regions			-		

control subjects, anxious patients showed widespread increased involvement of frontal-striatal regions. Whereas OCD patients, compared with control subjects, showed significantly increased activation of bilateral amygdala and left hypothalamus during color naming of OCD related words, PD and hypochondriasis patients did not (see Figure 2). In OCD patients, increased activation of right amygdala was found compared with PD patients, and of right VLPFC, temporal and supplementary motor cortex compared with hypochondriasis patients.

Figure 8.2. Increased BOLD signal in left and right amygdala during OCD related emotional interference in OCD patients compared with control subjects and plot of size of effect in this region in group by task interaction.

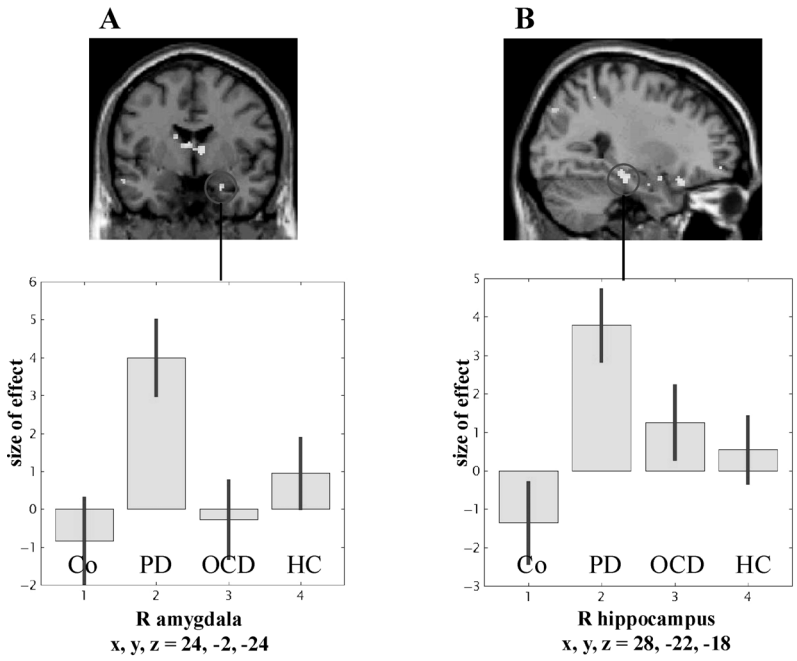


3. Panic related versus neutral

Across all groups, color naming of panic related words compared with neutral words (see Figure 8.1) was correlated with increased BOLD signal in bilateral frontal-striatal regions, temporal-parietal cortices, fusiform gyrus and thalamus, right hippocampus, right amygdala and left rostral brainstem. Group by task interactions are shown in Table 8.4. Compared with control subjects, PD patients showed increased activation bilaterally in the aPFC, ACC and inferior parietal cortex and right-sided in the DLPFC, VLPFC, OFC, thalamus, middle temporal cortex, amygdala (see Figure 3a) and hippocampus (see Figure 3b). Similar regions were identified when comparing PD with OCD; in addition, OCD versus control subjects did not yield significant interaction effects. In contrast, hypochondriasis patients

showed a similar activation pattern as PD patients, although no activation was found in amygdala and ACC.

Figure 8.3. Increased BOLD signal during panic related emotional interference in PD patients compared with control subjects in (a) right amygdala and (b) right hippocampus, and plot of size of effect in these regions in group by task interaction.



4. Effect of disease severity

No specific brain regions were found when correlations were investigated between OCD-interference related BOLD-responses and symptom severity in OCD as measured by the Y-BOCS and Padua-IR. Similarly, no correlation was found between panic-interference related activation patterns in PD patients and BSQ-scores. State anxiety was correlated with increased activation of right VLPFC (z =4.67) and left extrastriate cortex (z-=4.78) in OCD subjects during presentation of OCD related versus neutral words. No such state anxiety effects were found in PD or hypochondriasis patients.

Discussion

In the present study a cognitive and emotional Stroop task was used to investigate the neuroanatomical correlates of attentional bias across different anxiety disorders. The present results support the hypothesis of increased distractibility for irrelevant information

in patients with OCD, PD and hypochondriasis compared with healthy control subjects, associated with frontal-striatal and limbic involvement. Moreover, we found clear differences between OCD patients on the one hand and PD and hypochondriasis patients on the other. In particular, OCD patients displayed increased cognitive interference compared with healthy control subjects, which was accompanied by activation of posterior brain regions. Although OCD patients did not display an attentional bias in behavior during color naming of OCD related words relative to control subjects, there was a clear and specific neuronal response in mainly ventral and limbic brain regions. In contrast, patients with PD and hypochondriasis displayed no interference for incongruent versus congruent words, but showed an attentional bias for both panic and OCD related information, involving both ventral and dorsal brain regions.

In agreement with previous studies using the traditional color-word Stroop task, the present results showed the recruitment of prefrontal-striatal regions during cognitive interference. Prefrontal involvement implies the activation of various hierarchic subregions, known to subserve different executive subprocesses necessary to perform the task[83]. While activation of VLPFC may reflect lexical processing (word generation)[84] and working memory (maintenance of information)[85], activation of DLPFC and aPFC is associated with manipulation processes and complex executive control, respectively[85]. In addition to these prefrontal top-down control processes, the ACC is presumably involved in performance monitoring, response conflict and error-detection[24;27;28;31;33;34]. PD and hypochondriasis patients performed similarly during incongruent versus congruent color naming compared with control subjects, but performance in OCD patients was impaired. This finding is in agreement with Hartston & Swerdlow[37], who reported increased color-word interference in OCD patients compared with control subjects, but not with Moritz et al[38]. Increased regional brain activity associated with cognitive interference, in patients compared with controls, was observed in posterior regions only. This may reflect increased effort, i.e. enhanced visual processing (fusiform and parahippocampal gyrus), heightened arousal (insular cortex and brainstem) and attention (precuneus and parietal cortex). Compensation by recruitment of posterior regions has been found in OCD patients before[86]. Moreover, altered frontal-striatal functioning in OCD is not restricted to attentional bias, but has recently also been demonstrated during executive tasks [87].

Interestingly, our behavioral data showed improved performance for color naming of OCD related words compared with neutral words. This effect was found in all groups, but particularly in PD and hypochondriasis patients. It is inconsistent with the results of most, but not all[53], previous studies, reporting increased response times and error rates due to emotional interference[5]. Possibly, the use of supraliminal stimuli in a block design may have enabled subjects to devise strategies to facilitate task performance. Alternatively, anxiety disorder patients may engage in more extensive processing of threat stimuli compared with normal controls[88].

Across all groups, color naming of OCD related versus neutral words was correlated with activation of VLPFC, OFC and posterior (temporal and parieto-occipital) regions. As expected, all three patient groups showed increased involvement of these regions. Although this increased prefrontal-striatal recruitment during OCD related

emotional interference was not restricted to OCD patients but also present in patients with PD and hypochondriasis, important differences were also found between the patient groups. First, in OCD patients VLPFC was activated, whereas patients with PD and hypochondriasis mainly showed DLPFC, aPFC and medial prefrontal activation. Second, in OCD patients bilateral amygdala activation was found during color naming of OCD related words, while no amygdala involvement was found in the other patient groups, as shown in Figure 2. The role of the amygdala in the evaluation of the behavioral significance of external stimuli and affective responses[89;90] has been addressed in a number of studies using fear paradigms in normal controls[73;91-95]. Most previous imaging studies in OCD have observed amygdala involvement only in small samples and/or at lower statistical thresholds[96;97], or not at all[98-101], which may have been due to various methodological issues[102]. Strong reciprocal connections exist between the amygdala and widespread regions of frontal, insular, temporal and occipital cortices[103]. Although at present our understanding of the neuronal circuits underlying human emotion perception is limited, two interacting systems have been hypothesized to subserve the different subprocesses of emotional appraisal and behavior[90]. First, a ventral system, including the amygdala, insula, ventral striatum and ventral prefrontal regions (rostral ACC, VLPFC, OFC), is mainly important for the identification of the emotional significance of a stimulus and the generation of an autonomic, unconscious emotional response. Second, a dorsal system, consisting of the parahippocampal gyrus and dorsal regions of the frontal cortex (dorsal ACC, aPFC, DLPFC and medial PFC), is engaged in additional regulation of the initial emotional response by combining cognitive and emotional input. The present results in OCD patients are likely to reflect automatic processing of disease-specific emotional cues by the ventral system. Additional cognitive regulation seems to be reflected in the role of the dorsal ACC. Involvement of the dorsal ACC is consistent with the results of Shin et al[70]. They found dorsal ACC activation during emotional interference in their Vietnam veterans with PTSD, while rostral ACC activity was found in the non-PTSD group. Although OCD patients were capable of normal task performance, our behavioral data also showed that RTs as well as disease-specific neuronal responses in OCD subjects were correlated to subjective distress ratings. This suggests that cognitive function in OCD is vulnerable to high levels of state anxiety.

In contrast to OCD related words, color naming of panic related words was not associated with differences in error rate, either across groups or between groups. However, RTs were significantly longer in PD and hypochondriasis patients compared with OCD patients and normal controls. Our imaging data parallel these behavioral results. Compared with control subjects and OCD patients, PD patients showed increased activation of prefrontal areas (mainly right sided), right rostral ACC, amygdala, hippocampus, rostral brainstem and thalamus. When comparing hypochondriasis patients to OCD patients and controls, a similar pattern was observed, but without the involvement of the amygdala and rostral brainstem as was found in PD. However, contrasting PD and hypochondriasis subjects did not yield significant interaction effects. Structural[104;105] as well as functional (resting state[106;107] and challenge[108]) neuroimaging studies in PD, have repeatedly demonstrated the role of the amygdala and hippocampal region in the symptomatology of panic disorder. In addition, pharmacological challenge studies in PD have indicated a brainstem origin of

panic attacks[10]. So far, only two functional MRI studies were performed in PD. In these studies, PD patients showed increased activation of prefrontal, anterior cingulate and (para)hippocampal cortices in response to threat related words[88] or imagery exposure[109].

The widespread dorsal prefrontal involvement in PD and, to a lesser extent, hypochondriasis again suggests (cognitive) modulation of emotional responses. However, in contrast to OCD subjects during color naming of OCD related words, this seems to intervene with task demands. Whether this is due to stimulus characteristics (e.g. greater salience of panic related words relative to OCD related words) or reflects differences in processing of emotional material between OCD and PD/hypochondriasis is not yet clear. A post hoc comparison between neuronal activation patterns during color naming of disease-specific words in OCD and PD showed increased recruitment of the dorsal system in PD patients compared with OCD patients (data not shown). This might reflect increased cognitive modulation elicited by the initial emotional response in PD, and intervening with performance.

