Limbic changes detected by MRI involved in

memory, emotional and olfactory dysfunctions in Parkinson's disease.

Thesis presented by

Naroa Ibarretxe-Bilbao,

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Supervised by

Dr. Carme Junqué i Plaja, University of Barcelona

Dr. Pere Vendrell i Gomez, University of Barcelona

Medicine Doctorate Program

Department of Psychiatry and Clinical Psychobiology

School of Medicine, University of Barcelona

Dr. CARME JUNQUÉ I PLAJA and PERE VENDRELL I GÓMEZ, *Professors at University of Barcelona,*

CERTIFIE that they have supervised and guided the PhD thesis entitled "**LIMBIC CHANGES DETECTED BY MRI INVOLVED IN MEMORY, EMOTIONAL AND OLFACTORY DYSFUNCTIONS IN PARKINSON'S DISEASE**"*, presented by Naroa Ibarretxe-Bilbao. They hereby assert that this thesis fulfils the requirements to be defended for the Degree of Doctor.*

Signature,

Dr. Carme Junqué i Plaja
University of Barcelona

Signature,

Dr. Pere Vendrell i Gómez
University of Barcelona

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This thesis has been carried out in the Neuropsychological Group of the Psychiatry and Clinical Psychobiology Department of the School of Medicine, University of Barcelona. The group belongs to the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS).

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en que la utilizó Joan Guinovart)

Contents

Foreword

This thesis, presented to obtain the degree of Doctor by the University of Barcelona, is the result of five studies carried out during a 4-year period at the Department of Psychiatry and Clinical Psychobiology, School of Medicine, University of Barcelona. During this period, I have obtained the Diploma d'Estudis Avançats (DEA) through the Neurosciences Program of the School of Medicine at the University of Barcelona. The following articles have been published in international journals, as a result of the work performed, with a ***global impact factor (IF) of 14.441*** *(ISI of Knowledge, Journal Citation Reports 2008):*

Paper I:

Ibarretxe-Bilbao N, Ramirez-Ruiz B, Tolosa E, Marti MJ, Valldeoriola F, Bargallo N. Hippocampal head atrophy predominance in Parkinson's disease with hallucinations and with dementia. *Journal of Neurology* 2008; 255: 1324-1331. IF = 2.536

Paper II:

Ibarretxe-Bilbao N, Ramirez-Ruiz R, Junque C, Marti MJ, Valldeoriola F, Bargallo N, Juanes S, Tolosa E. Differential progression of brain atrophy in Parkinson disease with and without visual hallucinations. *Journal of Neurology, Neurosurgery and Psychiatry* (online first). IF = 4.622

Paper III:

Ibarretxe-Bilbao N, Junque C, Tolosa E, Marti MJ, Valldeoriola F, Bargallo N, Zarei M. Neuroanatomical correlates of impaired decision-making and facial emotion recognition in early Parkinson's disease. *European Journal of Neuroscience* 2009; 30: 1162-1171. IF = 3.385

Paper IV:

Olfactory impairment in early Parkinson's disease is related to fractional anisotropy reduction in central olfactory areas. A voxel-based diffusion tensor imaging (DTI) study. *(in preparation)*

Paper V:

Early alterations in brain functional networks during episodic memory retrieval task in Parkinson's disease. *(in preparation)*

Paper VI: *review*

Ibarretxe-Bilbao N, Tolosa E, Junque C, Marti MJ. MRI and cognitive impairment in Parkinson's disease. *Movement Disorders* 2009; 24: S748-S753. IF= 3.898

Glossary of Abbreviations

AD: *Alzheimer's disease*

ANOVA: *Analysis of Variance*

BA: *Brodmann area*

BDI: *Beck's Depression Inventory*

CBF: *Cerebral blood flow*

CSF: *Cerebral Spinal Fluid*

CPT: *Conner's Continuous performance*

DTI: *Diffusion Tensor Imaging*

DWI*:* *Diffusion Weighted Imaging*

DLB: *Dementia with Lewy Bodies*

FA*:* *Fractional Anisotropy*

fMRI: *Functional Magnetic Resonance Imaging*

GM: *Gray Matter*

HC: *Healthy controls*

IQ: *Intelligence Quotient*

ICA*:* *Independent Component Analysis*

LB: *Lewy bodies*

LN: *Lewy neurites*

MCI: *Mild cognitive impairment*

MD: *Mean diffusivity*

MDS*:* *Movement Disorders Society*

MMSE: *Mini-mental state examination*

MNI: *Montreal NeurologicaI Institute*

MRI: *Magnetic Resonance Imaging*

MSA: *Multisystem atrophy*

NPI: *Neuropsychiatric Inventory*

ICA: *Independent component Analysis*

IGT: *Iowa Gambling Task*

OFC: *Orbitofrontal cortex*

PD: *Parkinson's disease*

PET: *Positron Emisson Tomography*

POC: *Primary olfactory cortex*

PSP: *Progressive supranuclear paralisis*

RAVLT: *Rey's Auditory Verbal Learning test*

ROI: *Region of Interest*

SD: *Standard Deviation*

SPM: *Statistical Parametric Mapping*

TIV: *Total Intracranial Volume*

TBSS: *Tract-based spatial statistics*

UPDRS: *Unified Parkinson Disease Rating Scale*

VBM: *Voxel-Based Morphometry*

VH*:* *Visual hallucinations*

WAIS*:* *Wechsler Adult Intelligence Scale*

WM: *White Matter*

1. Introduction

1. Introduction

1.1. Parkinson's disease: a multisystem degeneration

Parkinson's disease (PD) has traditionally been considered a motor disorder characterized by tremor, rigidity, bradykinesia and postural instability. However, there is a growing interest in understanding the widespread, multisystem nature of the neurodegeneration that accounts for "non-dopaminergic" symptoms and its critical role in the quality of life of PD patients (Lang and Obeso. 2004). PD should no longer be considered as a disorder characterized solely by parkinsonism but as a brain disease with different manifestations such as cognitive and olfactory dysfunctions, dysautonomia, sleep fragmentation, rapid eye movement behavior disorder, mood and anxiety disorders, and depression (Tolosa, et al. 2009).

The increasing support for the conception of PD as a multiple system neurodegenerative disorder has emerged from research demonstrating the existence of considerable extranigral pathology (Del Tredici, et al. 2002). The non-dopaminergic or non-motor clinical manifestations are probably caused by the neuronal dysfunctions in many cortical, subcortical, brainstem, and peripheral autonomic sites (Ferrer. 2009, Lang and Obeso. 2004).

In summary, nowadays PD is considered a systemic disease of the nervous system with variegated clinical symptoms that appear even earlier than motor manifestations. Table 1 presents a synthesis of non-motor symptoms in PD.

Table 1. Non-motor symptoms in Parkinson's disease and their neuropathological substrates

Non-motor symptoms in PD	Presumed underlying brain structures
Cognitive dysfunction and dementia	Subcortical: Basal nucleus of Meynert, locus ceruleus Limbic system: Amygdala, hippocampus. Neocortex: associative frontal, temporal and parietal
Hallucinations, psychosis: mainly visual hallucinations	Amygdala, limbic cortex
Olfactory loss: Impairment in odor detection, discrimination and identification	Olfactory bulb, anterior olfactory nucleus, amygdala, piriform and entorhinal cortex.
Mood disorders: depression, anxiety	Locus ceruleus, raphe nuclei, amygdala
Sleep disturbances: REM behavior disorder, excessive daytime sleepiness, insomnia	Nucleus subceruleus, pedunculopontine nucleus, thalamus and hypothalamus.
Dysautonomia: Gastrointestinal disturbances, urinary dysfunction, sexual dysfunction, orthostatic hypotension.	Amygdala, dorsal nucleus of the vagus, intermediolateral column of the spinal cord, sympathetic ganglia, enteric and autonomic plexuses
Other non-motor symptoms: Pain, apathy, fatigue, mid-life obesity, impaired color discrimination, restless legs syndrome.	Unclear

This table is adapted from Tolosa, et al. 2009.

1.2. Neuropathology of PD

A massive dopaminergic denervation and depletion occurs in PD even before the first motor symptoms appear. The onset of clinical motor symptoms in PD occurs when 60–80% of striatal dopaminergic terminals and 40–50% of cell bodies in the substantia nigra pars compacta have been lost leading to reduced dopaminergic input to the striatum and both presynaptic and postsynaptic compensatory mechanisms in the nigrostriatal dopaminergic system (Ferrer. 2009, Lang and Obeso. 2004). In addition to the substantia nigra other nuclei are involved, such as the locus ceruleus, reticular nuclei of the brainstem, and dorsal motor nucleus of the vagus, as well as the basal nucleus of Meynert, the amygdala and hippocampus (Ferrer. 2009). A large proportion of the neurons in these areas have typical Lewy body (LB) inclusions that contain ubiquitin and alpha-synuclein (Spillantini, et al. 1997), and similar protein aggregations, which are known as Lewy neurites (LNs), are also seen in neuronal projections (Sandmann-Keil, et al. 1999).

It has been shown that the intracellular changes that lead to protein aggregation and formation of LBs and LNs may begin in the dorsal glossopharyngeal-vagus (IX/X) complex in the brainstem, the olfactory bulb and olfactory tract, the locus ceruleus, the caudal raphe nucleus even before any changes occur in the substantia nigra (Del Tredici, et al. 2002) and this theory fits well with the fact that non-motor symptoms such as olfactory dysfunctions precede the development of parkinsonism. Braak et al., 2003, proposed a six-stage system of brain pathology to indicate a predictable sequence of ascending LB pathology from the medulla oblongata and olfactory bulb to the midbrain, diencephalic nuclei, and finally the neocortex (Braak, et al. 2003). The stages described by Braak are as follows: The earliest lesions appear in the olfactory bulb, anterior olfactory nucleus, and dorsal IX/X motor nucleus (stage 1) followed by lesions in the lower raphe nuclei, the magnocellular portions of the reticular formation and the locus ceruleus (stage 2). In stage 3, the upward-moving process crosses the upper limit of the pontine tegmentum and enters the basal portions of the midbrain and forebrain, affecting the substantia nigra pars compacta and the central subnucleus of the amygdala, and from there extends into the basolateral nuclei. In stage 4, the transition zone between the allocortex and neocortex is drawn into the disease process including the temporal

mesocortex (transentorhinal region). In the final stages 5 and 6, the neurodegenerative process attains its greatest topographic extent. With the temporal mesocortex as its starting point, the inclusion body pathology gradually overruns the entire neocortex. Inclusion bodies appear first in the prefrontal and high-order sensory association areas of the neocortex (stage 5), then in the premotor and first order sensory association areas, and, finally, in some instances, even in the primary fields (stage 6).

Figure 1 shows the stages of PD-related pathology.

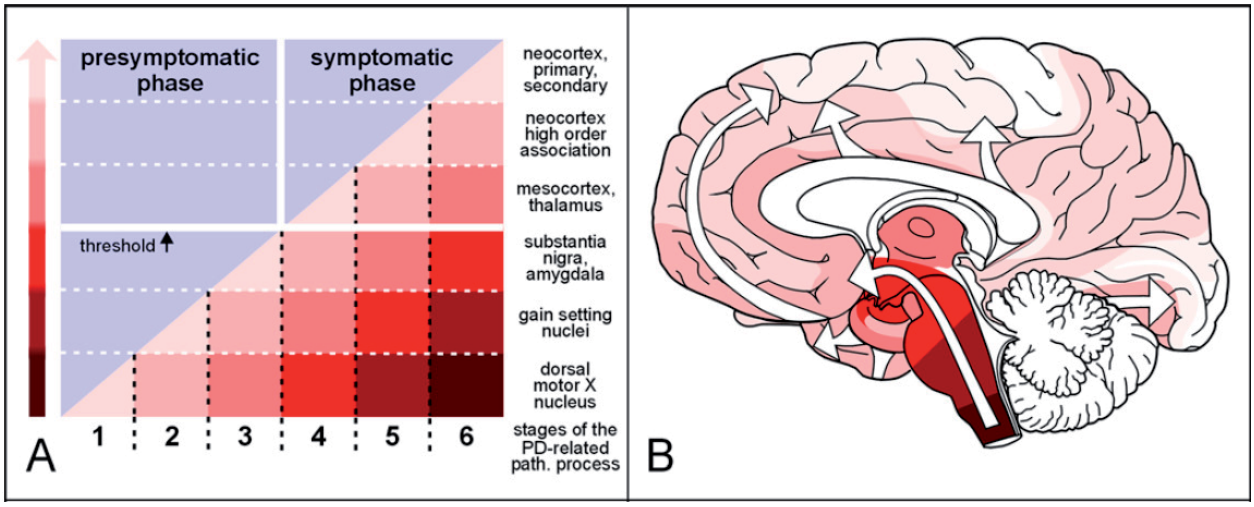

Figure 1. Diagram showing the ascending pathological process in Parkinson's disease. (Braak et al., 2004).