Concerning the issue of disease-specificity, our data indicate a clear distinction between OCD on the one hand and PD and hypochondriasis on the other hand, but not between PD and hypochondriasis. First, during incongruent versus congruent color naming, all patient groups recruited additional posterior brain areas relative to controls, but only in OCD performance was significantly impaired. Second, in OCD we observed increased recruitment of anterior cingulate and limbic regions only during color naming of OCD related versus neutral words, whereas in PD and hypochondriasis patients widespread activity in prefrontal, striatal and temporal regions was found during color naming of both OCD and panic related words. This is consistent with the hypothesis of a more generalized attentional bias to threat cues in PD. However, both in OCD and PD, amygdala activation was found to be disease-specific. Moreover, in OCD, state anxiety was correlated with longer RTs only during the disease-specific interference condition. Based on similarities between hypochondriasis and OCD, inclusion of hypochondriasis in the spectrum of obsessive-compulsive disorders has been suggested[110;111]. However, other distinctive features provide support for the association between hypochondriasis and PD[74]. While hypochondriasis and PD co-occur and overlap phenomenologically[112], this overlap is by no means complete[113]. The results from the present study apparently support the association between PD and hypochondriasis. We did, however, not include an extra category of disease-specific words for hypochondriasis, unrelated to panic and OCD.

To our knowledge, this study is the first to elucidate the neuronal correlates of color-word interference and attentional bias in three distinct, but related, disorders, i.e. OCD, PD and hypochondriasis. Strengths of the study are the inclusion of medication-free subjects, a large sample size permitting random effects analysis and the comparison across different patient groups. A limitation of the present study is that the hypochondriasis patients were significantly older than the other participants, although a post-hoc analysis of covariance with age as nuisance variable showed that the interaction effects, described in the present paper, persisted after regressing out age. Second, we did not specifically investigate diagnose subgroups, e.g. OCD patients with prominent washing or checking symptoms, or PD patients with agoraphobia or lactate sensitivity. A third issue that needs to be further

investigated, is the possible role of strategic differences in task performance, by comparing overt (supraliminal presented) versus covert (subliminal presented) stimuli. For example, decreased involvement of dorsal brain regions in OCD compared with PD, as observed in the present study, may reflect predominantly unconscious emotional stimulus processing in OCD[90]. Alternatively, it can be argued that condition effects in OCD may have been obscured by ongoing top-down control processes irrespective of stimulus type, reflecting the difference between tonic and phasic symptomatology in OCD and PD. Finally, future research is warranted to explore whether the behavioral and neuropsychological abnormalities observed in the present study are trait- or state dependent, for example whether these resolve after successful treatment.

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9

Summary & general discussion

Working in the field of neuroimaging is just like looking for a flower in a meadow.

The objectives of this work were to investigate the frontal-striatal and limbic circuits during emotional and cognitive processes in patients with obsessive-compulsive and related disorders and to determine the specificity of possible abnormalities for these disorders. To address these issues, task related changes in regional cerebral blood blow (rCBF) and blood oxygenation level dependent (BOLD) signal were measured, using both positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). The neuroimaging studies described in this thesis started in 2000. As can be seen from a review of this field (part I), almost half of the relevant studies have been published after 2000. This illustrates the tremendous developments within the field and stimulates to further innovation of imaging techniques and neuropsychological paradigms (part II). In this last chapter of the thesis, significance and limitations of the results of this thesis (part III) will be discussed. Subsequently, a view is presented on *where we are now* and *where we are going*.

Part I

Other people have traveled the meadow of neuroimaging before. They made maps of their routes and they described the flowers they picked on their way. Some of their paths appear to have a dead-end; others seem to promise infinite views. Before starting your walk, it is important to study the maps of your predecessors. Which paths look most promising? And are there any hidden paths that have never been walked before?

Part I of this thesis critically reviewed the results of neuroimaging studies in obsessive-compulsive disorder (OCD) and panic disorder (PD), and illustrated some of the strengths and limitations of the various imaging techniques available.

Chapter two reviewed two decades of neuroimaging research in OCD, ranging from morphological measurements to functional experiments at baseline, after symptom provocation, and during neuropsychological performance. The overview clearly shows a shift in thinking about OCD over time. Theoretical models were first based on the idea of an exclusive, or at least prominent, role of the striatum. Subsequent theories emphasized the functional significance of the frontal-striatal circuits[1], and the differentiation (mainly dorsal versus ventral) within these circuits. Recently, limbic structures, mainly the amygdala, have been implicated as well[2].

To date, imaging findings in OCD may be summarized as follows: 1) resting state in OCD patients is associated with increased activity of the ventral parts of frontal-striatal circuits - probably reflecting ongoing emotional and cognitive processing related to tonic symptomatology - and decreased activity in the dorsal parts of the prefrontal cortex, 2) during symptom provocation, activation of limbic structures (mainly the amygdala) and additional recruitment of ventral frontal-striatal regions may reflect the processing of salient information and emotional responses, and 3) exaggerated responses (anxiety and/or distress) may, at least in part, be the result of insufficient suppression or top-down control by the dorsal frontal-striatal circuit. The results of the present thesis provide a major contribution to this conclusion. Nevertheless, it should be realized that this description is a

simplification of the complex mechanisms underlying the disorder and that it does not fully answer the question about the disorder-specificity of the assumed pathophysiological processes.

In order to investigate the issue of disorder-specificity, comparisons between OCD and related disorders are warranted. The comparison with PD enables the differentiation between aspecific distress or anxiety related characteristics and OCD specific deficits. **Chapter three** illustrated the impact of neuroimaging experiments on the understanding of PD. Neuroimaging studies in PD, compared with those in OCD, originate from a different tradition. So far, the work in PD largely consists of pharmacological challenge studies in order to induce panic attacks, and receptor ligand studies, most of which have addressed the functioning of the GABA-benzodiazepine complex. Theoretical models of PD are based on the assumption that the main components of the illness – anticipatory anxiety, panic attacks and phobic avoidance - are linked to distinct neuroanatomical systems in the brain: the temporal lobe structures (mainly the amygdala, hippocampus and parahippocampal gyrus), the brainstem, and the prefrontal cortex, respectively[3;4]. Results of imaging studies in PD only partly support this hypothesis. As in most imaging studies a clear description of the emotional state at the time of data acquisition is lacking, proper neuroanatomical differentiation between panic attacks, anticipatory anxiety and other related symptoms is not yet possible.

Part II

Working in the field of neuroimaging is like looking for a rare flower in a meadow. The flower of interest is often overgrown by weeds. And the colorful spot seen in the distance might just as well be a plastic bag.

Noise and artifacts are the weeds and plastic bags of neuroimaging. It requires a critical attitude towards the experimental data acquired in order to improve signal-to-noise ratios and to differentiate between true and artificial findings. Only this attitude will lead to further innovation of imaging techniques. In Part II, two methodological aspects were investigated.

In **chapter four** an important methodological aspect of PET imaging has been investigated in detail, namely the effect of subject motion on the quality of the attenuation correction. Correction for tissue attenuation is a vital step in obtaining quantitative PET data. The most commonly used method to correct for tissue attenuation is by direct measurement using a separately acquired transmission scan. However, subject motion between transmission and emission scans may result in misalignment and, therefore, in erroneous attenuation correction. When standard attenuation correction is used, this mismatch can result in either underestimation or overestimation of regional activity concentrations. In case of random motion, transmission-emission mismatch will result in diminished signal-to-noise ratios and false-negative findings (type-II errors). On the other hand, as illustrated by a clinical case report, task related motion will result in systematic reconstruction artifacts and consequently in false-positive results (type-I errors). During

symptom provocation experiments in psychiatric patients task related motion may easily occur when the presented emotional stimuli elicit muscle contractions accompanying emotions of fear, disgust or discomfort. For that reason, head positions may differ between the provoked and neutral state.

It was investigated whether the implementation of an image registration (IR) method, which allows for motion-corrected attenuation correction, improved the accuracy of $H_2{}^{15}O$ PET analyses. The IR method, first described by Andersson et al.[5], is based on 3 assumptions: 1) the transmission scan and the first emission scan are well-aligned, 2) transformation matrices can be derived accurately from non-attenuation corrected emission scans, and 3) forward projected transmission scans result in the same attenuation correction factors as those derived directly from transmission scans when patient motion is absent. The implementation of the IR method consisted of the following five steps: 1) reconstruction of emission scans without attenuation correction, 2) calculation of transformation matrices between the first and the subsequent emission scans, 3) application of inverse transformation matrices to the reconstructed transmission scan, 4) forward projection of the transformed transmission scans to yield motion-corrected attenuation correction factors, and 5) application of these factors in the reconstruction of the emission scans. To evaluate the accuracy of this method, phantom studies (using a solid homogeneous 20-cm-diameter cylindrical phantom and a 3D brain Hoffman phantom) as well as studies in human subjects were performed. The results were compared with three alternative methods: 1) standard measured attenuation correction without motion correction, 2) calculated contour-based attenuation correction, and 3) no attenuation correction.

In case of subtraction rather than quantitative analyses, attenuation correction might not be necessary, thereby bypassing the problem of subject motion. Indeed, results of the clinical case report and human validation study confirmed that motion induced false-positive results, as obtained with standard attenuation correction, greatly reduced in case of subtraction analyses without attenuation correction. This method, however, is likely to generate extracranial (or border) artifacts, and is not suitable in the case of quantitative analysis (e.g. such as required in ligand studies). In addition, the calculated contour based method proved to be suboptimal, probably due to difficulties in accurately defining contours.

The elaborate evaluation of the IR method showed that this method reduces noise for the group subtraction analysis and removes type-I errors in case of task related motion, while improving the signal from expected activated areas. The phantom data showed excellent accuracy of the algorithms for image registration, reconstruction, and forward projection of the transmission scan data. Therefore, the IR method should be considered as the first choice for attenuation correction in PET activation studies. Although promising, the application of this method for dynamic ligand studies still needs validation.

Another domain of methodological consideration in neuroimaging research is the experimental paradigm used to visualize a specific cognitive and/or emotional state. An almost infinite battery of neuropsychological tasks can be used to investigate various neuropsychiatric disorders. To prevent contamination with aspecific findings, it is important to invest in the design of hypothesis driven paradigms. Comparison of task

related neuronal correlates between different groups requires a sensitive paradigm that specifically addresses the function of interest and incorporates a correction for potential differences in task performance.

In **chapter five** the design of a parametric self-paced pseudo-randomized event-related fMRI-version of the Tower of London task has been described. This planning task, originally developed by Shallice[6], is supposed to address the flexibility of the frontal-striatal and visuo-spatial circuits. A self-paced, parametric design allows for flexibility in response as well as for comparisons between subjects and/or groups at each task level, providing the opportunity to investigate groups with varying levels of performance. As performance is likely to deteriorate at higher complexity, control for performance differences is especially relevant for parametric designs. In addition to variation in reaction times, performance may also vary in hit frequency. Event-related analysis enables a selective analysis of correct responses, by separate modelling of the false events. Moreover, adequate randomization of trials is possible using an event-related design.