Though Lewy bodies (LB) are the pathologic inclusions that have traditionally been associated with PD, debate continues regarding their pathological significance (Lees, et al. 2009). Several theories regarding the role of LBs in the pathogenesis of PD have been proposed: i) they may be the consequence of a failed attempt to protect the damaged neuron from toxic protein species (Olanow, et al. 2004); ii) alternatively, the excessive quantities of toxic alpha-synuclein oligomers inside the inclusion may overwhelm the neuronal mechanisms responsible for aberrant protein clearance, disrupt the normal cell physiology, and lead to cell death (Rochet, et al. 2000) iii) finally LBs may simply be markers of cell damage but peripheral to the primary pathological process. Recent data demonstrate early involvement of the cerebral

cortex in PD due to the convergence of multiple metabolic defects such as mitochondrial abnormalities, oxidative stress, abnormal gene regulation and involvement of proteasomes and autophagosomes, and suggest that Lewy pathology is a relative late event, geared to isolate unremoved damaged protein, with little significance for cortical neurological deficits (Ferrer. 2009).

Braak's LB classification correlates with neurological deficits in the majority of patients with early onset and long duration of the disease (Braak, et al. 2005, Jellinger. 2004), but retrospective clinico-pathological studies have shown that there is no relationship between the staging and the clinical severity of PD, or, more specifically, cognitive impairment (Jellinger. 2008). In addition, some cases with a reasonable level of alpha-synuclein pathology in both brainstem and cortical areas are manifestly non-demented (Parkkinen, et al. 2005). It must be remembered that the degree of dementia is largely dependent on AD pathology rather than LB distribution. For example, the Sydney Multicenter Study of Parkinson's disease followed an initial group of 149 *de novo* PD patients for 20 years and demonstrated that the pathological evolution of those patients who presented the first PD symptoms before they were 65 years old (early onset) presented a caudorostral progression like the one described by Braak and developed dementia after 10 years of disease evolution. However, patients who developed PD after the age of 70 (late-onset) developed dementia within the first 10 years of disease evolution and did not follow the stages proposed by Braak. Late onset PD patients presented LBs in the cerebral cortex from the beginning and more AD-related pathology (Halliday, et al. 2008). The pathological substrate of dementia in PD remains a matter of controversy. Some studies emphasize the importance of AD-type pathology in the development of dementia in PD (Jellinger, et al. 2002), while others emphasize the involvement of Lewy type pathology in the limbic and cortical regions (Mattila, et al. 2000). However, the common co-occurrence of alpha-synuclein and AD-type pathology suggests that both may play an important role (Masliah, et al. 2001) and the heterogeneous cognitive deficits in PD probably reflect different forms of neuropathological involvement.

1.3 Cognitive dysfunctions in PD

1.3.1 Early cognitive dysfunctions

Though cognitive dysfunctions have been somewhat neglected in the study of Parkinson's disease, nowadays it is recognized that they occur even at early stages of the disease (Aarsland, et al. 2009, Elgh, et al. 2009, Foltynie, et al. 2004, Muslimovic, et al. 2005a). The first study to present the incidence of PD and the cognitive problems in a newly diagnosed cohort of patients using a community-based epidemiological approach was the one by Foltynie et al. in 2004, in which 36% of PD patients had evidence of cognitive impairment. Patients were classified as having predominant frontostriatal impairment if they performed poorly on the Tower of London task, predominant temporal lobe impairment if they performed poorly on a pattern recognition memory task or a global pattern of impairment if they performed poorly on tests in both domains. The different pattern of cognitive deficits seen among these patients suggests that sub-groups of patients categorized according to cognitive ability might be identifiable even in the early stages of disease, and that this may reflect regional differences in the underlying neuropathological processes (Foltynie, et al. 2004). In 2005 Muslimovic et al. reported that relative to controls, early PD patients performed significantly worse on most cognitive measures but the differences were mainly explained by measures of immediate memory and executive function. In addition, older age at disease onset was shown to be an important determinant of cognitive dysfunction in PD (Muslimovic, et al. 2005b). A recent study reported a twofold increase in the proportion of cognitive impairment in subjects with early, untreated PD compared to controls. The PD group was more impaired on all neuropsychological tests than controls, covering attention, psychomotor speed, verbal memory, and visuo-spatial and executive functions, but the largest effect size was found for verbal memory (Aarsland, et al. 2009). Similarly, cognitive dysfunction was recently reported in untreated patients in early PD, affecting attention, psychomotor function, episodic memory, executive function and category fluency (Elgh, et al. 2009). For a summary see table 2.

Table 2. Cross-sectional community based studies in early PD

	Sample	Age	Disease duration (months)	% of cognitive impairment	Type of cognitive dysfunction
Foltynie *et al.* 2004 UK CamPAIGN study	159	70.3	30	36%	Executive function and memory
Muslimovic *et al.* 2005 Netherlands study	115	66.2	18.8	24%	Executive function and memory
Aarsland *et al.* 2009 Norwegian Park West study	196	67.6	28	19%	Attention, psychomotor speed, verbal memory, visuo-spatial and executive functions.
Elgh *et al.* 2009 Umea population-based study	88	68.1	25	30%	Attention, psychomotor, episodic memory, executive function and category fluency

Two of these cross-sectional studies led into longitudinal studies. In the UK Cambridgeshire Parkinson's Incidence from GP to Neurologist (CamPAIGN) study, an assessment of 126 patients was performed after a follow-up of 3.5 years. At follow-up, 57% presented cognitive impairment and 10% dementia. The multiple regression analysis showed that the variables that predicted the appearance of dementia were: age = or > 72, non- tremor dominant, pentagon copying score, semantic fluency < 20 (Williams-Gray, et al. 2007). The Netherlands study also performed a follow-up after 3 years to examine the evolution and predictors of cognitive decline in PD, reporting that 48% of the patients had developed cognitive impairment and 9% developed dementia (Muslimovic, et al. 2009). Measures that decreased significantly over time in newly diagnosed patients were psychomotor speed and attention and, to a lesser though still significant extent, impairment was reported in tests of memory, visuo-spatial skills, and executive functions. In contrast to the previous longitudinal studies, none of the baseline features predicted cognitive change in newly diagnosed patients.

To understand the focus of the present thesis, it is important to note that cognitive dysfunction in early stages of PD is much more extended and not only limited to executive dysfunction, as was previously

believed. Initial studies reported that cognitive dysfunction in early PD only affected fronto-striatal circuits, provoking a marked executive dysfunction (Owen, et al. 1992). Memory impairment in early PD was thought to depend on a problem in retrieving the storage information and therefore also reflecting fronto-striatal dysfunction (Cooper, et al. 1991). However, there is increasing evidence of medial temporal lobe atrophy in PD that may be responsible for memory dysfunction in PD (Camicioli, et al. 2003, Junque, et al. 2005, Tam, et al. 2005).

In addition, other functions that have recently been reported to be altered in early PD are decision-making and recognition of emotions. Studies using the Iowa gambling task (Bechara, et al. 1997) (for a description of the task, see methods section) have shown impairment in decision-making in advanced PD (Mimura, et al. 2006, Pagonabarraga, et al. 2007) but also in early PD (Kobayakawa, et al. 2008, Perretta, et al. 2005). On the other hand, several studies have reported impairment of recognition of facial expressions of emotions in PD. Though some authors failed to detect differences between patients and controls (Adolphs, et al. 1998, Pell and Leonard. 2005), a general impairment in the recognition of all emotions (Yip, et al. 2003) or specific impairment in some types of emotion have been described (Ariatti, et al. 2008, Clark, et al. 2008, Kan, et al. 2002, Lawrence, et al. 2007, Sprengelmeyer, et al. 2003, Suzuki, et al. 2006). Specifically, Sprengelmeyer et al. reported that *de novo* PD patients scored significantly lower in recognizing sadness and fear in comparison with healthy controls (Sprengelmeyer, et al. 2003). These results suggested that recognition of these emotions is impaired very early in the disease and is independent of treatment.

To understand the differences in the studies assessing cognitive function in newly diagnosed patients we must consider the heterogeneity in PD. There is a consensus that PD is a heterogeneous disease, and data-driven approach studies have identified distinct subgroups of patients in early PD. For example, Lewis et al., 2005, identified four main subgroups: patients with an earlier disease onset; a tremor dominant subgroup; a non-tremor dominant subgroup with significant levels of cognitive impairment and mild depression; and a subgroup with rapid disease progression but no cognitive impairment (Lewis, et al. 2005). Post et al., 2008, also reported heterogeneity in newly diagnosed PD, and proposed three distinct

subgroups: a younger onset group; an intermediate older onset group with more anxiety and depressive symptoms; and an oldest onset group with more motor impairment and higher rate of progression (Post, et al. 2008). In addition, the confluence of factors such as depression, which is known to be frequent in PD, must be taken into account (Stefanova, et al. 2006).

1.3.2 Mild cognitive impairment (MCI)

All the studies reported in the previous section (see table 2) highlight cognitive impairment in PD as a key feature from the time of diagnosis onwards. The use of the MCI concept in PD has generated some controversy. Methodological issues may influence the outcome of studies of MCI in PD, and thus may contribute to the discordant results (Aarsland, et al. 2009). First, and most importantly: what is considered as MCI in PD?

1.3.2.1 Definition of MCI

MCI as a clinical diagnostic entity was first defined to designate an early, but abnormal, state of cognitive impairment which refers to a transitional zone between normal cognitive function and clinically probable Alzheimer's disease (AD) (Petersen. 2004).

The specific recommendations for the general MCI criteria include the following: (i) the person is neither normal nor demented; (ii) there is evidence of cognitive deterioration, shown by either objectively measured decline over time and/or subjective report of decline by self and/or informant in conjunction with objective cognitive deficits; and (iii) activities of daily living are preserved and complex instrumental functions are either intact or minimally impaired (Winblad, et al. 2004). In addition, different subtypes of MCI have been defined: If memory is the only domain impaired, then the classification is amnestic MCI-single domain; if other domains are impaired in addition to memory, the classification is amnestic MCI-multiple domain; similarly, if memory is not impaired, non-amnestic MCI is the classification, and again the determination is then made of either a single non-memory domain or multiple

non-memory domains being impaired, yielding non-amnestic MCI-single domain or non-amnestic MCI-multiple domain, respectively (Petersen. 2004).

As previously mentioned, though MCI was first defined in AD context, an increasing number of researchers use these criteria for defining cognitive impairment in other disorders with neurodegenerative, vascular, psychiatric or traumatic etiology. The definitions are essentially common with regard to their aim and theoretical framework in that they (i) refer to non-demented persons with cognitive deficits measurable in some form or another, and (ii) represent a clinical syndrome that can be used to classify persons who do not fulfill a diagnosis of dementia, but who have a high risk of progressing to a dementia disorder (Winblad, et al. 2004). However, as the literature on MCI has expanded, there has been some confusion concerning the specific boundaries of the condition, and unfortunately, this is the case in Parkinson's disease.

1.3.2.2 Caveats and classification challenges

The definition and proportion of patients with MCI in PD depends heavily on the choice and number and type of tests used to measure cognitive function and cutoffs used to define impairment (1, 1.5 or 2 standard deviations (SD) below the control group). The inclusion of a control group is crucial to adjust the effect and should form part of all studies estimating the proportion of MCI in PD. Another problem with the definition of MCI in PD is how to ensure that the functional decline is due to the cognitive impairment and not to the motor symptoms of the disease, and finally, the effect of drugs and psychiatric features (Aarsland, et al. 2009). Therefore, future research needs standardized criteria for diagnosis of MCI in PD, which is currently being provided by the Movement Disorders task force, longitudinal studies and studies of the validity of subtypes.