To evaluate this version of the Tower of London task, 22 healthy control subjects were investigated. Compared with baseline, planning activity was correlated with increased blood oxygenation level dependent (BOLD) signal in the dorsolateral prefrontal cortex (DLPFC), striatum, pallidum, (pre)motor cortex, supplementary motor area (SMA), and visuo-spatial system (precuneus and inferior parietal and parietal-occipital cortex). Task load was associated with increased activity in the same regions, and additional recruitment of the left anterior prefrontal cortex, a region supposed to be specifically involved in 3rd order executive functioning.

Two different aspects need to be considered when interpreting these results. First, compared with the main effects (i.e. planning versus baseline contrast), task load effects are more specific, although less sensitive, in their measurement of planning related changes in BOLD response. Second, while increasing task load, other processes, not specific for planning, might also increase (e.g. working memory). Therefore, the investigation of the neuronal correlates of planning might be best visualized by the combination of main and task load effects.

Another methodological issue in neuroimaging of psychiatric conditions is the experimentally induced state of interest. Although not addressed in part II, probably the most important questions remain: in which state is the patient at the moment of data acquisition and to what extent do researchers succeed in inducing and maintaining a desired emotional and/or cognitive state in subjects within an experimental setting? It is questionable whether the usual subtraction designs really succeed in isolating only the neuropsychological function of interest, even with a carefully matched baseline condition (e.g. for stimulus complexity and motor demands). Moreover, aspecific factors, such as arousal or distress, may disturb the visualization of the task related neuronal correlates.

Being aware of the difficulty of capturing the mental state of interest, an attempt was made to develop proper paradigms in order to investigate three different mental states: 1) emotional perception during provoked contamination fear, 2) higher-order cognitive function during executive performance, and 3) the interaction between emotional and cognitive processes during a paradigm addressing attentional bias.

Part III

We started our walk. And how lucky we were! We found beautiful flowers on our way.

Chapter six described the neurophysiological correlates of experimentally induced symptoms of contamination fear in OCD patients. This was not the first study to investigate the symptomatic state in OCD patients. Inconsistencies in earlier experimental results due to various methodological concerns (lack of control groups, medicated subjects, idiosyncratic tactile stimuli, off-on designs, limited sample size), however, asked for replication of the findings in 1) a homogeneous group, 2) of medication-free OCD patients, 3) in comparison with healthy control subjects, 4) during a randomized design, 5) with standardized visual stimuli. To this end, a symptom provocation experiment was performed in 11 medication-free OCD patients with contamination fear and 10 healthy control subjects. Using oxygen-15 water ($H_2{}^{15}O$) PET, task related changes in rCBF were measured during a randomized block design containing visual presentations of 'dirty' and 'clean' surroundings. To prevent type-I errors due to task related, subject motion induced transmission-emission mismatches, the IR method (as described in chapter 4) was used to reconstruct images.

Behavioral data showed that the provocation design was successful in inducing both subjective distress and obsessionality. Whereas obsessionality scores increased in OCD patients as well as in healthy control subjects, subjective distress scores only increased in the patient group. In other words, subjects in both groups experienced 'ritualism', but only OCD patients also became anxious. Moreover, OCD patients became sensitised rather than habituated as a result of the contamination related stimuli. Imaging findings corresponded with these behavioral results. Healthy control subjects showed provocation induced increased rCBF in the left DLPFC and right caudate nucleus. The recruitment of frontal-striatal regions in controls in response to emotional stimuli seems to reflect 'normal ritualism' or top-down control of the emotional response. In contrast, in OCD patients the provoked symptomatic state was correlated with increased rCBF in the left amygdala. Moreover, in this group a time by condition interaction effect was found in the right amygdala, reflecting sensitization. The involvement of the amygdala in OCD during the symptomatic state is in agreement with the literature on the central role of the amygdala in evaluating the emotional significance of external stimuli and fear responses[7;8]. In OCD research, however, the role of the limbic system, mainly the amygdala, in the pathophysiology of the symptomatic state has been underestimated so far. Based on these results, it is suggested that the observed differences between OCD patients and controls reflect a failure of the frontal-striatal circuitry in OCD patients to control the processing of negative disease-relevant stimuli, resulting in an inadequate fear response, involving both amygdalae.

The flexibility of the frontal-striatal system in OCD during an emotionally neutral state was investigated using an fMRI version of the Tower of London task, as described in **chapter seven**. To control for differences in performance between OCD patients and healthy control subjects, and to increase the specificity of the experimental effect of interest, a parametric self-paced pseudo-randomized event-related design (as described in chapter 5) was used.

Twenty-two medication-free OCD patients and 22 healthy control subjects were included. Behavioral data showed decreased performance scores across all levels in OCD patients compared with control subjects, whereas reaction times (RTs) were significantly longer in OCD patients only during the two easiest task levels. Within groups, performance was not significantly correlated with symptom severity and subjective distress. Compared with controls, imaging results showed decreased task-associated activation in OCD patients in several regions previously found to be involved in planning, in particular DLPFC, basal ganglia and parietal cortex. Task load correlated with increased activity in the left DLPFC in control subjects compared with OCD patients. In contrast, the OCD group showed increased - presumably compensatory and/or stress-related - involvement of bilateral cingulate, ventrolateral prefrontal and parahippocampal cortices, left anterior temporal cortex and dorsal brain stem. The decreased responsiveness of the frontal-striatal system, described in OCD patients, was not correlated with ratings for symptom severity and subjective distress.

The results further support the involvement of a frontal-striatal dysfunction in the pathophysiology of OCD. Some alternative explanations might, however, be postulated in relation to the present differences between OCD patients and healthy control subjects, and some important concerns remain. First, it might be argued that only the parametric task load contrast represents a valid and specific comparison between different groups, since the main effects for task might have been confounded by differences in baseline activity. Baseline differences may result from both resting state differences between patients and controls, and dissimilar cognitive or emotional processes during the baseline task in patients compared with controls. Second, the finding of decreased frontal-striatal responsiveness during planning performance might be at least partly explained by poor matching for intelligence. Although only correct responses were selected to control for performance differences, and in spite of the fact that post-hoc analyses of covariance with regard to educational level were performed (showing that the crucial fMRI task by group interaction effects in the striatum and DLPFC persisted after correcting for differences in education), the present findings need replication in an appropriately matched sample. Third, the question remains whether the frontal-striatal dysfunction during planning is specific for OCD, or whether it reflects an aspecific characteristic of anxiety or even neuropsychiatric disorders in general. This issue can be addressed by comparing the task related activation patterns across different patient groups. The present data set enables a comparison between OCD, PD and hypochondriasis, and this analysis will be performed in the near future. If patients with PD and hypochondriasis show normal frontal-striatal recruitment during planning, similar to controls, it might be concluded that the observed frontal-striatal dysfunction in OCD is specific for the pathophysiology of OCD. In contrast, if patients with PD and/or hypochondriasis also have decreased frontal-striatal responsiveness during planning performance, in common with OCD patients, a closer look at the shared features will contribute to a better understanding of common characteristics across disorders and /or aspecific factors confounding the task related activation patterns.

In **chapter eight**, addressing attentional bias, emotional and cognitive processes are strongly interwoven. Difficulty in inhibiting irrelevant information is a key feature of OCD. Because most of their attentional resources are allocated to threat cues related to their

concerns, OCD patients are limited in their ability to selectively attend to relevant stimuli, whilst simultaneously ignoring irrelevant competing stimuli. As the critical process of gating, i.e. inhibiting irrelevant information, has been linked to frontal-striatal function and the evaluation of emotional stimuli to limbic function, the investigation of attentional bias to disease-relevant emotional cues might contribute to the understanding of the altered function of both frontal-striatal and limbic circuits in OCD. Moreover, comparisons across related disorders are needed to address the specificity of the dysfunction.

To investigate the neuronal correlates of attentional bias across related anxiety disorders, cognitive and emotional Stroop task related BOLD responses were measured in medication-free patients with OCD (N=16), PD (N=15), and hypochondriasis (N=13), and the behavioral and imaging results of these patient groups were compared with those of 19 healthy control subjects. Contrasts of interest were the cognitive interference effect (incongruent versus congruent color words), the OCD related emotional interference effect (OCD related negative words versus neutral words) and the panic related emotional interference effect (panic related negative words versus neutral words).

Cognitive interference in all patient groups relative to controls was correlated with recruitment of additional posterior brain regions, but performance was impaired only in OCD patients. In OCD, color naming of disorder specific (OCD related) words only was associated with increased activation of ventral frontal-striatal and limbic regions, including bilateral amygdala, although performance was not abnormal. In contrast, patients with PD and hypochondriasis showed increased activation of ventral and widespread dorsal frontal-striatal regions during both OCD and panic related words. In addition, in PD patients, the speed of color naming panic related words was significantly reduced, which was associated with increased activation of the right amygdala and hippocampus.

These results imply clear differences between OCD patients on the one hand and PD and hypochondriasis patients on the other. The disorder specific neuronal response in OCD mainly involves ventral brain regions, which are assumed to be implicated in emotional appraisal of emotional cues and unconscious fear responses. Attentional bias was found to be more generalized in patients with PD and hypochondriasis, involving both ventral and dorsal brain regions, which reflects not only unconscious emotional stimulus processing, but also increased cognitive elaboration of the initial emotional response.

Where we are now?

Now that we've returned from our journey the time has come to ask ourselves what we actually found. Did we really pick the specific beautiful flowers we were looking for, or have we been seduced by the painted flowers of van Gogh?

Taking together the results described in part III, it can be hypothesized that altered dorsal frontal-striatal function in OCD patients is responsible for 1) decreased inhibition of ventral frontal-striatal and limbic recruitment in response to disease-relevant emotional cues and 2) decreased executive performance. This hypothesis, if confirmed, leaves several questions to be answered.

First, the term frontal-striatal appears to be too broad to capture the subtle alterations of brain functioning in OCD during the various emotional and/or cognitive conditions of interest. A distinction between dorsal and ventral frontal-striatal circuits is generally accepted in both the neuroanatomical and neuropsychiatric literature. However, the results of the present study do not allow us to unequivocally associate any of the studied OCD emotional and cognitive deficits to a specific striatal region or a particular prefrontal cortical circuit. A key issue seems to be how cognitive and emotional functions interact or, in other words, dorsal and ventral frontal-striatal circuits might influence each other. Moreover, there appear to be differences in interpretation as to connectional characterization of the dorsal and ventral striatum in the neuroanatomical and the neuropsychiatric literature. Whereas in neuroanatomical models the limbic (or ventral) striatum is defined as the area that receives hippocampal and amygdaloid input and is associated with emotional and motivational functions[9], in psychiatric models the hippocampus belongs to the dorsal circuit[8]. Close collaboration between psychiatrists and anatomists is required in developing future theoretical models of psychiatric conditions.