1.3.2.3 MCI in PD

Using the modified Petersen's criteria for MCI in a cohort of 72 non-demented PD patients a total of 38 PD patients were diagnosed with MCI (amnestic, n= 6; single non-memory domain, n= 17; multiple

domains, n= 15). Interestingly, 62% of the patients with PD and MCI developed dementia during a 4-year period follow-up (Janvin, et al. 2005). In another study, 21% of the sample met criteria for MCI, the frontal/executive dysfunction being the most frequently abnormal cognitive domain (33%) followed by amnestic deficits (22%), multiple domain MCI without amnestic deficit (22%) and finally multiple domain MCI with amnestic deficit (11%) (Caviness, et al. 2007). In a recent study of 196 non-demented drug-naive patients, 37 patients with PD (18.9%) fulfilled the criteria for MCI. Twenty-three (62.2%) of the PD patients with MCI had non-amnestic single domain MCI, one (2.7%) had non-amnestic MCI multiple domain, 9 (24.3%) amnestic single domain MCI, and 4 (10.8%) had amnestic multiple domain MCI (Aarsland, et al. 2009). It has been suggested that the transition from MCI to dementia in PD is characterized by the addition of cortical type cognitive defects upon a progressive fronto-subcortical impairment and efforts are being made to develop scales aimed at measuring cortical cognitive deficits, such as the recently published Parkinson's Disease–Cognitive Rating Scale (PD-CRS) (Pagonabarraga, et al. 2008).

1.3.3 Dementia in PD

1.3.3.1 Definition

Dementia has been increasingly accepted as a common feature in patients with PD, especially in old age. Dementia in PD is characterized by impairment in attention, memory, executive and visuo-spatial functions. Behavioral symptoms such as affective changes, hallucinations, and apathy are frequent (Emre, et al. 2007). As previously mentioned (see 1.2. Neuropathology of PD), previous work on the clinico-pathological correlations in patients with PD and dementia can be broadly classified into those studies suggesting a correlation of dementia, with brainstem pathology, those suggesting that limbic and cortical LB-type degeneration is the main correlate, and those suggesting that co-incident Alzheimer type pathology is the main correlate of dementia. However, specific criteria for the clinical diagnosis of dementia associated with PD were lacking until 2007, when the Movement Disorder Society (MDS)

recruited a Task Force to define the clinical diagnostic criteria for dementia in PD. Studies previous to this used the criteria from the Diagnostic and Statistical Manual of Mental Disorders as a guide, along with the administration of Folstein's Mini-Mental State Examination (MMSE). A score of 23 or lower (depending on the study) and DSM items were required to fulfill dementia criteria. Nowadays, the clinical diagnostic criteria of dementia in PD depend on the consensus from the MDS. The diagnosis of dementia is based on a two-level process depending upon the clinical scenario and the expertise of the evaluator involved in the assessment: **Level I** is aimed primarily at the clinician with no particular expertise in neuropsychological methods, but who requires a simple, pragmatic set of tests that are not excessively time-consuming. **Level II** is used in concert with level I when there is the need to specify the pattern and the severity of the dementia and when the diagnosis remains uncertain or equivocal (Dubois, et al. 2007).

Criteria for the diagnosis of dementia in PD (level I)
1. A diagnosis of PD based on the Queen's Square Brain Bank criteria for PD
2. PD developed prior to the onset of dementia (> 1 year)
3. MMSE below 26
4. Cognitive deficits severe enough to impact daily living
5. Impairment in at least two of the following tests:
• Months reversed or Seven backward
• Lexical fluency or Clock drawing
• MMSE pentagons
• 3-Word recall

Criteria for the diagnosis of dementia in PD (level II)	
COGNITIVE DOMAIN	**NEUROPSYCHOLOGICAL TESTING**
Global efficiency	Mattis Dementia Rating Scale
Executive functions:	
• Working memory	• Digit span, spatial spam, digit ordering.
• Conceptualization	• Similarities, WCST.
• Set activation	• Verbal fluency
• Set shifting	• Trail making test (TMT)
• Set maintenance	• Stroop test
• Behavioral control	• Prehension behavior
Memory	RAVLT (free and cued recall test)
Instrumental functions:	
• Language	• Boston naming test
• Visuo-constructive	• Copy of the clock
• Visuo-spatial	• Benton line orientation test, Cube analysis (VOSP)
• Visuo-perceptive	• Benton face recognition test, Fragmented letters (VOSP)
Neuropsychiatric functions:	
• Apathy	• Apathy scale
• Depression	• MADRS, Hamilton, BDI, GDS-15
• Visual hallucinations	• PPQ 6
• Psychosis	• NPI

Tables are adapted from Dubois, et al. 2007

1.3.3.2 Epidemiology

Large community-based studies have reported that the point prevalence of dementia in PD is close to 30% and that the incidence rate is 4-6 times higher than in controls. In addition, as the disease progresses a substantial proportion of patients will develop dementia. The cumulative prevalence is very high: at least 75% of the PD patients who survive for more than 10 years will develop dementia (for the latest review of epidemiological studies, see Aarsland and Kurz, 2009).

Aarsland et al. performed a systematic review in 2005 including 13 cross-sectional studies with a total of 1767 patients of whom 554 were diagnosed with dementia, a prevalence of 31.3% (Aarsland, et al. 2005a). The results of studies published since that review corroborate these findings, reporting rates of dementia in PD of between 22% and 48% (Athey, et al. 2005, de Lau, et al. 2005, Hobson and Meara. 2004). In newly diagnosed patients the prevalence of dementia has been reported to be between 8% and 16% (Reid, et al. 1996, Foltynie, et al. 2004). However, it should be noted that this studies were published previous to the consensus criteria for diagnosis of dementia with Lewy Bodies (DLB) which advocates a diagnosis of DLB if dementia onset is before or within 1 year after onset of PD (McKeith, et al. 2005).

Longitudinal studies make it possible to report the cumulative proportion of PD patients who develop dementia over time, providing a more accurate estimate of the frequency of PD. The Sydney Multicentre Study of Parkinson's disease followed 136 newly diagnosed PD patients over more than 20 years: At base-line, 17% were classified as having dementia; after 3 and 5 years, 26% and 28% were demented (Reid, et al. 1996); after 15 years, 48% were demented; and at 20 years follow-up, dementia was present in 83% of the survivors (Hely, et al. 2005, Hely, et al. 2008). Another longitudinal study is the Stavanger study which examined the 8-year prevalence, characteristics and risk factors of dementia in patients with PD (mean duration of the disease when entering the study was 9.2 years). At baseline, 28% had dementia. After 8 years, the cumulative prevalence of dementia was found to be 78% (Aarsland, et al. 2003).

1.3.3.3 Risk factors for dementia in PD

Dementia is associated with a higher health care burden (Spottke, et al. 2005), a higher frequency of institutionalization (Parashos, et al. 2002) and reduced quality of life in patients and caregivers (Aarsland, et al. 1999). Knowledge of the risk of dementia is therefore crucial for patients, caregivers, and health care planners (Buter, et al. 2008).

The clinical predictors of dementia in PD have been reported to be: age (Aarsland, et al. 2001, Hughes, et al. 2000, Mahieux, et al. 1998), the severity of motor symptoms (Aarsland, et al. 2001, Levy, et al. 2000), rigid-akinetic PD subtype (Alves, et al. 2006, Levy, et al. 2000, Rajput, et al. 2009) rapid eye movement (REM) sleep disorder (Gagnon, et al. 2009, Sinforiani, et al. 2008), visual hallucinations (Aarsland, et al. 2003, Galvin, et al. 2006) and cognitive dysfunction (Aarsland, et al. 2001, Janvin, et al. 2006) including impaired verbal fluency (Jacobs, et al. 1995, Mahieux, et al. 1998), memory (Levy, et al. 2002) and executive dysfunction (Janvin, et al. 2006, Levy, et al. 2002, Mahieux, et al. 1998).

In the following subsections I will discuss the role of visual hallucinations and cognitive dysfunctions in the development of dementia in PD, which are of special relevance for the present thesis.

1.3.3.1.1 Visual hallucinations in PD

Psychosis in PD is frequently associated with death, nursing home placement, development and progression of dementia, and persistence of psychosis and it is characterized mainly by the presence of visual hallucinations (VH) (Factor, et al. 2003). The prevalence of VH in PD has been reported to be between 6% and 60% (Cummings. 1991, Diederich, et al. 2005). A retrospective clinico-pathological study reported a prevalence of 50% (Williams and Lees. 2005). Hallucinations, like other non-motor features of PD, are not well recognized in routine clinical practice. A lack of uniform definition and classification of symptoms, inclusion of different populations in the studies and different methodologies may account for disparities in prevalence between studies (Papapetropoulos and Mash. 2005).

The most widely used classification distinguishes between simple and complex VH. Minor hallucinations include presence and passage hallucinations and major or complex hallucinations usually consist in well-

formed persons, animals or objects (Fenelon, et al. 2000). The visual hallucinations most often reported in PD are complex (Barnes and David. 2001). The typical hallucination experience occurs while the person is alert and with eyes open, generally in dim surroundings. Patients describe a blurry image that appears suddenly without voluntary effort, filling an area of the visual field. The hallucination is present for a few seconds, typically moves while present, and then suddenly vanishes (Barnes and David. 2001). Even though some of the images appear real, seem to occur externally, and are not under voluntary control, most patients know that they are hallucinating. Insight into the hallucinatory nature of the phenomenon is normally maintained in all the patients without dementia and in more than half of those with dementia (Fenelon, et al. 2000).

VH generally occur during the second half of the disease's course (Williams and Lees. 2005) and have a persistent and progressive nature (Goetz, et al. 2001). Hallucinations have been consistently associated with combined levodopa/dopamine agonist therapy, suggesting that these drugs may play an important role in the pathophysiology of hallucinations (Goetz, et al. 2001). However, VH are most likely of multifactorial origin. Although higher doses of levodopa are known to be related clinically to hallucinations in individual patients, several underlying characteristics of patients with Parkinson's disease (disease severity, dementia, depression, worse visual acuity) may be more important determinants of which patients experience hallucinations (Barnes and David. 2001, Holroyd, et al. 2001, Holroyd and Wooten. 2006).

Cross-sectional studies have reported that non-demented PD patients with VH present greater neuropsychological impairment than those without VH in domains such as verbal (Grossi, et al. 2005, Ramirez-Ruiz, et al. 2006, Sinforiani, et al. 2006) and visual memory (Barnes, et al. 2003, Ramirez-Ruiz, et al. 2006), language comprehension (Ramirez-Ruiz, et al. 2006) and visuo-spatial (Sinforiani, et al. 2006) and visuo-perceptive functions (Barnes, et al. 2003, Ramirez-Ruiz, et al. 2006). Frontal dysfunction has also been described in PD with VH, including deficits in verbal fluency (Barnes, et al. 2003, Grossi, et al. 2005, Imamura, et al. 2008, Ramirez-Ruiz, et al. 2006), sustained attention (Meppelink, et al. 2008), and inhibition (Barnes and Boubert. 2008, Imamura, et al. 2008). Moreover, longitudinal studies have

shown that the presence of VH is a significant predictor of dementia in PD (Aarsland, et al. 2003, Galvin, et al. 2006, Santangelo, et al. 2007) and is associated with a more rapid general cognitive decline assessed by MMSE (Aarsland, et al. 2004, Sinforiani, et al. 2008). A longitudinal study reported that 45% of PD patients with VH developed dementia after one year's follow-up and showed a significant progressive decline in visual memory and visuo-perceptive functions (Ramirez-Ruiz, et al. 2007).