Second, it is questioned whether anxiety is the key feature of OCD, leading to the repetitive behaviors, or whether OCD is a primary 'cognitive' disorder and anxiety just the spin-off. In other words, which level of emotional processing is most abnormal: the appraisal of the emotional significance of a stimulus, the subsequent fast and unconscious behavioral response, or the higher-order evaluation and modification of this initial response? This issue is hard to investigate and longitudinal designs are needed to differentiate between cause and effect. The present working hypothesis implies a primary failure (hypofunction) in the dorsal frontal-striatal circuit rather than a primary deficit (hypersensitivity) of the limbic or ventral frontal-striatal circuits. Support for a primary deficit in the DLPFC stems from imaging studies in children, showing altered maturation of the DLPFC in pediatric OCD[10]. Although replication is needed, this suggests a primary 'cognitive deficit'. To date, however, it has not been possible to prove this theory and the opposite hypothesis still cannot be rejected. As stated by Damasio[11] and LeDoux[7], trying to disentangle the issue might lead to a Cartesian error. Separation of emotion and cognition implicitly involves an artificial divorce of two closely interacting partners. Interactions between the different functional neuronal circuits subserving emotional and cognitive processes and the effects of the various neurotransmitters, from early development to the mature adult brain, are poorly understood and need further elaboration.

Third, the hypothesis suggests a direct top-down control mechanism from the DLPFC to the limbic circuit (mainly the amygdala). Although this sounds attractive, neuroanatomical evidence for projections descending directly from the DLPFC to the amygdala is rather weak. Whereas connections of the amygdala with orbitofrontal and medial prefrontal areas are robust and bi-directional, connections with lateral prefrontal areas are sparse, uni-directional and primarily ascending[12;13]. One possible way in which diverse streams of information could guide behavior would be through the rich interconnections between prefrontal areas[9], involving cortico-cortical and cortico-thalamo-cortical connections. Whereas the latter have long been thought to be organized in a strict reciprocal manner, recent evidence indicates that different cortical areas might be interconnected via the thalamus[14]. However, little is known about the complex direct and indirect connections subserving the communication between emotion and cognition. An

important problem with the interpretation of neuroanatomical tracing studies is that these are at best carried out in non-human primates necessitating the extrapolation of the results to the more complex human brain. It is assumed that humans differ from animals in the way cognition is used to modify instinct-driven behavior. Possibly, humans differ from non-human primates in the complexity of the neuroanatomical connections between dorsolateral prefrontal areas and limbic structures (e.g. the amygdala). The same reasoning applies to the opposite direction of the reciprocal interactions between emotion and cognition. Although psychiatrists and psychologists are easily inclined to assign a role for anxiety in executive impairment in patients, it is unclear in which way the amygdala might influence dorsolateral prefrontal functioning.

Fourth, OCD is not the first or only psychiatric disorder in which a frontal-striatal dysfunction has been implicated. Not only anxiety disorders, but also psychotic disorders (e.g. schizophrenia), movement disorders (e.g. Tourette's Syndrome) and mood disorders (e.g. major depressive disorder) have been attributed to an imbalance of the frontal-striatal circuits. This phenomenon might be explained in two different ways. On the one hand, different subregions of the frontal-striatal circuits, subserving different emotional and/or cognitive processes, might be involved in different neuropsychiatric disorders. Even in the case of a disorder specific dysfunction, the question remains whether the neurophysiological alterations reflect either state or trait characteristics of the disorder. Longitudinal follow-up measurements, for instance before and after treatment, may contribute to a better understanding of this issue. On the other hand, various neuropsychiatric disorders might share a common feature. These features might be related to the illness (e.g. feelings of stress and hopelessness) or to aspecific demographic and behavioral characteristics (e.g. educational level, unemployment, and smoking). Another common feature might be temperament, i.e. the inborn psychological profile. Schwartz et al.[15;16] showed that adults who, in the second year of their life, had been categorized as inhibited, display amygdala hyperresponsiveness to novel versus familiar faces in comparison with those previously categorized as uninhibited.

Where are we going?

To date, almost all neuroimaging results in OCD have been based on comparisons between OCD patients and healthy control subjects. There are two questions with respect to the issue of disorder-specificity: 1) is a neurophysiological differentiation possible between OCD subcategories, and 2) are the frontal-striatal and limbic activation patterns found in OCD specific for this disorder or is there overlap with other anxiety, mood and even psychotic disorders? To answer these questions, two opposite approaches are needed: 'splitting', i.e. contrasting subcategories within OCD, and 'lumping', or grouping together of OCD patients with patients suffering from other neuropsychiatric disorders, thereby focusing on functional dimensions rather than on DSM-categories. The latter approach appears to be the most promising one. Similarities between anxiety disorders seem to be more pronounced than differences. In addition, reported dysfunctional neuronal circuits are implicated in general aspects of human, and even non-human, behavior. First, it is necessary to understand the main processes underlying normal and pathological emotional perception,

and fear responses and inhibition. Only then, will it be possible to interpret, sometimes subtle, neurophysiological differences between related disorders.

Using the strategy of 'lumping', an important methodological question remains to be addressed: how to select patients and control subjects independent of the DSM-based criteria? The answer to this question depends on the aspect of the disorder to be investigated. A first step in the dimensional approach is to combine different related disorders as compared with healthy control subjects. Another possibility for subject inclusion, both in patients and in controls, is the use of a cut-off score on a scale developed to measure a specific dimension, for instance state anxiety, compulsiveness, inhibition, uncertainty, disgust, etc. The between-subject variance in the dimensional scores might be used to perform analyses of covariance with the BOLD response of interest. It will be interesting to use this approach to reanalyze the data presented in chapters seven and eight. Instead of grouping patients by diagnosis (OCD, PD and hypochondriasis), the score on the Padua Inventory Revised[17;18] or a visual anxiety scale may be used to investigate the role of a possible common feature on the task related BOLD response. Moreover, the score on the Whitely Index[19], a measure for obsessionality to diseases, might be used to further investigate similarities and differences in neuronal response to OCD and panic related negative words across the different patient groups.

As proposed by Phillips et al.[8], in a model on emotional perception, altered behavioral responses to emotional information might be investigated at roughly three different levels. The first level concerns the identification of the emotional significance of a stimulus. It might be possible that perceptual processing of emotional stimuli is impaired in anxiety disorders due to altered amygdala function. Vuilleumier et al.[20] have shown that emotion can directly affect sensory processing at an early stage of perception. If so, anxious people may not only have a different interpretation of what they see, but may also literally perceive these stimuli as abnormal themselves[21]. The second and third levels of emotional perception relate to the production and regulation of an affective response to this emotional information. When focusing on anxiety disorders, these processes might best be investigated by studies on fear responses, fear extension learning and higher-order modulation of behavior. Whereas almost nothing is known about the role of the DLPFC in the modulation of fear responses, studies in extinction learning, both in animals and in humans, have contributed to the understanding of the role of the ventromedial PFC in the inhibition of learned fear. Direct interconnections between the ventromedial PFC (or subgenual anterior cingulate cortex) and the amygdala have been implicated in fear extinction, which is not simply a process of 'unlearning', but rather one of 'new learning'[22-24]. Understanding how learned fears are diminished and how extinction learning is changed in patients with anxiety disorders might be an important step in translating neurobiological research to diagnosis and treatment of these patients.

As mentioned above, there is a lack of understanding of the modulation of emotional responses by higher-order cognitive processes. Therefore, probably one of the key issues for the coming years is to integrate research on emotion and cognition. With regard to the complex interactions between emotion and cognition, there appear to be two important questions: 1) which direct and indirect descending connections from the DLPFC to the amygdala play a role in the presumed top-down control of the amygdala response, and 2) in which way can activation of the amygdala directly or indirectly influence

executive functioning? More insight in the functional neuronal circuits connecting emotion and cognition, underlying normal and pathological behavior, necessitates a multidisciplinary approach, combining lesion studies, structural measurements, and functional imaging paradigms in human and non-human primates. Useful comparisons between human and non-human experiments require neuropsychological paradigms that are applicable across various species.

Finally, future research in this field may benefit most from a longitudinal and multimodal approach. Probably the most interesting cohort consists of young children, with and without obsessive-compulsive and related disorders. Longitudinal follow-up of these children into adolescence and adulthood enables visualization of natural history as well as the evaluation of long-term effects of environmental influences and treatment strategies. Multimodal designs can also contribute to a better differentiation between cause and effect. For instance, altered morphology may be related to an imbalance of interacting neurotransmitter systems, and differences in task related BOLD responses may be confounded by early maturation deficits. If we succeed in mapping neurophysiological profiles relevant for the symptomatology of patients, these profiles might be used as endophenotypes for subsequent genetic investigations.

Concluding remarks

OCD is an interesting neuropsychiatric disorder, particularly because of the simultaneous presence of cognitive and emotional processes underlying the characteristic, pathological behavior. The specificity of research findings and the sensitivity of experimental paradigms, however, seem to be limited. This may be explained by the congeniality with other neuropsychiatric disorders, and the lack of a strict dividing line between normal and pathological obsessive-compulsive behavior (or between adequate and inadequate fear responses). At presence it is not clear, which research approach will be superior in its attempt to understand the specific underlying mechanisms of the symptomatology. In view of the heterogeneity of the clinical phenomena in OCD, the main challenge for neuroimaging researchers is to define and map the clinically most relevant and specific psychopathological features of the disorder. The findings described in this thesis contribute to the knowledge about the underlying mechanisms of some of the psychopathological processes that are basic to neuropsychiatric diseases.