However, the brain mechanisms underlying VH in PD are not fully understood. Structural and functional abnormalities within the primary visual system and visual association areas, including ventral and dorsal pathways, have been reported in PD with VH (Diederich, et al. 2009). Our group previously reported that non-demented PD patients with VH showed areas of gray matter reduction in the parietal and temporal associative regions in comparison with non-hallucinating PD patients (Ramirez-Ruiz, et al. 2007). Functional imaging studies also reported perfusion abnormalities in occipital, temporal and parietal areas (Boecker, et al. 2007, Matsui, et al. 2006, Oishi, et al. 2005, Okada, et al. 1999). In addition to the dysfunction in posterior areas, frontal dysfunctions were also reported in fMRI studies in response to simple (Stebbins, et al. 2004) and complex (Ramirez-Ruiz, et al. 2008) visual stimuli. The involvement of the frontal lobe in VH has also been confirmed by PET studies showing a pattern of frontal hypermetabolism in patients with PD and VH (Nagano-Saito, et al. 2004). The presence of VH has also been associated with Lewy Body (LB) pathology in medial temporal areas. While VH are considered a characteristic feature of PD and DLB, they are rarely reported in other parkinsonian disorders such as Corticobasal Degeneration or Supranuclear Palsy (Williams and Lees. 2005). Neuropathological studies showed the association between VH and the presence of high densities of LBs in medial temporal areas (Harding, et al. 2002). The involvement of all these areas (visual associative, frontal and medial temporal areas) is indicative of brain abnormalities in PD patients with VH prior to dementia.

1.3.3.1.2 Early cognitive dysfunctions are predictive of dementia

Studying the cognitive deficits in early PD is clinically very relevant because these deficits affect goal directed behavior and therefore have a great impact on the quality of life of the patients (Schrag, et al. 2000) and of the carers (Aarsland, et al. 1999). In addition, early cognitive deficits are predictive of dementia (Jacobs, et al. 1995, Janvin, et al. 2005, Levy, et al. 2002, Mahieux, et al. 1998, Williams-Gray, et al. 2007) and determining these early changes by neuropsychological evaluation may facilitate early identification of patients at risk for dementia. For the moment, although we are far from being able to modify the course of dementia in PD, at least with an early identification of patients at risk for dementia we can suggest appropriate plans for treatment.

It has been reported that memory (Levy, et al. 2002), verbal fluency (Jacobs, et al. 1995, Mahieux, et al. 1998) and executive dysfunction (Janvin, et al. 2005, Mahieux, et al. 1998) are predictors of dementia in PD. In 2003 Woods et al. reported that relative to a non-demented PD group, PD patients with dementia demonstrated significantly poorer performance on digits backward (Wechsler Memory Scale-Revised), word list learning and recognition (California Verbal Learning Test), and perseverative errors on the Wisconsin Card Sorting Test (Woods and Troster. 2003). Each of these baseline neuropsychological variables exhibited adequate diagnostic classification accuracy for predicting demented and non-demented PD group membership at follow-up (Woods and Troster. 2003). In addition, in the work by Janvin et al., 2005, twenty-five (42%) new cases of dementia were diagnosed after 4 years, and time to complete the third card of the Stroop test was the only variable that was independently associated with dementia (Janvin, et al. 2005). These results suggest that subtle frontal/executive dysfunction is evident during the immediate dementia prodrome and may be of prognostic value in identifying PD patients at risk for dementia.

However, posterior-based neuropsychological deficits are proving to be even more important predictors of dementia than executive functions. In the follow-up study of the sample collected by Foltynie et al in 2004 (see *1.3.1 section*); the most important clinical predictors of global cognitive decline following correction for age were neuropsychological tasks with a more posterior cortical basis, including semantic

fluency and ability to copy an intersecting pentagons figure at the baseline assessment. This work clarifies the profile of cognitive dysfunction in early PD and demonstrates that the dementing process in the illness is heralded by cognitive deficits with a posterior cortical basis, reflecting probable non-dopaminergic cortical Lewy body pathology (Williams-Gray, et al. 2007).

1.4 Olfactory dysfunction in PD

One of the non-motor manifestations in PD which is receiving increasing attention from researchers and clinicians and which is known to start in the premotor phase of the disease is the olfactory dysfunction. Olfactory loss occurs in up to 90% of PD patients. Patients present marked deficits in odor detection (Doty, et al. 1988, Mesholam, et al. 1998, Tissingh, et al. 2001), discrimination (Mesholam, et al. 1998, Tissingh, et al. 2001) and identification (Doty, et al. 1988, Doty, et al. 1992, Hawkes, et al. 1997, Mesholam, et al. 1998, Stern, et al. 1994, Tissingh, et al. 2001), and these deficits appear early in the disease course (Doty, et al. 1992, Tissingh, et al. 2001). Olfactory dysfunction is independent of the magnitude of motor impairment, cognitive status, medication, disease duration, and disease severity (Doty, et al. 1988) and precedes classic motor symptoms in PD serving as a preclinical marker or predictor of PD (Ponsen, et al. 2004). For example, an association between impaired olfaction and the future development of PD was found in a population-based prospective study (Ross, et al. 2008). The olfactory dysfunction has also been observed in some asymptomatic relatives of patients with either familial or sporadic forms of PD (Berendse, et al. 2001, Ponsen, et al. 2004, Siderowf, et al. 2007). Other studies have also found that hyposmia is frequently present in patients with REM sleep behavior disorder (RBD), a sleep disorder that is also well established as a premotor symptom of PD (Postuma and Montplaisir. 2006). All these studies strongly suggest that olfactory loss is an early feature of PD, which develops before parkinsonian signs are detectable. Olfactory testing is easy to perform, and is therefore likely to have a role as a biomarker in future strategies aimed at detecting subjects at risk for developing PD or who are in the premotor phase of the disease (Tolosa, et al. 2009).

Olfactory information is transmitted from peripheral olfactory structures (the olfactory epithelium) to more central structures including the olfactory bulb which, through olfactory tracts and without thalamic relay, connects with primary olfactory cortex which involves the piriform cortex, amygdala and entorhinal cortex. The orbitofrontal cortex (OFC), the main neocortical target of the primary olfactory cortex, is also involved in odor identification (Price, 2004). Deficits in odor detection may be the consequence of peripheral defects in the olfactory pathway, whereas deficits in "higher order" olfactory functions, such as odor discrimination and identification, may result from the involvement of more central olfactory structures (Doty. 2009, Tissingh, et al. 2001).

Braak et al. suggested that the PD-related pathological process advances in a predictable sequence beginning in the olfactory bulb (Braak, et al. 2003), and proposed that sporadic PD could be caused by a neurotropic pathogen entering the brain via the nasal route with anterograde progression into the temporal lobe which could also explain the impairment in olfaction described in the prodomal period (Hawkes, et al. 2007). However, it was recently suggested that although the pathogens may enter the brain via the olfactory nerve, which may cause the olfactory dysfunction in PD, evidence that they initiate or cause the neurodegenerative disease is circumstantial (Doty. 2008). In addition, it has not yet been demonstrated that hyposmia unequivocally precedes neuronal loss in the substantia nigra, as would be expected on the basis of Braak staging.

So far, neuroimaging studies have not found a consistent relation between olfactory deficits and cerebral changes in PD. One SPECT study reported no correlation between dopamine uptake and olfactory measures, but found a correlation with disease severity (Lehrner, et al. 1995). However, another study found a significant correlation between dopamine transporter uptake in the striatum, mostly the putamen, and UPSIT scores in early PD patients (Siderowf, et al. 2005). Structural studies in healthy humans related the olfactory function to the depth of the olfactory sulcus (Hummel, et al. 2003) but there were no differences between PD patients and healthy controls in this measure (Kim, et al. 2007). Using voxel-wise analysis of diffusion weighted imaging increased diffusivity in the olfactory tract of early PD patients was reported and the authors suggested that this fact could explain hyposmia in PD (Scherfler, et al. 2006). A

recent study using DTI reported a correlation between diffusion indices in the cerebellum and thresholds of olfactory identification (Zhang, et al. 2009). Finally, olfactory dysfunction and related brain activity in PD has also been studied using fMRI, which found a lack of activation in right hippocampus and amygdala of PD patients (Westermann, et al. 2008). One of the main objectives of the present thesis consists trying to identify the neuroanatomical correlates of the olfactory dysfunction in early stages of the disease by means of DTI (paper IV). Therefore, this issue will be extensively discussed later on in the discussion section.

1.5 Neuroimaging studies in PD

1.5.1. T1-weighted structural MRI studies

The methods most widely used to assess structural MRI changes in PD have been volumetric region-of-interest (ROI) and voxel-based morphometry (VBM). The ROI method consists in measuring manually delineated and anatomically defined regions within the brain based on an a priori hypothesis. ROI approach takes into consideration the variability across subjects, but as it also depends on the subjective criteria of the investigator, time-consuming inter- and intra-rater validations are mandatory. VBM is a fully automated whole brain measurement technique which maps the statistical probability of differences in regional tissue volume or density between groups (Ashburner and Friston. 2000). It provides a non-biased measure of regions that may be neglected in hypothesis-based studies using ROI. However, the normalization stage within the VBM analysis, which is required to ensure that the same brain regions can be compared between subjects, transforms the shape of the brain image and may distort the abnormal tissue and artificially inflate the atrophied areas (Mechelli et al., 2005). Other structural techniques which have proved successful in detecting atrophy are surface-based and 3D-modeling analyses (Apostolova, et al. 2006a, Scher, et al. 2007) but to date there are no published studies in PD.

1.5.1.1 Structural MRI studies in PD patients with dementia

Manual ROI studies in PD patients have mainly focused on medial temporal lobe structures because these areas are known to present atrophy in other dementias such as Alzheimer's disease (AD) and dementia with Lewy bodies (DLB), and because differences in the degree of atrophy between dementias may have important implications for diagnosis (Barber, et al. 2000, Hashimoto, et al. 1998). In PD with dementia atrophy of the hippocampus (Bouchard, et al. 2007, Camicioli, et al. 2003, Junque, et al. 2005, Laakso, et al. 1996) and amygdala (Bouchard, et al. 2008, Junque, et al. 2005) have been reported; this atrophy remains statistically significant after controlling for global cerebral volume. In addition, reduced entorhinal cortex volume has been reported in PD patients with dementia compared to controls (Kenny, et al. 2008). These studies show that, although medial temporal lobe atrophy is characteristic of AD (Scheltens, et al. 1992), it may also underlie dementia in PD.

In a previous study using VBM our group observed that PD patients with dementia showed gray matter loss in hippocampus, parahippocampus and anterior cingulate gyrus as well as the basal ganglia in comparison with healthy controls (Summerfield, et al. 2005). Interestingly, the hippocampus (Apaydin, et al. 2002, Churchyard and Lees. 1997) and cingulate gyrus (Kovari, et al. 2003, Mattila, et al. 2000) are known to be major targets for Lewy body (LB) inclusions in PD. Another VBM study with a large number of patients provided further evidence of medial temporal and basal ganglia atrophy, but also reported greater widespread neocortical atrophy (Burton, et al. 2004). In that study, atrophy of the medial temporal lobe structures was more pronounced in AD than in demented PD and there were no differences between DLB and PD with dementia. However, another group reported that DLB patients showed greater neocortical atrophy than PD patients with dementia in the temporal, parietal and occipital lobes (Beyer, et al. 2007a).

The degree of atrophy in demented PD patients may vary depending on the time of the occurrence of dementia in the course of the disease (i.e., early versus late). Early development of dementia has been associated with more severe degeneration of cortical and sub-cortical structures in neuropathological

(Ballard, et al. 2006) and neuroimaging studies (Beyer and Aarsland. 2008). Overall, neuroimaging studies are in agreement with findings in post-mortem neuropathological studies in demented PD, which report morphological changes in limbic (Churchyard and Lees. 1997), paralimbic (Kovari, et al. 2003) and neocortical areas (Apaydin, et al. 2002, Mattila, et al. 2000) and have the advantage of assessing these changes *in vivo*. The fact that changes in medial temporal lobe areas are common to dementia in PD and to other neurodegenerative diseases such as AD and DLB raises the question of whether AD or cortical Lewy body type changes underlie cognitive dysfunctions in PD (Jellinger. 2006); unfortunately, the structural MRI neuroimaging techniques are still some way from resolving this matter.

1.5.1.2. Structural MRI studies in PD without dementia

The first study using a manual ROI approach to assess the hippocampus in non-demented PD patients reported decreased hippocampal volume in PD patients without dementia compared to controls (Laakso, et al. 1996). However, these results should be viewed with caution, because some patients may have met criteria for mild cognitive impairment, although it was not detected. Another study looked for differences in several structures including the hippocampus, parahippocampus, temporal lobe, frontal lobe and parieto-occipital areas, and found that only corrected hippocampal volumes differed in non-demented PD patients and controls ($p = 0.004$), even though the effect size for non-demented PD patients was only 0.66, compared with effect sizes of 1.22 and 1.81 for demented PD and AD groups respectively (Camicioli, et al. 2003). It has also been reported that non-demented PD patients presented volume reductions of 11% in the amygdala and 10% in the hippocampus compared with controls; volumes of both structures in non-demented PD patients had values between those of demented patients and controls, although the differences did not reach statistical significance (Junque, et al. 2005). In addition, significant age-associated hippocampal atrophy in PD was found in one study in which hippocampal volumes in old (> 70) non-demented patients differed from controls, but not in younger cases (Bouchard, et al. 2008).

Studies using visual evaluation of atrophy in PD, in which scores of zero represented absence and scores of four high severity (Scheltens, et al. 1992), reported that early stage non-medicated PD patients had

atrophy in the bilateral prefrontal cortex and hippocampus. The right hippocampus atrophy score was 1.15 in PD versus 0.45 in controls, and the left hippocampus score was 1.05 in PD versus 0.64 in controls (Bruck, et al. 2004). Another study from the same group in a sample of non-demented PD patients but at a more advanced stage of the disease and with impairment in several cognitive domains also reported atrophy in the hippocampus and the prefrontal cortex compared with controls (Jokinen, et al. 2009). Rated visually, more severe medial temporal atrophy has also been reported in PD subjects versus controls, but less than in subjects with DLB and AD (Tam, et al. 2005).

Results of VBM studies in PD patients without dementia are heterogeneous but most findings suggest the involvement of neocortical areas. In these patients atrophy has been reported in caudate nucleus (Brenneis, et al. 2003), frontal areas and insula (Burton, et al. 2004), prefrontal cortex and parahippocampus (Nagano-Saito, et al. 2005), hippocampus and anterior cingulate (Summerfield, et al. 2005), left intraparietal sulcus (Cordato, et al. 2006), superior temporal and frontal gyrus (Beyer, et al. 2007b, Ramirez-Ruiz, et al. 2007) and cerebellum (Camicioli, et al. 2009). A possible explanation for this high variability may be the heterogeneous characteristics of PD patients who are often considered as one uniform group, without differentiating between cognitively intact patients and those with neuropsychological deficits or with hallucinations.

1.5.1.3 Structural MRI in PD with cognitive impairment and hallucinations

As described in previous sections cognitive impairment is common even in newly diagnosed PD patients, occurring in 25-30% of cases (Muslimovic, et al. 2005), and patients with cognitive deficits have an increased risk of developing dementia (Janvin, et al. 2005, Williams-Gray, et al. 2007). One study found that PD patients with mild cognitive impairment (MCI) diagnosed according to the criteria proposed by Petersen et al. 2001 had gray matter reductions in temporal and frontal areas compared with patients without MCI (Beyer, et al. 2007b). Another imaging study that identified MCI subtypes before conversion to various kinds of dementia found that the subtype most closely associated with conversion to dementia in PD was characterized by third ventricular enlargement and similar, though less severe, atrophy of the

medial temporal lobe compared with MCI patients who converted to AD. Corrected hippocampal volumes in MCI patients converting to AD were 0.084 mm^3 (left) and 0.078 mm^3 (right) compared with values of 0.109 mm^3 (left) and 0.099 mm^3 (right) in MCI patients converting to PD (Meyer, et al. 2007)(Meyer et al., 2007). Unfortunately, MCI criteria for PD are not well defined, and modified criteria used for AD (Petersen, et al. 2001)(Petersen et al., 2001) have mainly been used for diagnosis. New criteria have recently been proposed for the diagnosis of dementia in PD (Dubois, et al. 2007), and similar efforts are being made to create standardized criteria for MCI diagnosis in this condition.

Patients with visual hallucinations (VH) present greater neuropsychological impairment than those without, and longitudinal studies have pointed out the presence of VH as a predictor of dementia in PD (Aarsland, et al. 2003, Galvin, et al. 2006, Santangelo, et al. 2007). We found that non-demented PD patients with VH had gray matter loss in occipito-parietal regions compared to patients without VH (Ramirez-Ruiz, et al. 2007). These results suggest that pathological changes occurring in PD with VH are more marked and severe than those occurring in non-hallucinating PD patients.

1.5.1.4 Neuroanatomical correlates of neuropsychological dysfunctions

Manual ROI studies also provide evidence that specific cognitive deficits are related to specific structural changes in PD. Hippocampus volumes have been reported to correlate with memory scores (Bouchard, et al. 2008, Camicioli, et al. 2003, Junque, et al. 2005) and overall cognitive performance scores (Camicioli, et al. 2003, Junque, et al. 2005), but not with frontal functions (Riekkinen, et al. 1998). In addition, amygdalar volumes have been reported to correlate with scores on these cognitive tests (Bouchard, et al. 2008, Junque, et al. 2005). The atrophy of medial temporal structures probably runs in parallel and may underlie the memory dysfunctions associated with PD.

VBM is also a useful tool for assessing the neuroanatomical correlates of neuropsychological dysfunctions in PD. Correlations between semantic fluency and gray matter of temporal, frontal and cerebellar areas have recently been reported (Pereira, et al. 2009a) suggesting that semantic fluency impairment may reflect structural gray matter changes in regions involved in language. Correlations between visuo-spatial performance and GM density have been found in the superior parietal lobules and the superior occipital gyrus of PD patients. Poor performance on visuo-perceptual tests was also found to be significantly associated with GM decreases in the fusiform, the parahippocampus, and the middle occipital gyrus (Pereira, et al. 2009b).

1.5.1.5 Longitudinal structural MRI studies in PD

The progression of regional brain atrophy with VBM in PD has only been investigated in two studies (Brenneis, et al. 2007, Ramirez-Ruiz, et al. 2005). The first study showed limbic and temporo-occipital areas of gray matter reduction after a 25-month follow-up (Ramirez-Ruiz, et al. 2005), but the other study found no areas of gray matter loss in PD patients after a follow-up period of 1.4 years (Brenneis, et al. 2007). The differences in these results may be explained by longer disease duration, increased age of patients, more prolonged follow-up and use of uncorrected p values in the study in which atrophy was documented. In agreement with the first study, an earlier report by Hu et al. found that the annual brain volume loss was greater in PD patients than in controls and that these changes correlated with cognitive decline (Hu, et al. 2001). However, other serial MRI studies using measures of global atrophy for monitoring disease progression reported no differences in atrophy rates between controls and non-demented PD patients (Burton, et al. 2005, Paviour, et al. 2006), but found significantly increased atrophy in PD patients with dementia compared to non-demented PD patients and controls (Burton, et al. 2005).

1.5.2. Diffusion studies in PD

DTI allows *in vivo* examination of the microstructural integrity of white matter (WM). DTI is sensitive to water diffusion characteristics, such as the determination of directionality and the magnitude of water diffusion. DTI information can be assessed using different parameters, such as fractional anisotropy (FA) or mean diffusivity (MD). Apart from quantification of FA and MD, recent studies have shown that directional diffusivities such as axial diffusivity (presumed to be the diffusivity along the axon) and radial diffusivity (presumed to be the diffusivity perpendicular to the axon) are more specific to underlying biological processes, such as myelin and axonal changes (Song, et al. 2002). In general, the relationship between these diffusivities, FA and MD, is such that FA increases when radial diffusivity decreases and/or axial diffusivity increases, while MD increases when axial diffusivity and/or radial diffusivity increases, and vice versa (Qiu, et al. 2008). The present thesis (for more information see paper IV) will mainly focus on the FA values which provide information on the degree of diffusion directionality of the fibers and range from 0 (isotropic diffusion) to 1 (anisotropic diffusion), being sensitive to the presence and integrity of WM fibers (Assaf and Pasternak. 2008). A decrease in FA value of white matter areas may be a sensitive indicator of histological abnormality, even if the values are derived from normal-appearing tissue by conventional MRI (Roosendaal, et al. 2009).

1.5.2.2 Diffusion-weighted imaging studies (DWI) in PD

Most MR imaging studies of the diffusion of water protons (DWI) in patients with PD to date used ROI analysis and focused on subcortical gray nuclei, mainly in search of features useful for differential diagnosis with other parkinsonisms (Kollensperger, et al. 2007, Nicoletti, et al. 2006, Paviour, et al. 2007, Schocke, et al. 2004, Seppi, et al. 2003). In most of these studies, no significant differences were found in apparent diffusion coefficient values in the subcortical GM between patients with PD and controls. Similarly, in a recent study that used voxel-based analysis of the mean apparent diffusion coefficient, no evidence of brain-tissue damage was found in patients with PD except an isolated increase in diffusivity in the olfactory tracts (Scherfler, et al. 2006).

1.5.2.3 Diffusion Tensor Imaging (DTI) studies in PD

Table 3 presents a summary of all the studies using DTI in PD. Reductions of FA values in the substantia nigra and its projections have been reported in PD (Chan, et al. 2007, Yoshikawa, et al. 2004) even in *de novo* PD patients (Vaillancourt, et al. 2009). Other areas that have shown decreased FA values in early PD compared to controls are motor areas and the cingulum (Karagulle Kendi, et al. 2008). Recent studies also reported decreased FA or increased MD, localized bilaterally in the cerebellar and orbitofrontal cortex of PD patients (Zhang, et al. 2009); and in the genu of the corpus callosum and superior longitudinal fasciculus (Gattellaro, et al. 2009). PD patients with cognitive deficits, specifically executive dysfunctions, showed reduced FA in the right frontal and left parietal white matter (Matsui, et al. 2007b); and in PD patients with dementia, reductions have been reported in bilateral posterior cingulated bundles compared to PD without dementia(Matsui, et al. 2007a).

At present, the pathological interpretation of FA reduction in the brain with neurodegenerative disease is not clear (Assaf and Pasternak. 2008). One possibility is that degeneration of the somata of neurons may secondarily produce axonal loss in the underlying WM. Indeed, the administration of MPTP in a murine model of PD demonstrated that measures derived from DTI were significantly correlated with the number of SN dopaminergic neurons lost following intoxication (Boska, et al. 2007). Another possible interpretation for FA changes is the presence of cerebrovascular disease. WM hyperintensities (WMH) have been described in PD related to dementia (Beyer, et al. 2006) but not in early PD patients (Dalaker, et al. 2009). Finally, it is also possible that FA changes reflect a demyelinating process as a consequence of overexpression of synuclein proteins which has been previously associated with demyelinating changes in PD (Galvin, et al. 1999). These and other possibilities will be addressed in depth in paper IV. Though it is not clear whether reductions in FA can be attributed to physiological factors including edema, demyelination, gliosis, and inflammation (Assaf and Pasternak. 2008), FA provides unique clues concerning the structure and geometric organization of tissues (Le Bihan, et al. 2001) and may highlight subtle anomalies in the organization of white matter tracks that are not visible with plain, anatomical MRI (Assaf and Pasternak. 2008).