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Nederlandse samenvatting

Beeldvormend hersenonderzoek bij obsessieve-compulsieve en aanverwante stoornissen en de rol van de fronto-striatale en limbische circuits

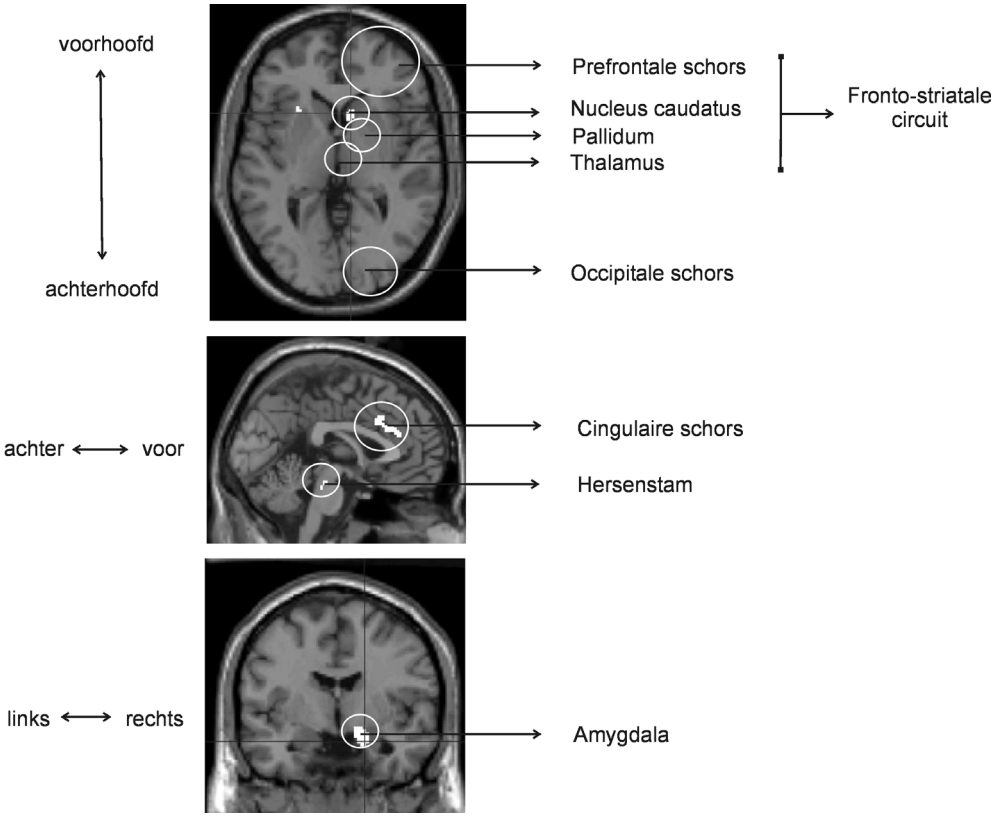
De achtergrond

De dwangneurose (ook wel obsessieve-compulsieve stoornis of OCS genoemd) wordt gekenmerkt door dwanggedachten (obsessies) en/of dwanghandelingen (compulsies). Obsessies zijn hardnekkige, hinderlijke repetitieve gedachten of beelden die angst of spanning veroorzaken. Vaak hebben deze gedachten te maken met de angst besmet te worden, met een extreme twijfel over de juistheid van het eigen handelen of de overtuiging iets belangrijks te hebben nagelaten en daardoor verantwoordelijk te zijn voor mogelijke rampzalige gevolgen. Gedachten kunnen ook voortkomen uit de behoefte tot symmetrie of perfectionisme of een vastomlijnd gevoel over 'hoe het moet zijn'. Karakteristieke thema's zijn verder seksualiteit en agressie. Compulsies zijn rituele gedragingen (soms uiterlijk waarneembaar, soms slechts mentaal aanwezig) die steeds op stereotiepe wijze worden uitgevoerd om de angst of de spanning, ontstaan door de obsessies, te doen afnemen. Vaak voorkomende dwanghandelingen zijn wassen, controleren, ordenen, tellen, herhalen en verzamelen.

OCS is een 'verscholen' chronische psychiatrische ziekte: mensen met zelfs ernstige dwangklachten vertonen in contact met anderen vaak weinig afwijkend gedrag en zij blijven meestal jarenlang buiten het zicht van hulpverleners. Dit ondanks de vaak ernstige beperkingen in dagelijks, sociaal en beroepsmatig functioneren. Uit epidemiologisch onderzoek blijkt dat OCS een niet-zeldzame stoornis is, met vaak een chronisch recidiverend beloop. OCS wordt sinds de introductie van de derde editie van de Diagnostic and Statistic Manual (DSM-III) van de American Psychiatric Association in 1980 geclassificeerd als een angststoornis. Andere angststoornissen zijn onder meer de paniekstoornis, de posttraumatische stressstoornis, de sociale fobie, de specifieke fobie (b.v. hoogtevrees, vliegangst, angst voor spinnen) en de generaliseerde angststoornis. Het klinisch beeld van OCS vertoont ook overeenkomsten met andere, niet binnen het angstcluster geclassificeerde stoornissen. Zo zijn obsessieve-compulsieve klachten frequent aanwezig bij mensen met het syndroom van Gilles de la Tourette en neurologische aandoeningen zoals Sydenham's chorea en morbus Huntington. Ook vertoont het klinisch beeld overeenkomsten met de somatoforme stoornis hypochondrie, ofwel de preoccupatie met de vrees een ernstige ziekte onder de leden te hebben.

Hoewel uitgebreide behandelingmogelijkheden bestaan om dwangklachten te doen afnemen, is over de werking van de hersenen bij deze aandoening nog relatief weinig bekend. Inzicht in de neurobiologische achtergrond van OCS zou op termijn kunnen bijdragen aan nieuwe behandelstrategieën voor therapieresistente dwangklachten. De verwantschap met de neurologische ziektebeelden Sydenham's chorea en morbus Huntington brachten het onderzoek naar de neurobiologische achtergrond van OCS op de betrokkenheid van specifieke prefrontale en subcorticale (= onder de hersenschors gelegen) gebieden van de hersenen (zie Fig. 1). Deze samenwerkende hersengebieden worden ook wel aangeduid met de term fronto-striatale circuits. Tevens werd een verstoorde functie van limbische structuren (met name de amandelkern of amygdala) vermoed, gezien diens rol in normale angstreacties bij dieren. Het onderzoek naar de betrokkenheid van de fronto-striatale en limbische circuits bij de etiologie van OCS is de laatste decennia in een stroomversnelling geraakt met de komst van nieuwe technieken voor beeldvormend onderzoek, met name positron emissie tomografie (PET) en magnetic resonance imaging (MRI, kernspinresonantietomografie). Hiermee bleek het mogelijk om op weinig belastende

Figuur 1.



wijze *in vivo* afbeeldingen van zowel structuur als functie van de hersenen te verkrijgen. Dergelijke technieken maken gebruik van subtiele regionale veranderingen in bloeddoorstroming, welke gepaard gaan met de taak-gerelateerde veranderde neuronale activiteit in de overeenkomende hersenengebieden.

De vraagstelling

Het doel van het onderzoek, zoals beschreven in dit proefschrift, was het verkrijgen van inzicht in de rol van verschillende neuronale circuits, met name de fronto-striatale en limbische circuits, bij cognitieve en emotionele processen die ten grondslag liggen aan de symptomen van OCS. Met andere woorden, is er sprake van aantoonbare afwijkingen in de activiteit van deze neuronale circuits bij OCS-patiënten in vergelijking met gezonde mensen? Een bijkomend doel was om te onderzoeken in hoeverre deze eventuele afwijkingen specifiek zijn voor OCS-patiënten of dat deze op ook optreden bij mensen met aanverwante stoornissen, zoals paniekstoornis of hypochondrie.

Beeldvormend hersenonderzoek is een relatief nieuwe tak van onderzoek die zich razendsnel ontwikkelt. De studie is van start gegaan in het jaar 2000. Zoals ook blijkt uit het literatuuroverzicht (deel I van het proefschrift), is ongeveer de helft van de relevante studies in dit veld gepubliceerd na het jaar 2000. Dit geeft aan hoe belangrijk de ontwikkeling en innovatie van scantechnieken en neuropsychologische experimenten is geweest tijdens de periode waarin deze studie is uitgevoerd (deel II van het proefschrift). De resultaten van het onderzoek naar de rol van de fronto-striatale en limbische circuits tijdens verschillende cognitieve en emotionele processen in OCS (deel III van het proefschrift) bevestigen in belangrijke mate de hypothese van een verstoorde functie van deze circuits bij OCS en dragen bij aan verder inzicht in de overeenkomsten en verschillen tussen aanverwante stoornissen.

De opbouw van het proefschrift

Het proefschrift bestaat uit drie delen. Deel I bevat een uitgebreid literatuuroverzicht: de resultaten van twee decennia beeldvormend onderzoek in OCS en paniekstoornis. De literatuur is ingedeeld naar type experiment en/of gebruikte techniek. In het overzicht zijn ook de eigen bevindingen opgenomen om ze zo te plaatsen binnen de grotere context.

Deel II van het proefschrift beschrijft de bijdrage die binnen deze promotiestudie is geleverd aan de methodologie van beeldvormend hersenonderzoek in het algemeen. Dit betreft 1) het onderzoek naar het effect van ongewenste hoofdbeweging van de proefpersoon op de kwaliteit van PET-scans en, 2) het geschikt maken van een neuropsychologische taak voor gebruik bij onderzoekspopulaties met ongelijk prestatievermogen.

Deel III van het proefschrift beschrijft de achtergrond en de resultaten van drie patiëntgebonden experimenten. De resultaten bevestigen de hypothese van een verstoorde functie van de fronto-striatale en limbische circuits bij OCS. De resultaten dragen ook bij aan verder inzicht in de overeenkomsten en verschillen tussen OCS en aanverwante stoornissen.

De samenvatting van de resultaten

Deel I: literatuuroverzicht (hoofdstukken 2 en 3)

Het literatuuroverzicht van beeldvormend onderzoek in OCS, zoals beschreven in **hoofdstuk 2**, laat duidelijk een verschuiving zien in het denken over deze aandoening. Theoretische modellen waren in eerste instantie gebaseerd op een exclusieve, of op zijn minst prominente, rol van het striatum (met name de staartkern of nucleus caudatus). Latere theorieën benadrukten de functionele betekenis van de fronto-striatale circuits en de verdere ventrale versus dorsale differentiatie binnen deze circuits. Recent is ook de rol van het limbische systeem (met name de amygdala) in de modellen opgenomen.
Onderzoek naar structurele afwijkingen bij OCS heeft veel inconsistente resultaten opgeleverd. Een mogelijke uitzondering is de globaal verlaagde wit/grijs-verhouding in de

dorsale prefrontale schorsgebieden, suggestief voor OCS als neuronale ontwikkelingsstoornis. Functioneel beeldvormend onderzoek heeft daarentegen meer eenduidig afwijkingen in de fronto-striatale en limbische gebieden laten zien en zou als volgt kunnen worden samengevat: 1) in rust is met name verhoogde ventrale en verlaagde dorsale fronto-striatale activiteit kenmerkend voor OCS, 2) gedurende symptoomprovocatie neemt de activiteit toe in de limbische structuren (waaronder de amygdala) en – in wisselende mate – de ventrale fronto-striatale gebieden, 3) toegenomen respons (angst en spanning) op emotionele stimuli zou deels verklaard kunnen worden door verminderde onderdrukking van de limbische activiteit vanuit de dorsale fronto-striatale gebieden, en 4) bij cognitieve taken gaat verminderde activering van de dorsale fronto-striatale circuits samen met verhoogde activiteit van de anterieure cingulaire schors en posterieure schorsgebieden (zoals de parahippocampale schors). Beeldvormend onderzoek naar de neurochemische veranderingen bij OCS laat verder betrokkenheid zien van diverse interacterende neurotransmitter systemen, waaronder serotonine, dopamine, glutamaat en acetylcholine.