Study *First author and year*	**Parkinson's disease sample** N and disease stage	**DTI acquisition and analysis**	**Summary of main findings**
Yoshikawa et al., 2004	N= 12 PD (7 early PD and 5 advanced PD); 7 PSP; 8 controls	1.5 T, 6 directions Extracting FA values from 15 manual ROIs	PD patients had decreased FA in the nigrostriatal projections regardless of the clinical stage of the disease. In advanced cases subcortical WM FA reduction was also reported.
Nilsson et al., 2007	N= 2 PD, 4 MSA and 3 PSP (pilot study)	3 T, 32 directions Fibre tracking	Qualitative comparisons between PD patients and controls did not reveal any differences in visualized tracts.
Chan et al., 2007	N= 73 PD patients and 78 controls.	1.5 T ROIs of basal ganglia and substantia nigra	In PD the FA value of the substantia nigra was lower compared with controls. Inverse correlation between FA and clinical severity.
Matsui et al., 2007a	N= 21 PD (6 with and 15 without executive dysfunction)	1.5 T, 6 directions FA values of 8 white matter ROIs.	PD patients with executive dysfunction showed reduced FA values in the right frontal and left parietal white matter.
Matsui et al., 2007b	N= 26 PD and 11 PD with dementia	1.5 T, 6 directions FA values of 8 white matter ROIs.	PD patients with dementia showed reduced FA in bilateral posterior cingulated bundles compared with PD.
Tessa et al., 2008	N= 27 PD novo (13 tremor dominant type, 11 akinetic-rigid type and 3 mixed type)	1.5 T, 6 directions Brain histograms from global MD and FA maps	In *de novo* PD there is an increase of global FA values (unexpected results)
Gattellaro et al., 2009	N= 10 PD and 10 controls	1.5 T, 12 directions ROIs in major fiber bundles.	In PD patients, FA was decreased in the genu of the corpus callosum and in the superior longitudinal fasciculus.
Zhang et al., 2009	N= 25 PD patients and 25 controls	3 T, 12 directions Voxel-based analysis of FA and MD	Decreased FA in cerebellum bilaterally and right gyrus rectus. Increased MD in bilateral OFC and inferior temporal gyri. Correlation between FA in the cerebellum and olfactory identification
Tir et al., 2009	N= 19 PD patients, 14 MSA-P and 14 controls.	1.5 T, 32 directions Voxel-based analysis of FA and MD	No differences between PD, MSA-P and controls. MSA-P reduced FA versus controls in primary motor cortex and cerebellum.
Menke et al., 2009	N= 10 PD and 10 controls	3 T, 60 directions	Slightly lower but not significant FA values in PD patients.
Vaillancourt et al., 2009	N= 14 early untreated PD and 14 controls	3 T, 27 directions Rostral, middle and caudal ROI	FA of the substantia nigra, caudal more than rostral, was reduced in PD patients.
Karagulle Kendi et al., 2009	N= 12 PD and 13 controls	3 T, 12 directions Voxel-based analysis of FA and MD	Decreased FA in PD in the frontal lobes (motor supplementary area and cingulum). Diffusion abnormalities were not associated with WM or GM changes

1.5.3 Functional studies

1.5.3.1. PET studies and cognitive dysfunctions

PET studies have mainly been focused on basal ganglia uptake by using F-dopa PET, which was the first neuroimaging technique validated for the assessment of presynaptic dopaminergic integrity. A striatal annual PD disease progression ranging from 4.0% to 6.6% of the baseline regional ^{18}F-dopa uptake has been reported (Hilker, et al. 2005a). However, not only the striatal but also the orbitofrontal and amygdalar presynaptic dopaminergics functions are altered in early PD (Ouchi, et al. 1999).

However, of particular interest to the present thesis are the studies using PET to assess cortical activity in PD patients with cognitive dysfunctions. Using18F-fluorodeoxyglycose PET, decreased prefrontal (BA9) and parietal (BA39) uptake has been reported in PD cases with mild cognitive deficits in comparison with patients without cognitive deficits (Huang, et al. 2007, Huang, et al. 2008). In PD patients with MCI, there are extensive areas of hypometabolism in the posterior cortical regions, including the temporo-parieto-occipital junction, medial parietal, and inferior temporal cortices suggesting that posterior cortical dysfunction is the primary neuroimaging feature of PD patients at risk for dementia (Hosokai, et al. 2009). Posterior blood flow reductions are also reported in non-demented hallucinating patients (Matsui, et al. 2006, Oishi, et al. 2005, Oishi, et al. 2005, Oishi, et al. 2005, Okada, et al. 1999). In PD with dementia extensive anterior and posterior reductions have been reported (Wallin, et al. 2007). In addition, loss of cholinergic neurons in the basal nucleus of Meynert has been observed in postmortem PD brains, and has been thought to contribute to cognitive decline in PD (Whitehouse, et al. 1983). Brain cholinergic function can be estimated by measuring acetylcholinesterase (AChE) activity in the brain with PET. A significant reduction in cortical AChE activity in PD (Hilker, et al. 2005b, Shimada, et al. 2009) and a more sizeable decrease in cortical AChE activity in PD with dementia (Hilker, et al. 2005b) and DLB than in AD has been reported (Shimada, et al. 2009). PET findings are important for understanding the correspondence between atrophic areas assessed by structural MRI or activations and deactivations

found in PD patients using fMRI. In the future, the combination of MRI, fMRI and PET studies using different tracers (FDG, MPPF, and FDDNP) may prove essential for a better understanding of Parkinson's disease.

1.5.3.2. Functional magnetic resonance (fMRI) studies

fMRI has several advantages over PET for brain mapping, including much greater temporal resolution and slightly greater spatial resolution. In addition, fMRI allows cerebral activity to be evaluated without the injection of radioactive tracers. fMRI is based on the blood-oxygen level dependent (BOLD) signal. Briefly, the BOLD signal is the result of the following process. Deoxyhemoglobin is paramagnetic, and therefore acts as an endogenous contrast agent that causes changes in the T2 signal. When local cerebral blood flow (CBF) increases after an increase in local neuronal firing, there is a mismatch between the increase in CBF and the increase in oxygen consumption. Paradoxically, the CBF increase exceeds the increase in oxygen consumption, which results in a reduction in deoxyhemoglobin concentration, and hence, in the BOLD signal. Therefore, by using pulse sequences sensitive to the BOLD signal, one can map changes in neuronal activity. The available evidence suggests that the local BOLD signal is a function of the synaptic inputs into a brain area and of local neuron processing, including the activity of excitatory and inhibitory interneurons ((Logothetis. 2002, Logothetis and Pfeuffer. 2004).

fMRI studies allow analysis of the activity associated with a particular task (motor, sensorial or cognitive functions) and/or resting state brain. The results of the first study using fMRI to analyze motor function in PD (Sabatini, et al. 2000) are in agreement with the findings using PET/SPECT. PD patients show areas of deactivation in rostral motor supplementary area (MSA) but areas of increased activation in premotor and parietal cortex and cerebellum in comparison with healthy controls while performing a motor task. The greater resolution of fMRI makes it possible to differentiate between caudal and rostral MSA (Sabatini, et al. 2000). When administering levodopa, the medial frontal circuitry is recovered and there is no need to recruit frontal lateral and parietal cortices; consequently the activation in these "compensating" areas disappears (Haslinger, et al. 2001).

Olfactory dysfunction and related brain activity in PD have also been studied using fMRI (Takeda, et al. 2009, Westermann, et al. 2008). Lack of activation was reported in the right hippocampus and amygdala (Westermann, et al. 2008) and precentral gyrus (BA6/6) and middle temporal gyrus (BA19/39) (Takeda, et al. 2009) in PD patients while perceiving an odorant stimulus.

Finally, cognitive related fMRI studies in PD have assessed brain activity related to working memory (Lewis, et al. 2003, Mattay, et al. 2002), reward processing (Schott, et al. 2007), perceptual processing of fearful stimuli (Tessitore, et al. 2002) and implicit memory (Moody, et al. 2004). Default network deactivation failure in non-medicated PD while performing an executive task has also been reported (van Eimeren, et al. 2009).

2. APPROACH AND GENERAL OBJECTIVES

2. OBJECTIVES AND HYPOTHESIS

2.1. Background

According neuropathological research limbic changes occur in Parkinson's disease with and without dementia (Aarsland, et al. 2005b, Apaydin, et al. 2002, Braak, et al. 2003, Braak, et al. 2004, Braak, et al. 2005, Churchyard and Lees. 1997, Del Tredici, et al. 2002, Sandmann-Keil, et al. 1999, Sandmann-Keil and Braak. 2005) and precede neocortical impairment (Braak, et al. 2003, Braak, et al. 2004, Del Tredici, et al. 2002).

Structural MRI studies using both manual ROI and VBM techniques reported atrophy of limbic structures including amygadala and hippocampus, in both demented and non-demented PD patients (Bouchard, et al. 2008, Burton, et al. 2004, Camicioli, et al. 2003, Junque, et al. 2005, Laakso, et al. 1996, Summerfield, et al. 2005); and a progression of limbic atrophy over time (Ramirez-Ruiz, et al. 2005). In addition, atrophy of paralimbic areas (i.e. paracingulate gyrus) (Summerfield, et al. 2005) and limbic association cortex (i.e orbitofrontal cortex) has also been reported in PD (Burton, et al. 2004, Feldmann, et al. 2008, Nagano-Saito, et al. 2005).

Functional studies have also reported limbic dysfunctions in Parkinson's disease. PET studies reported that orbitofrontal and amygdalar presynaptic dopaminergic functions are altered in early PD (Ouchi, et al. 1999) and a significant decrease in the metabolism of the medial OFC in a follow-up study of patients with early PD (Huang, et al. 2007). fMRI studies showed an abnormal amygdalar response in PD while recognizing fearful faces (Tessitore, et al. 2002) and lack of activation in the right hippocampus and amygdala while perceiving odorant stimuli inside the scanner (Westermann, et al. 2008).

Neuropsychological studies pointed out that the functions that are known to depend on the integrity of limbic system are impaired in Parkinson's disease even in the early stages of the disease. Specifically, it has been reported and impairment in verbal memory (Aarsland, et al. 2009, Elgh, et al. 2009, Foltynie, et al. 2004, Muslimovic, et al. 2005, Troster. 2008), recognition of facial expressions of

emotions (Ariatti, et al. 2008, Assogna, et al. 2008, Clark, et al. 2008, Kan, et al. 2002, Sprengelmeyer, et al. 2003, Yip, et al. 2003), decision-making (Kobayakawa, et al. 2008, Mimura, et al. 2006, Pagonabarraga, et al. 2007, Perretta, et al. 2005) and olfactory function (Doty, et al. 1988, Doty, et al. 1992, Hawkes, et al. 1997, Mesholam, et al. 1998, Stern, et al. 1994, Tissingh, et al. 2001). However, the neuroanatomical and neurofunctional correlates of these dysfunctions are not known or are poorly investigated. For memory functions, hippocampal and amygdalar volumes have been reported to correlate with memory impairment (Bouchard, et al. 2008, Camicioli, et al. 2003, Junque, et al. 2005). For decision making and recognition of emotions there are no studies of its correlates; and for olfactory dysfunctions one study reported a correlations between this impairment and a reduced fractional anisotropy in the cerebellum (Zhang, et al. 2009).

2.2. General objective

The general aim of this thesis is to investigate the neuroanatomical and neurofunctional correlates of declarative memory, decision-making, recognition of emotions and olfactory dysfunctions in PD. We hypothesized that all these dysfunctions are due to the limbic degenerative changes associated with PD. We used structural MRI (T1-weighted MRI and DTI), functional MRI (fMRI) and neuropsychological testing to assess declarative memory, emotional processing and decision-making, and olfactory function.

Firstly, we focused on the hippocampal atrophy putatively related to declarative memory dysfunctions and PD, and a possible neuroradiological marker for the evolution to dementia. This issue was investigated in papers I and II. Secondly, we aimed to investigate structural correlates of deficits in the recognition of emotions, decision-making and olfactory dysfunction reported early in the disease course by means of VBM and DTI (paper III and IV). Finally in paper V the functional correlates of recognition memory were assessed using fMRI.

2.3 Specific objectives and hypotheses

Paper I and III

Background

There is controversy whether memory deficits in PD depend on the integrity of fronto-striatal circuitry or the hippocampus. Memory impairment in PD is very relevant because it is seen even in the early stages of the disease (Muslimovic et al., 2005), it progresses over time (Muslimovic et al., 2007), it is more pronounced in patients with visual hallucinations (Grossi et al., 2005; Ramirez-Ruiz et al., 2006), and it predicts evolution to dementia. Patients with dementia present higher densities of LBs and LNs in the CA 2-3 region of the hippocampus, and the degree of cognitive impairment correlates with the density of LNs in the CA2 hippocampus field (Churchyard et al., 1997). In addition, visual hallucinations are clinical predictors of dementia in Parkinson's disease and are also associated with medial temporal lobe changes (Williams and Lees. 2005).