Onderzoek naar de ziektespecificiteit van de beschreven afwijkingen bij OCS is mogelijk door middel van vergelijking met aanverwante stoornissen. De vergelijking met de paniekstoornis maakt het mogelijk te differentiëren tussen aspecifieke stress- en/of angstgerelateerde karakteristieken en voor OCS specifieke afwijkingen. **Hoofdstuk 3** belicht de bijdrage van beeldvormend onderzoek aan de kennis over de paniekstoornis. Beeldvormend onderzoek naar de paniekstoornis kent een andere geschiedenis dan die in OCS. Vooralsnog werd deze vooral bepaald door farmacologische provocatiestudies en ligandstudies naar de werking van het GABA-benzodiazepinecomplex. Theoretische modellen waren gebaseerd op de veronderstelling dat de verschillende componenten van de ziekte (anticipatieangst, paniekaanvallen en fobische vermijding) geassocieerd zijn met verschillende neuronale systemen in de hersenen (respectievelijk amygdala, hersenstam en prefrontale schors). Deze hypothese wordt slechts deels gedragen door de bevindingen van beeldvormend onderzoek, mogelijk door de beperkte mogelijkheid de precieze emotionele toestand van de proefpersoon op het moment van dataverzameling te bepalen of te beïnvloeden.

Deel II: bijdragen aan de methodologie van beeldvormend hersenonderzoek (hoofdstukken 4 en 5)

Het gebruik van beeldvormende technieken is te vergelijken met het zoeken van een zeldzame bloem in een veld: de gezochte bloem is vaak overwoekerd met onkruid en een kleurige stip gezien in de verte zou voor hetzelfde geld een rondslingerende plastic zak kunnen zijn. Ruis en artefacten zijn het onkruid en de plastic zakken van beeldvormend hersenonderzoek. Het vereist een kritische houding bij het bewerken en interpreteren van de experimentele data. In **hoofdstuk 4** wordt een belangrijk methodologisch aspect, het effect van ongewenste beweging van het hoofd van de proefpersoon tijdens het experiment, in detail uitgewerkt. Gedurende de analyses van de PET data werd duidelijk dat beweging van de proefpersonen geleid zou kunnen hebben tot artefacten. Bij nadere bestudering bleken deze te maken te hebben met een onjuiste correctie voor attenuatie (verzwakking van het signaal door opname in het weefsel). Correctie voor attenuatie is een essentiële stap bij het

verkrijgen van kwantitatieve data. De bekendste methode (de zogenaamde gemeten attenuatiecorrectie) maakt gebruik van directe meting via een transmissiescan, welke wordt gemaakt direct voorafgaand aan de experimentele emissiescans. Beweging van het hoofd tussen de afzonderlijke emissiescans door leidt tot ten opzichte van elkaar verschoven posities van de transmissie en emissiescans. Wanneer de beweging willekeurig is, zal de verschuiving leiden tot een afgenomen signaal-ruis-verhoudingen en vals-negatieve bevindingen. Wanneer de beweging echter taak-gerelateerd plaatsvindt, bijvoorbeeld door toegenomen spanning in het lichaam tijdens het kijken naar angstprovocerende stimuli, zal de verschuiving leiden tot systematische reconstructieartefacten en daardoor vals-positieve resultaten. Onderzocht werd of de implementatie van een alternatieve methode voor attenuatiecorrectie, de zogenaamde 'image registration method' of IR-methode, zou leiden tot toegenomen kwaliteit van de PET beelden. Gebruik makend van zowel fantoomstudies als humane experimenten, werd de IR-methode vergeleken met de gemeten attenuatiecorrectie en twee andere benaderingen (geen correctie voor weefselverzwakking en berekende of 'contour-based' attenuatiecorrectie). De resultaten lieten zien dat de IR-methode superieur is ten opzichte van de andere methoden, door een toegenomen signaal-ruis-verhouding en een reductie van vals-positieve bevindingen. Gebruik van deze methode kan daarom gezien worden als 1ste keus bij PET activatiestudies.

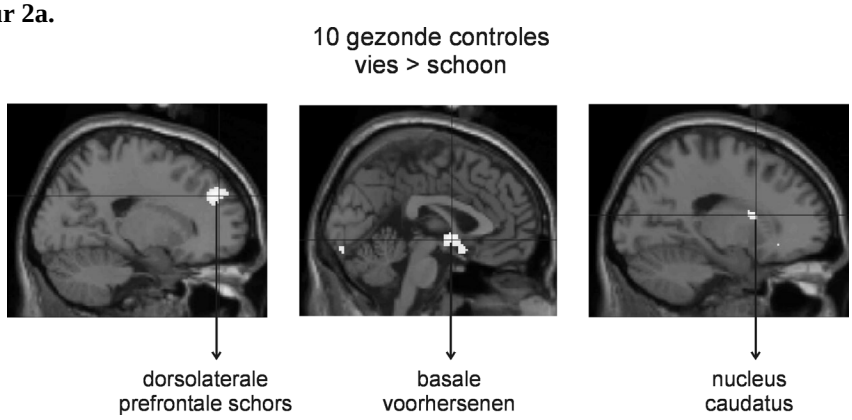
Een ander methodologisch aspect van belang is de aanpassing van het neuropsychologische paradigma voor gebruik in de scanner bij onderzoekspopulaties met een variabel niveau van prestatie. **Hoofdstuk 5** beschrijft een fMRI-versie van de *Tower of London*, een planningstaak. De Tower of London bestaat uit een plankje met 3 stokjes van respectievelijk 1, 2 en 3 bal-lengten en drie verschillend gekleurde ballen. In beeld worden twee configuraties getoond, de beginconfiguratie en de doelconfiguratie. Aan de proefpersonen wordt gevraagd om het minimaal aantal stappen te tellen dat nodig is om van begin- naar doelconfiguratie te komen. Men mag daarbij slechts 1 bal tegelijkertijd van plaats veranderen en een bal kan alleen verplaatst worden als er geen bal bovenop ligt. In de controleconditie moeten proefpersonen het totaal aantal blauwe en gele ballen tellen. De fMRI-versie van deze taak bestond uit de volgende elementen: parametrische condities (variërend van 1 tot 5 stappen) in pseudo-gerandomiseerde aanbieding, flexibele responssnelheid en separate analyse van goede en foute antwoorden. Voorafgaand aan toepassing in patiënten werd het paradigma getest bij 22 gezonde controles. Iedere 3 seconden werden MRI-beelden vervaardigd (433 scans/proefpersoon in totaal). Vergeleken met de controleconditie ging planning gepaard met verhoogde activiteit in de dorsolaterale prefrontale schors, motorische schors, visuospatiële gebieden (pariëtale en occipitale schorsgebieden) en het striatum en pallidum. Toename in complexiteit van de taak correleerde met extra activering van dezelfde gebieden en additionele activiteit in de linker anterieure prefrontale schors, een gebied dat een speciale rol toegekend heeft gekregen in 3de-orde executieve functies. Op basis van deze resultaten kan geconcludeerd worden dat de taak zeer geschikt is voor het bestuderen van het executief vermogen in OCS-patiënten: de taak brengt specifiek het dorsale fronto-striatale systeem in beeld en maakt het mogelijk te corrigeren voor verschillen in prestatieniveau.

Deel III: resultaten van drie patiëntgebonden experimenten (hoofdstukken 6, 7 en 8)

Dit deel van het proefschrift bevat de achtergrond en de resultaten van drie patiëntgebonden experimenten. Het beschrijft de neuronale correlaten van normale en pathologische emotionele en/of cognitieve processen in patiënten met OCS in vergelijking met zowel gezonde controleproefpersonen als patiënten met aanverwante aandoeningen (namelijk paniekstoornis en hypochondrie). Drie experimenteel geïnduceerde mentale condities worden achtereenvolgens beschreven: 1) informatieverwerking van emotionele stimuli tijdens de provocatie van smetvrees, 2) executief cognitief functioneren tijdens de uitvoering van een planningstaak en 3) de interactie tussen emotionele en cognitieve processen gedurende een aandachtstaak met ziekte-specifieke woorden.

Wij waren niet de eersten die de smetvreesprovocatie gebruikten om de bij OCS betrokken neuronale circuits te bestuderen. Echter, de studie beschreven in **hoofdstuk 6** verschilt van voorgaande studies door enkele methodologische verbeteringen: de selectie van een homogene groep medicatievrije smetvreespatiënten, de inclusie van een controlegroep, en het gebruik van gestandaardiseerde visuele stimuli die gerandomiseerd aan de proefpersonen is aangeboden. Met behulp van licht radioactief gelabeld water werden PET-scans gemaakt in een actieve conditie (vieze plaatjes) en een rustconditie (schone plaatjes). In de controlegroep was een toegenomen activiteit zichtbaar in de linker dorsolaterale prefrontale schors, basale voorhersenen en de rechter nucleus caudatus tijdens het kijken naar de vieze plaatjes (zie Fig. 2a). Bij deze groep lijkt de bijsturing van de emotionele reactie vanuit het dorsolaterale circuit verantwoordelijk voor het voorkómen van een angstreactie.

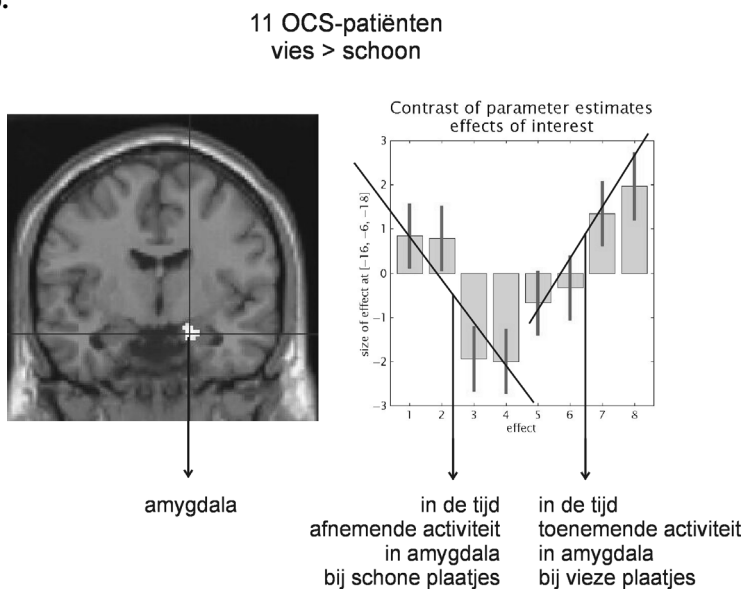
Figuur 2a.