Objectives

Paper I was a cross-sectional study with the following specific objectives:

1. To examine the differences in hippocampal atrophy in PD patients with dementia and in non-demented patients with and without visual hallucinations in comparison with healthy controls by means of VBM technique.
2. To investigate the reductions of individual cases to determine the frequency of hippocampal atrophy.
3. To describe the patterns of hippocampal atrophy.
4. To assess the neuroanatomical correlates of verbal memory dysfunctions in PD.

Paper II was a longitudinal study of the previous sample with the following specific objectives:

1. To study the course of memory deficits and evolution to dementia.
2. To establish the progression of hippocampal atrophy and other cerebral structures.

Hypotheses

In the first study we expected that:

1. Since PD patients with VH are at a risk of developing dementia they may present hippocampal atrophy similar to demented patients.

2. The frequency of hippocampal atrophy would be higher in PD patients with VH compared to PD patients without VH.

3. The atrophy of the hippocampus would affect the hippocampal CA2 field.

4. Memory dysfunctions in PD depend on degeneration of the limbic system. Hippocampal atrophy would be related to a poorer performance in memory.

In the longitudinal study we expected that:

5. PD patients with VH would develop dementia and marked memory deterioration.

6. The brain atrophy in PD patients with VH would progressively affect the hippocampus showing a pattern of atrophy similar to demented patients.

7. In addition to the hippocampus, other brain regions including limbic, paralimbic and neocortical areas, which are reported to be involved in dementia in PD, would show progressive atrophy.

Paper III and IV

Background

Limbic dysfunction occurs early in the disease course. Previous studies have reported decision-making (Kobayakawa, et al. 2008, Perretta, et al. 2005), facial recognition of emotions (Assogna, et al. 2008, Sprengelmeyer, et al. 2003) and odor identification deficits (Doty, et al. 1992, Tissingh, et al. 2001) in early PD patients. Lesion and functional studies have identified the amygdala and OFC as key structures for decision-making and recognition of emotions functions. Olfactory information is transmitted from peripheral olfactory structures to more central structures including the olfactory bulb which, through the olfactory tracts, connects with the primary olfactory cortex comprising the piriform cortex, the anterior cortical nucleus of the amygdala, and the rostral entorhinal cortex. The OFC represents the main target of the primary olfactory cortex and is also involved in odor identification. Neuronal loss and LBs are known to occur in bulb and central olfactory areas (Braak et al., 2003; Silveira-Moriyama et al., 2008).

Objectives

1. In paper III we aimed to study the neuroanatomical correlates of dysfunctions in facial emotion recognition and decision-making in early PD by means of VBM.
2. In paper IV we aimed to investigate the neuroanatomical correlates of olfactory dysfunction by means of DTI.

Hypotheses

In paper III we expected that:

1. Patients in the early stages of the disease would show atrophy in limbic and paralimbic areas, and dysfunction in decision-making and recognition of emotions.

2. There would be a correlation between gray matter loss in OFC and amygdala and deficits in recognition of emotions and decision-making.

In paper IV we expected that:

3. Patients in the early stages of the disease would show abnormal structural connectivity between limbic and neocortical areas, and dysfunction in odor identification.

4. Olfactory dysfunction in early PD would be associated with reduced fractional anisotropy of the connections between the primary (medial temporal areas) and secondary olfactory cortex (orbitofrontal cortex).

Paper V

Background

Cognitive functions are processed by means of highly distributed, interacting cortical networks. Functional connectivity is determined by the coordinated functional activation of different brain regions. In early stages of PD, cortical metabolic changes are known to occur.

Objectives

In the current experiment we aimed to:

1. Investigate alterations in the pattern of functional brain connectivity for episodic memory retrieval by means of Independent Component Analysis in early PD.

Hypotheses

1. There will be synchronous activation and deactivation of different brain areas to adequately perform the task.

2. PD patients will show overactivation of preserved brain areas as a way of compensating for the dysfunction of malfunctioning areas in comparison with healthy controls.

3. METHODS

3. Methods

The present thesis consists of five studies examining the neuroanatomical basis of non-motor dysfunctions in patients with Parkinson's disease. We studied different samples and used different neuropsychological and MRI acquisition procedures and neuroimaging analyses. All the studies were approved by the institutional ethics committee, and written informed consent was obtained from the patients (or their caregiver in the case of patients with overt cognitive impairment) and from healthy controls after full explanation of the procedures involved in the study. A detailed description of the methodological approaches, sample characteristics, clinical, neuropsychological and/or behavioural tests and MRI methods can be found in each study. The main methodological aspects are summarized here:

3.1. Study samples

The PD patients involved in the papers I and II were from a previously studied initial sample forming part of a larger project carried out at the Neuropsychology Group (Department of Psychiatry and Clinical Psychobiology, Faculty of Medicine) at the University of Barcelona, Spain (*http://www.ub.edu/neuropsychology/html/portada.html*) in collaboration with the Parkinson Disease and Movement Disorders Unit at the Hospital Clinic. Indeed, this sample has been the basis for several publications in scientific journals (Ramirez-Ruiz et al. 2006a, b, Ramirez-Ruiz et al., 2007). In the first study, one hundred subjects participated divided into four groups: nine PD patients with dementia, 16 non-demented patients with visual hallucinations (VH), 19 non-demented PD patients without VH and 56 healthy controls (for more details see paper I). The second study (paper II) consisted in a follow-up of the previous sample. Twelve PD patients with VH, 14 PD patients without VH and 12 controls participated in the follow-up assessment which was carried out after 29.91 months (S.D= 5.74; range= 26-32).

For the third, fourth and fifth studies, twenty-four early PD patients and 24 healthy controls matched for age, gender and years of education were recruited from the Parkinson's Disease Movement Disorders Unit, Neurology Service, Hospital Clinic, Barcelona. All patients fulfilled the UK PD

Society Brain Bank (PDSBB) diagnostic criteria for PD (Daniel and Lees. 1993). Other inclusion criteria for patients were: i) age 40-65 years; ii) Hoehn and Yahr stage ≤ II; iii) disease duration ≤5 years; and iv) absence of motor fluctuations. Exclusion criteria for all subjects were: i) the presence of dementia, ii) the presence of other neurological or psychiatric disorders such as depression, iii) the presence of visual hallucinations assessed by the Neuropsychiatric Inventory Questionnaire (NPI-Q).

Table 4. Brief summary of subjects included and techniques used in each of the papers.

Paper number and type	Characteristics of the study samples	Neuroimaging analysis
Paper I Prospective, cross-sectional	9 PD patients with dementia 16 PD patients with VH 19 PD patients without VH 56 healthy controls N = 100	VBM with SPM2 (hippocampal ROI) MR 1.5 T
Paper II Prospective, longitudinal	12 PD patients with VH 14 PD patients without VH 12 healthy controls N = 38	VBM with SPM5 MR 1.5 T
Paper III Prospective, cross-sectional	24 early PD patients 24 healthy controls matched for age, gender and years of education. N =48	VBM with FSL (OFC and amygdala ROI) MR 3 T
Paper IV prospective, cross-sectional	24 early PD patients divided into hyposmic and anosmic groups. 24 healthy controls matched for age, gender and years of education. N =48	TBSS with FSL MR 3 T
Paper V Prospective, cross-sectional	24 early PD patients 24 healthy controls matched for age, gender and years of education. N =48	MELODIC with FSL MR 3 T

Abbreviations: MR: magnetic resonance; VH: visual hallucinations

3.2. Clinical and neuropsychological assessment

3.2.1 Clinical assessment

In all the studies an extensive clinical and neurological assessment was carried out to determine the characteristics of Parkinson's disease. We recorded the following variables:

- *Demographical variables:* Age, gender, years of education and handedness.
- *Illness severity:* Hoehn and Yahr staging and motor subscale from the Unified Parkinson's Disease Rating Scale (UPDRS) were used. Duration of illness and predominance of the disease were also recorded.
- *Mood assessment:* We used the scores of Hamilton Depression Rating Scale (HDRS) (paper I and II) and Beck Depression Inventory (BDI-II) (paper III, IV and V).
- *Hallucinations:* To evaluate the phenomenology of hallucinations we used a structured interview designed at our hospital which comprised items covering modality (visual, auditory, tactile and olfactory), content (animals, people, objects) and temporal aspects (time of the day, frequency and duration). The severity of the VH was rated using the Spanish version of the neuropsychiatric inventory (NPI) (subscale hallucinations).
- *Dementia:* The diagnosis of dementia was initially based on an interview with the patient and the caregiver using the Diagnostic and Statistical Manual of Mental Disorders, Revised, Fourth Edition (DSM IV-TR) along with the administration of Folstein's Mini-Mental State Examination (MMSE score =< 23). After the publication of the diagnostic criteria for Parkinson Disease Dementia by the Movement Disorder Society in 2007 we followed the proposed algorithm (level I) which required: i) a diagnosis of PD, ii) the development of PD prior to the onset of dementia, iii) MMSE below 26, iv) presence of cognitive deficits severe enough to impact daily living, v) impairment in more than one cognitive domain including attention, executive function, visuo-constructive ability and memory.

3.2.2 Neuropsychological assessment

The following neuropsychological tests were administered in the present thesis:

- The Vocabulary, Information and Similarities Subtest from the Wechsler Adult Intelligence Scale-Third Edition (WAIS-III) for assessment of premorbid IQ and general cognitive ability.
- Rey's Auditory Verbal Learning Test (RAVLT) for verbal memory: learning, delayed recall and recognition.
- Warrington Recognition Memory for Faces for visual memory.
- Visual Form Discrimination Test and Benton Facial Recognition Test for assessment of visuoperceptive functions.
- Phonetic (words starting by "p" in 1 minute) and semantic fluencies (animals in 1 minute).
- Token Test for language comprehension.
- Boston Naming Test for visuo-verbal language skills.
- Iowa Gambling task for decision-making.
- Ekman 60 Faces test to assess recognition of facial expressions of emotions.
- Conners' Continuous Performance Test II (CPT II), a test to measure sustained attention.
- University of Pennsylvania Smell Identification Test (UPSIT) for odor identification.

3.3. MRI acquisition

Image acquisitions for all the studies were performed in the Centre de Diagnòstic per la Imatge (CDIC), Neuroradiology Section, Radiology Service, at the Hospital Clínic (Barcelona, Spain) in accordance with the specific study protocol.

3.3.1 T1-weighted structural images

In papers I and II, all scans were obtained from a 1.5 T GE Nvi/Cvi 8.4 machine (GE, Milwaukee, WI, USA). The imaging protocol included an axial 3D IR Prep SPGR (Inversion Recovery Prepared Spoiled Gradient-echo) sequence of the entire brain and the following parameters: TR (Repetition

Time) = 17; TE (Echo Time) = 5; TI (Inversion Time) = 300; 1.5 mm thickness; FoV (Field of View) = 24x24; 256x256; 1 NEX (Number of Excitations).

In papers III, IV and V images were acquired using a TIM TRIO 3T scanner (Siemens, Germany). A set of high-resolution 3-dimensional T1-weighted images was acquired with a MPRAGE sequence in sagittal orientation (TR/TE= 2300/2.98 ms; TI= 900ms; 256×256 matrix, 1mm isotropic voxel).

3.3.2 Diffusion Tensor Imaging (DTI)

For paper IV, DTI was obtained in a sagittal orientation in an anterior-posterior phase direction using a single-shot EPI sequence (TR= 5533 and TE= 88) with diffusion encoding in 30 directions (b values 0 and 1000 s/mm^2). The reconstructed voxel size was 1x1x1 mm^3 and 44 slices were acquired. The acquisition time for the DTI scan was 3 min and 10 s. Immediately after the DTI, a T2-weighted scan was acquired in the same geometry.