Bij de OCS-patiënten daarentegen, was de actieve conditie gecorreleerd met verhoogde activiteit in de linker amygdala. Bovendien was een in de tijd toenemende activiteit zichtbaar in de rechter amygdala gedurende de actieve conditie (ook wel sensitisatie-effect genoemd, zie Fig. 2b). De amygdala staat bekend om haar rol in emotionele informatieverwerking en angst. De resultaten suggereren dat het verschil in emotionele

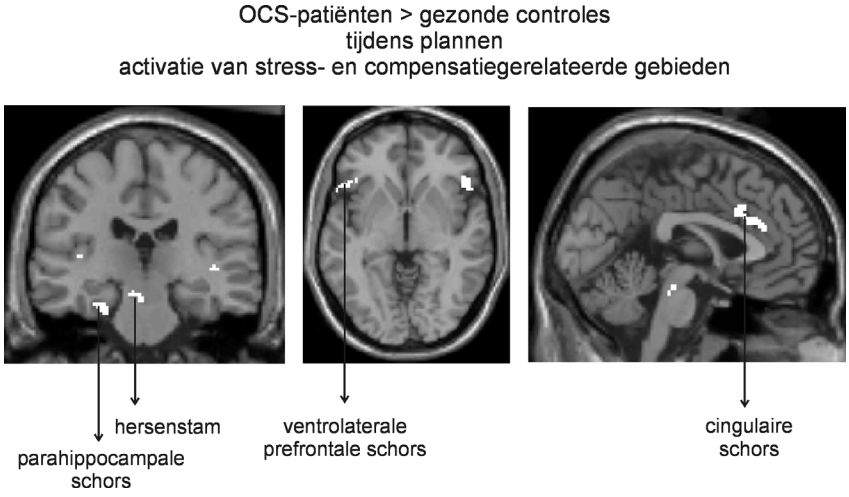
reactie op de aangeboden plaatjes (de patiënten reageren met een significante toename in angst, de controles niet) is te verklaren door een bij OCS verminderde controle op het alarmsysteem (amygdala) vanuit het dorsale fronto-striatale circuit, resulterend in een inadequate angstreactie en sterke activering van beide amygdalae.

Figuur 2.b.



De bevinding van het tekortschieten van het dorsale fronto-striatale systeem in OCS gedurende emotioneel beladen condities, doet vermoeden dat ditzelfde circuit verantwoordelijk is voor verstoring van bepaalde denkfuncties (bijvoorbeeld het leervermogen, planning, aandacht en besluitvorming). De bestudering van de flexibiliteit van het dorsale fronto-striatale systeem in OCS gedurende de uitvoering van een planningstaak (*Tower of London*) wordt beschreven in **hoofdstuk 7**. In totaal deden 22 medicatievrije patiënten met OCS en 22 gezonde controles mee aan de studie. Vergeleken met gezonde controles maakten patiënten met OCS significant meer fouten tijdens de uitvoering van de taak. Tijdens het plannen worden verschillende gebieden van het fronto-striatale systeem geactiveerd, zowel bij gezonde controles als bij patiënten met OCS. Echter, vergeleken bij controles is dit fronto-striatale systeem bij mensen met OCS minder actief. De verminderde reactiviteit van deze hersengebieden lijkt gecompenseerd te worden met verhoogde activiteit in andere gebieden (waaronder de cingulaire en parahippocampale schors). Tevens is in de OCS-groep verhoogde activiteit zichtbaar in stressgerelateerde gebieden (zie Fig. 3). Samengevat laat deze studie zien dat een verminderd planningsvermogen in OCS samen gaat met verminderde reactiviteit van het dorsale fronto-striatale systeem en verhoogde activiteit in stress- en compensatiegerelateerde hersengebieden.

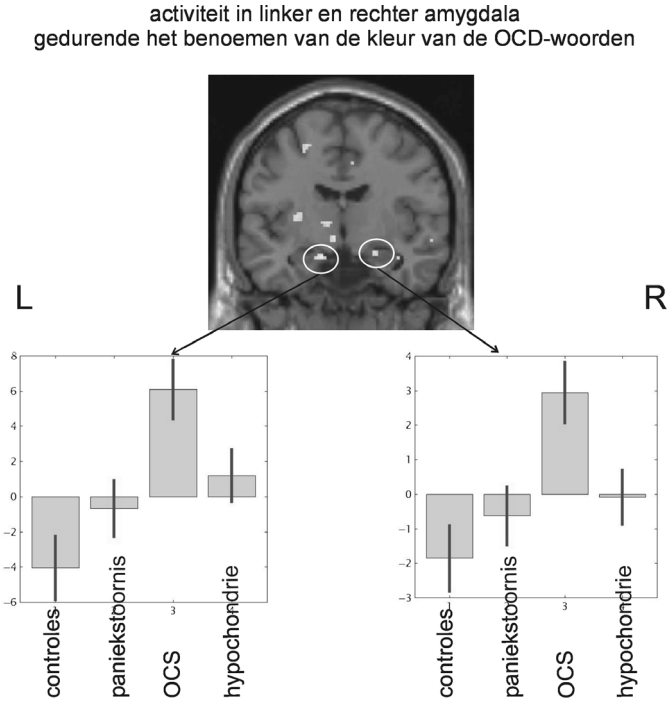
Figuur 3.



In het experiment van **hoofdstuk 8** zijn emotie en cognitie sterk met elkaar verweven. Het is bekend dat angstige mensen minder goed in staat zijn irrelevante informatie te negeren, onder meer doordat zij de neiging hebben de aandacht te richten op elementen die te maken hebben met hun angsten. Met behulp van de *Stroop*, een aandachtstaak, wordt gekeken in hoeverre het een proefpersoon lukt de aandacht te richten op de kleur van de inkt van getoonde woorden, onafhankelijk van de inhoud van die woorden. Bij deze taak is het de bedoeling zo snel mogelijk de knop in te drukken die correspondeert met de kleur van de inkt. Wanneer het antwoord beïnvloed wordt door de betekenis van het woord (zowel in reactietijd als in juistheid van het antwoord) is er sprake van interferentie. De taak bestond uit een zuiver cognitief deel en een ziekte-gerelateerd, emotioneel deel. De cognitieve condities bestonden uit congruente (bijvoorbeeld het woord ‘rood’ in rode inkt geschreven) en incongruente (bijvoorbeeld het woord ‘rood’ in blauwe inkt geschreven) woorden. Tijdens de emotionele condities werden woorden getoond die betrekking hebben op OCS (bijvoorbeeld de woorden ‘twijfel’ en ‘vies’) en paniekstoornis (bijvoorbeeld de woorden ‘menigte’ en ‘hartaanval’). Prestatie op deze ziekte-gerelateerde woorden werd vergeleken met die tijdens neutrale woorden (bijvoorbeeld de woorden ‘tafel’ en ‘ovaal’). Met het doel de fronto-striatale en limbische circuits te bestuderen tijdens de uitvoering van deze aandachtstaak, en bijkomend de specificiteit van de mogelijke afwijkingen te onderzoeken, werden met behulp van fMRI 4 onderzoekspopulaties vergeleken: medicatievrije patiënten met OCS (N=16), paniek (N=15) en hypochondrie, en een gezonde controlegroep (N=19). Vergeleken met controles en patiënten met paniek en hypochondrie, hadden OCS-patiënten slechtere scores op het cognitieve deel van de taak, maar de uitvoering van dit deel ging bij alle patiëntengroepen (vergeleken met gezonde controles) gepaard met verhoogde activiteit in compensatiegerelateerde hersengebieden. Tijdens de uitvoering van het emotionele deel van de Stroop was een duidelijk verschil zichtbaar tussen OCS-patiënten enerzijds en patiënten met een paniekstoornis en hypochondrie anderzijds. Patiënten met OCS hadden normale scores bij het benoemen van de kleur van OCS-woorden, maar dit ging gepaard

met verhoogde activiteit in het ventrale fronto-striatale systeem en beide amygdalae (zie Fig. 4).

Figuur 4.



Patiënten met een paniekstoornis hadden slechtere scores op de paniek-woorden. Terwijl OCS-patiënten heel specifiek reageerden op OCS-woorden en niet op paniek-woorden, vertoonden patiënten met een paniekstoornis zowel op OCS-woorden als op paniek-woorden een verhoogde activiteit in het fronto-striatale systeem. Echter, alleen tijdens de paniek-woorden was ook de verhoogde amygdala-activiteit zichtbaar. Tevens waren de geactiveerde fronto-striatale gebieden in patiënten met een paniekstoornis uitgebreider dan bij OCS-patiënten. Patiënten met hypochondrie vertoonden een beeld dat sterk leek op die van patiënten met een paniekstoornis. Zij lieten echter geen activiteit in de amygdala zien.

De conclusies

Op basis van de bevindingen beschreven in deel III kan de volgende hypothese geformuleerd worden: verstoord dorsaal fronto-striataal functioneren in OCS-patiënten ligt ten grondslag aan 1) de verminderde remming op de ventrale fronto-striatale en limbische activiteit als reactie op ziekte-specifieke emotionele stimuli en 2) een verminderd cognitief functioneren, welke deels kan worden gecompenseerd door het aanspreken van alternatieve hersengebieden of strategieën.

Toekomstig onderzoek zal zich moeten richten op het in kaart brengen van de interacties tussen de verschillende fronto-striatale en limbische circuits om zo antwoord te kunnen geven op de volgende vragen: 1) welke directe en indirecte connecties vanuit de dorsale fronto-striatale gebieden op het limbische circuit spelen een rol in de bijsturing van de emotionele respons en 2) op welke manier beïnvloedt activiteit in de amygdala direct of indirect het hoger cognitief functioneren? Het vergt een multidisciplinaire aanpak, waarbij laesiestudies, structurele metingen en functionele beeldvorming worden gecombineerd in mens en dier. Tevens is een grote winst te verwachten van longitudinaal patiëntgebonden onderzoek, zodat meer inzicht ontstaat in de vroege kenmerken, het natuurlijk beloop en het effect van behandeling.

Bloed, zweet en tranen

of de emotie achter de cognitie
en de mensen die deze in goede banen hebben geleid

Dank ben ik verschuldigd aan alle proefpersonen, met of zonder angst, die zich lieten overhalen mee te werken aan de soms belastende experimenten. Dank dat ik een kijkje kon nemen in uw denkende en voelende brein. Zonder uw onbaatzuchtige bijdrage aan de wetenschap was het onderzoek onmogelijk geweest.

Dit boekje is een kind van gemengde afkomst. Met een stevig neuronaal fundament (vakgroep anatomie) en groot gebracht in een fysisch milieu (PET-centrum en MRI-afdeling), is het gedreven door een levendige nieuwsgierigheid in de angstige geest (angstpolikliniek). Het heeft vele vaders, enkele moeders en een groot aantal ooms en tantes. Ik dank allen die op welke wijze dan ook hebben bijgedragen.

Een aantal mensen wil ik graag nog persoonlijk bedanken.

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Beste Richard, jij hebt een belangrijke rol gespeeld als supervisor op ‘nabije afstand’: nabij door je persoonlijke interesse in mijn ontwikkeling als jonge onderzoeker en op afstand door de inhoudelijke vrijheid die je me gaf. Jij zag de soms lastige wisselwerking tussen emotie en cognitie en hebt als meester in de cognitieve en diplomatieke bijsturing, en met de nodige humor en relativering, menig emotioneel moment helpen overwinnen. Ik heb jouw vaardigheden afgekeken en ik hoop deze in de toekomst, wanneer ik het zonder jouw supervisie moet doen, nog vaak te gebruiken.

Beste Dick, jij begon niet aan een makkelijke taak toen je jouw eerste promovendus eind 1999 de hand schudde. De intensieve begeleiding die vanaf dat moment vorm kreeg laat zich niet in drie zinnen samenvatten. We hebben allebei een zeer verschillende stijl, maar het doel en de gedrevenheid dat doel te bereiken was altijd gelijk. Ik heb veel geleerd van jouw technische kennis en jouw perfectionisme bij het verkopen van de oogst. Toen je in de vorm van twaalf vrijwillige injecties radioactief gelabeld water, twee uur met flitsbril op je neus, meeging in mijn behoefte tot het uitroeien van bewegingsartefacten, wist ik dat je me steunde. Straks, als eerste Nederlandse professor in de psychiatrische beeldvorming, wacht jou de uitdagende taak gedachte en gevoel steeds verder te integreren.

Beste Adriaan, jouw bereidheid asiel te verlenen aan de psychiatrie heeft inmiddels ernstige vormen aan genomen. Eerst was een enkele verdwaalde geest nog makkelijk weg te stoppen in een stralingsgevaarlijke telkamer. Nu, vele gigabytes neurodata verder, wacht familie ‘psycho-1’ een uitzettingsprocedure. Maar de fysica en de psychiatrie zijn inmiddels te zeer met elkaar verstrengeld om het ooit nog zonder elkaar te moeten stellen.

Beste Henk, jij hebt als geen ander de deur altijd open staan. Zelfs als jouw agenda overvol was, was je bereid mee te denken over de betekenis van de vlekjes op het scherm en had je conceptmanuscripten steeds binnen korte tijd van grondig commentaar voorzien. Ik hoop in de toekomst nog vaak bij je binnen te kunnen vallen, want de functionele modellen die gangbaar zijn in de psychiatrie missen al te vaak neuroanatomische evidentie. Naast de inhoudelijke inbreng vormde je samen met Menno Witter ook een aangenaam

pleegadres. Vanaf het allereerste begin, toen de J-vleugel nog dun bevolkt was, hebben jullie me opgenomen in de anatomische familie. Zo hebben jullie me ongemerkt wegwijs gemaakt over de neuronale paden. En we zijn nog lang niet uitgewandeld.

Een belangrijke stuwende kracht achter het onderzoek was de onderzoeksgeest van de angstpolikliniek, waar geen patiënt onopgemerkt aan een protocol kan ontsnappen. Ik wil Daniëlle Cath bedanken voor haar voortdurende enthousiaste inbreng. Daniëlle, ik waardeer jouw gedrevenheid je stem te laten horen in het sterk masculien georiënteerde academische gezelschap. Richard vergeleek ons wel eens met twee hanen, maar ik denk dat de term moederkloeken beter recht doet aan onze sterke emotionele betrokkenheid bij het reilen en zeilen van het onderzoek. Daarnaast heb jij samen met Ton van Balkom, Patricia van Oppen, Noor Tromp en alle behandelaren van de polikliniek ervoor gezorgd dat de instroom van medicatievrije proefpersonen vlot verliep.

Het vinden van gemotiveerde patiënten voor deelname aan het onderzoek werd verder gestimuleerd door de actieve patiëntenverenigingen onder de bezielende leiding van Josine van Hamersveld (Angst, dwang en fobie stichting) en Hans Eijsackers (Nederlandse Tourette vereniging). Jille Merkelbach kan ik helaas niet meer persoonlijk bedanken voor zijn geleverde strijd bij het vinden van patiënten met hypochondrie. Zo vaak kreeg ik optimistische emailtjes van je als het je weer was gelukt iemand bereid te vinden voor deelname aan het onderzoek. Jille, ik betreur het zeer dat jij het einde van onze gezamenlijke onderneming niet meer mee hebt kunnen maken. Julie van Hartskamp dank ik voor haar grote inzet bij de afronding van de dataverzameling in de periode dat ik weer fulltime in de kliniek werkte. Marjan Nielen bleek een grote aanwinst voor de ‘angst&depressie-imaging’-groep door haar neuropsychologische kennis van OCD-zaken. Marijke van ter Tholen bedank ik voor haar hulp bij het vergaren van oude en recente literatuur.

Het boekje bestaat voor een belangrijk deel uit water, waarvan de bron ligt onder de parkeerplaats van het VU medisch centrum. Met de symptoomprovocatiestudie werd voor het eerst in Nederland de productie van $H_2{}^{15}O$ voor patiëntgebonden onderzoek in werking gezet. Ondanks de onvoorspelbare grillen van het cyclotron en de extreem korte levensduur van het gelabelde water ($t_{½}$ = 2 min) was het met vereende krachten mogelijk een tamelijk ingewikkeld protocol uit te voeren. Ik dank hen die dit kostbare, radioactieve vocht maakten (Gert Luurtsema, Henri Greuter, Joke Eyndhoven, Patricia Ophemert) en zij die als trouwe waterdragers (Suzette van Balen, Bas Hoving, Rob Koopman, Annemarie de Wildt) de proefpersonen begeleidden tijdens de vaak spannende en belastende scansessies. Ronald Boellaard en Catalina Mesina dank ik voor de prettige samenwerking bij het optimaliseren van de technische aspecten van de dataverwerking. Pieper *391 (Remi Schmeits) was steeds als baken in het duister op te roepen wanneer het digitale huis ineen leek te storten (eenmaal was het kwaad al geschied). Secretariële ondersteuning (inclusief vrijwillige onderwerping aan het MRI experiment) kwam van Amanda Kalwij en Jaap van der Kuij.

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Knowledge from oversee came from Ray Dolan (Wellcome Department of Cognitive Neuroscience, London, UK) and Scott Rauch (Massachusetts General Hospital, Harvard, Charlestown, USA). Dear Ray, I would like to thank you for your contribution to our work. I hope we will once continue the stimulating discussion about what the big thing is in OCD. Dear Scott Rauch, most of the studies described in this thesis are largely inspired by your work in OCD. I am very honored that you reviewed my thesis and that you will participate in the opposition during the graduation ceremony. I am looking forward to meet you in May.

Gedeelde smart is halve smart. Mocht voorheen de AGIKO een zeldzame soort zijn en slechts zelden in staat te overleven, inmiddels vormen we een waar elftal (Peter, Marijke, Christel, Daniel, Arne, Gerben, Eric, Ritsaert, Saskia en Neeltje). Dé plaats om de twijfels, frustraties en cirkelredenaties teruggespiegeld te krijgen was aan de eettafel tijdens de 6-wekelijkse intervisies. De rector gaat niet akkoord met 11 paranimfen, maar ik weet me gesteund door jullie allemaal. Peter, jouw boek volgt. Ik voel me 12 mei gesterkt met de wetenschap dat jij de verdediging in geval van nood kan overnemen.

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Schoonpapa, ouwe reus, ik verleng hierbij voor het leven mijn abonnement op de exclusieve psychiatrie&wetenschap-knipselkrant.

Harold, liefste para-elf, de geest van de kunstenaar blijkt nauw verwant met die van de wetenschapper. Met jouw brede interesse, ijzersterk geheugen en wetenschappelijke geest, verruilde jij de geologie voor de gitaar. Maar onderzoeker ben je in hart en nieren. Jij hebt als onzichtbare coauteur een groot deel van het manuscript van kritisch commentaar voorzien en met al jouw zorgzaamheid en levenslust steeds weer mijn accu opgeladen. Nu is de beurt aan jou jouw geesteskind op de wereld te zetten.

Tot slot, Ada, lieve kleine meid, jij hebt me de belangrijkste titel gegeven die er bestaat. Jouw vrolijke nieuwsgierigheid is aanstekelijk en ik hoop dat je die eigenschap altijd behoudt. Dit boekje is voor jou: je mag het in 1000 stukjes scheuren.

Odile

Amsterdam, 3 maart 2005

Curriculum Vitae

Odile van den Heuvel werd geboren op 8 februari 1973 in Saverne, Frankrijk, als jongste in een gezin met vier kinderen. Haar jeugd bracht zij door in het Brabantse land. Zij behaalde in 1991 het eindexamen VWO aan het Augustinianum te Eindhoven en ging vervolgens geneeskunde studeren aan de Erasmus Universiteit Rotterdam. Gedurende de doctoraalfase van haar studie raakte ze, ondermeer door een onderzoekstage op de afdeling Kinder- en Jeugdpsychiatrie van het Sophia Kinderziekenhuis, enthousiast voor wetenschappelijk onderzoek. Vervolgens deed ze een epidemiologische studie onder vrouwen op het Zimbabwaanse platteland naar het gebruik van de lokale gezondheidzorg tijdens zwangerschap en bevalling. Na het behalen van het artsexamen in 1998, vertrok ze naar Amsterdam en begon als arts-assistent niet in opleiding (AGNIO) op de kliniek Stemmingstoornissen van het Academisch Medisch Centrum. Na een jaar haalde prof. dr. R. van Dyck haar naar het VU Medisch Centrum voor het onderzoek beschreven in dit proefschrift. Hiermee begon ze begin 2000 als arts-assistent in opleiding tot klinisch onderzoeker (AGIKO), met een stipendium van het NWO (MW 940-37-018). Sindsdien werkte ze afwisselend in de kliniek en op de universiteit. Het klinische deel van de opleiding tot psychiater zal ze nog voortzetten tot medio 2007. Odile is getrouwd met Harold Berghuis. Samen kregen ze op 17 april 2004 een dochter, Ada.

Odile van den Heuvel was born in Saverne, France, on the 8th of February 1973. She is the youngest in a family of four children. She was raised in the province of Noord-Brabant, in the South of the Netherlands. In 1991 she graduated from the Augustinianum high school, Eindhoven. The same year she started her study at the medical school of the Erasmus University in Rotterdam. She became attracted to scientific psychiatry during a training post at the Department of Child Psychiatry in the Sophia Children Hospital, Rotterdam. Afterwards, she made an epidemiological study in rural Zimbabwe on the use of health care during pregnancy and delivery. After she graduated from medical school in 1998, she moved to Amsterdam to work as a Junior Resident in Psychiatry in the Department of Mood Disorders at the Academic Medical Centre. One year later, prof. dr. R. van Dyck asked her to join the Free University (VU University Medical Center) to start the research project described in this thesis. She started this project at the beginning of 2000, and was awarded an NWO-scholarship (MW 940-37-018). Since this date she has worked concurrently at the hospital and at the university. She will finish her specialization in psychiatry in 2007. Odile is married to Harold Berghuis. On April 17th 2004 their daughter, Ada, was born.