3.3.3 fMRI

Data acquisition was also performed on the TIM TRIO 3T scanner (Siemens, Germany), using multi-slice gradient-echo EPI sequence [repetition time (TR): 2000 ms; echo time (TE): 30 ms; 36 x 3 mm axial slices] providing whole brain coverage. We used a 20-block design task with alternating *activation* and *contrast* conditions (10 blocks each). The whole experiment had a duration of 400 s (20s per block) obtaining a total of 200 volumes

Participants first viewed 35 items (duration 2 sec; intertribal interval (ITI), 1 sec) outside the scanner with instructions to study the items for a later test. The stimuli were words in capital letters from the Lexesp-Corco database (Sebastián Gallés et al. 2000) presented with VisuaStim Digital MRI Compatible Hig Resolution Stereo 3D glasses (Resonance Technology, Inc.) and Presentation ® version 10.1 (Neurobehavioral Systems) running in Windows XP. Once the subject was placed in the scan, the task started with an activation block which consisted of the presentation of seven words, of which three had been previously memorized outside the scan and four were not. Following this block,

participants were presented seven letters of which three were the letter “AAAAA” and the other four were random concatenation of letters. Using their right hands, participants pressed a button on a response box to indicate that the item was studied (target) or did not press it to indicate that it was not previously studied (foil). In total, 70 items (35 studied targets and 35 non-studied foils) were presented (for more details see paper V).

3.4. Neuroimaging techniques

3.4.1 Voxel-based morphometry

VBM is an automatic technique which briefly consists in the following: The MRI scans are segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) by a computer algorithm, transformed into stereotaxic space, smoothed, and submitted to standard statistical methods (i.e., two-sample t-test comparisons, paired t-test, ANOVA and multiple regression) to evaluate changes in concentration and/or volume of GM and/or WM tissues.

In the first paper, VBM group comparisons and individual analyses of unmodulated GM images (concentration or density) were carried out following a standard protocol described by Mechelli et al. 2005 using the SPM2 software (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, University College London, UK, http://www.fil.ion.ucl.ac.uk/spm) running in Matlab 6.5 (MathWorks, Natick, MA). In the second paper, group comparisons of modulated GM images (volume) were obtained using SPM5 software running in Matlab 7.0. Gray matter volumes were analyzed in a factorial design (3x2) ANCOVA. Our main interest was the contrast between base-line versus follow-up for each of the groups, specifically for the progression of volume loss in PD with VH patients over time. All results were thresholded at a cluster and voxel level of $p < 0.05$ corrected for multiple comparisons by False Discovery Rate (FDR).

In the third study, T1-weighted structural data were analyzed with FSL-VBM (FMRIB's Software Library, http://www.fmrib.ox.ac.uk/fsl/). Differences in gray matter volume between patients and

controls in the selected ROIs and correlations with neuropsychological scores were analyzed in a voxelwise fashion using FSL's randomise (which combines General Linear Model testing with permutation inference statistics). A corrected cluster size significance level of $p > 0.05$ was used to correct for multiple comparisons controlling for family-wise error (FWE).

3.4.2 TBSS

Voxelwise statistical analysis of the FA data was carried out using the TBSS (Tract-Based Spatial Statistics) part of FSL. First, FA images were created by fitting a tensor model to the raw diffusion data using FDT, and then brain-extracted using BET. All subjects' FA data were then aligned into a common space using the nonlinear registration IRTK. Next, the mean FA image was created and thinned to generate a mean FA skeleton which represents the centre of all tracts common to the group. Each subject's aligned FA data was then projected onto this skeleton and the resulting data fed into voxelwise cross-subject statistics. TBSS method improves the analysis by means of 1) carefully tuned non-linear registration, 2) projection onto an alignment–invariant tract representation (the `mean FA skeleton´)

3.4.3 MELODIC

The analysis was carried out using Tensorial Independent Component Analysis as implemented in MELODIC (Multivariate Exploratory Linear Decomposition into Independent Components) Version 3.05, part of FSL (FMRIB's Software Library, http://www.fmrib.ox.ac.uk/fsl/). This results in a three-way decomposition that represents the different signals and artefacts presented in the data in terms of their temporal, spatial, and subject-dependent variations. This approach is able to extract plausible activation maps, time courses, and session/subjects modes and provides a rich description of additional processes of interest such as image artefacts or secondary activation patterns. The resulting data decomposition gives simple and useful representations of multisubject FMRI data that can aid interpretation of group FMRI studies beyond what can be achieved using model-based analysis techniques.

4. Results

PAPER I

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ORIGINAL COMMUNICATION

Naroa Ibarretxe-Bilbao
Blanca Ramírez-Ruiz
Eduardo Tolosa
Mª Jose Martí
Francesc Valldeoriola
Nuria Bargalló
Carme Junqué

Hippocampal head atrophy predominance in Parkinson's disease with hallucinations and with dementia

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N. Ibarretxe-Bilbao, MSc · B. Ramírez-Ruiz, PhD · C. Junqué, PhD (✉)
Dept. of Psychiatry and Clinical Psychobiology
University of Barcelona
IDIBAPS
Casanova 143
08036 Barcelona, Spain
Tel.: +34-93/402-4570
Fax: +34-93/403-5294
E-Mail: cjunque@ub.edu

N. Ibarretxe-Bilbao, MSc · B. Ramírez-Ruiz, PhD · E. Tolosa, MD · M. J. Martí, MD · F. Valldeoriola · N. Bargalló, MD · C. Junqué, PhD
Institute of Biomedical Research August Pi i Sunyer (IDIBAPS)
Barcelona, Spain

E. Tolosa, MD · M. J. Martí, MD · F. Valldeoriola
Parkinson's disease and Movement Disorders Unit
Neurology Service
Institut Clínic de Malalties del Sistema Nerviós
Hospital Clínic de Barcelona, Spain

N. Bargalló, MD
Centre de Diagnòstic per la Imatge
Hospital Clinic de Barcelona (CDIC), Spain

Abstract We studied regional gray matter density in the hippocampus in Parkinson's disease (PD) patients. We obtained magnetic resonance scans in 44 PD patients (PD patients with dementia (PDD) = 9, non-demented PD patients with visual hallucinations (PD+VH) = 16, and PD patients without dementia and without visual hallucinations (PD-VH) = 19) and 56 controls matched for age and years of education. A region of interest (ROI) of the hippocampus following voxel-based morphometry (VBM) procedures was used to perform group comparisons, single-case individual analysis and correlations with learning scores. Group comparisons showed that PDD patients and PD+VH patients had significant hippocampal gray matter loss compared to controls. In PDD patients, hippocampal gray matter loss involved the entire hippocampus and in PD+VH this reduction was mainly confined to the hippocampal head. 78 % of PDD patients, 31 % of PD+VH patients and 26 % of PD-VH patients had hippocampal head gray matter loss when compared to controls. These results suggest that in PD the neurodegenerative process in the hippocampus starts in the head of this structure and later spreads to the tail and that, in addition, memory impairment assessed by Rey's Auditory Verbal Learning Test (RAVLT) correlates with hippocampal head gray matter loss.

Key words Parkinson's disease · dementia · hallucinations · MRI · hippocampus

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder frequently associated with the development of dementia [20]. Brain pathology in PD is characterized by cell loss and synuclein deposition in the form of Lewy bodies (LB) and neurites (LN) in numerous brain regions. Dementia is thought to occur when synuclein pathology extends to the cortex, but Alzheimer-type pathology probably contributes to the cognitive deterioration in many instances [8]. The hippocampal changes occur in patients with PD and are present in relatively early stages of the disease [7] and seen in both demented and non-demented patients, but in different degrees [10]. Patients with dementia have higher densities of LBs

and LNs in the CA-2-3 region of the hippocampus than patients without dementia. Moreover the degree of cognitive impairment correlates with the density of LNs in the CA2 hippocampus field [10].

Magnetic resonance imaging (MRI) studies in PD using the manual volumetric approach [9, 22] and VBM technique [5, 30, 34] have reported reduction of the hippocampus in demented as well as in non-demented PD patients. In contrast with other neurodegenerative diseases such as Alzheimer's disease, the regional predominance of hippocampal atrophy has been poorly investigated. Recently, Bouchard et al. [6] found that the hippocampal head in PD differed from controls in aged patients and that demented and non-demented PD also differed in the hippocampal head but not in the body or tail. Localizing in vivo the structural changes in the hippocampal formation in patients with PD might reflect the pathological basis of memory deficits. In this sense, it has been reported that verbal recall was associated with volume loss in the hippocampus head [6].

On the other hand, MRI hippocampal atrophy could be a marker to predict the development of dementia. Several studies have pointed the presence of visual hallucinations as a clinical predictor of dementia [1, 17]. In the study reported here, we considered a PD+VH group as patients at risk of dementia and hypothesized that this subgroup of patients could present hippocampal atrophy similar to demented patients.

We used the VBM technique and applied a ROI (region-of-interest) of the hippocampus in order to look for specific changes in this structure. In addition to group comparisons, we investigated the reductions of individual cases to determine the frequency of hippocampal atrophy and their regional predominance in patients with PD and dementia and in non-demented patients with and without visual hallucinations.

Methods

Subjects

One hundred subjects between 55 and 84 years of age participated in the study. This study is a part of a larger project investigating risk factors for dementia in Parkinson's disease carried out at the Parkinson's Disease Movement Disorders Unit, Neurology Service, Hospital Clinic in collaboration with the Department of Psychiatry and Psychobiology, University of Barcelona. In this investigation we studied four groups of individuals: 9 PDD, 16 PD+VH, 19 PD-VH, and 56 healthy controls matched to patients by age and years of education, without known current or passed major psychiatric or neurological disorders. All patients fulfilled the UK PD Society Brain Bank (PDSBB) criteria for PD [11] and the diagnosis of dementia was made by the neurologist based on an interview with the patient and the caregiver using the Diagnostic and Statistical Manual of Mental Disorders, Revised Fourth Edition (DSM IV-TR) [2] as a guide, along with the administration of Folstein's Mini-Mental State Examination (MMSE) [14]. Subjects needed a MMSE score of 23 or lower and DSM-IV-TR items to fulfill dementia criteria. Neurological assessment included the Unified Parkinson's Disease Rating Scale, motor subscale (UPDRS, part III) [13] and Hoehn and Yahr Rating Scale [21]. Visual hallucinations (VH) in our PD sample consisted of well-formed images of people, faces or animals and they were assessed by a structured interview developed in our hospital that comprised items covering the type (visual, auditory, tactile and olfactory) and temporal aspects of the hallucinations (time of the day, frequency and duration). Depression was evaluated by means of the Hamilton's Depression Scale [19]. Part of the present sample participated in other previous studies [29–32].

The study was approved by the institutional ethics committee. Written informed consent was obtained from the patients or their caregiver in patients with overt cognitive impairment after having fully explained the procedures involved in the study.

Neuropsychological assessment

Neuropsychological assessment consisted of MMSE to evaluate global cognitive dysfunction and Rey's Auditory Verbal Learning Test (RAVLT) [25]. The RAVLT consists of 15 words read aloud for five consecutive trials, each trial being followed by a free recall test. Learning is measured by the total number of words recalled over these five acquisition trials. After a 20 minute delay period, each subject is again required to recall the words in the list. Recognition is assessed by the proportion of words correctly recognized from a list that contains words from the original list and distracters. Learning, forgetting and recognition were selected as memory variables to be correlated with hippocampal atrophy.

Statistical analysis

Analyses were carried out by the Statistical Package for Social Sciences V12.0 [SPSS Inc, Chicago (IL), USA]. For normally distributed variables with homogeneity of variance, we performed one-way analysis of variance and post-hoc Tukey tests. For those variables that did not meet the homogeneity of variances requirement, we used a non-parametric Kruskal-Wallis test, which provides an χ^2 statistic and we also performed a post-hoc Mann-Whitney U test. Differences between groups were considered to be statistically significant at $p < 0.05$.

MRI acquisition and analysis

All scans were obtained from a 1.5 T GE Nvi/Cvi 8.4 machine (GE, Milwaukee, WI, USA). The imaging protocol included an axial 3D IR Prep SPGR (Inversion Recovery Prepared Spoiled Gradient-echo) sequence of the entire brain and the following parameters: TR (Repetition Time) = 17; TE (Echo Time) = 5; TI (Inversion Time) = 300; 1.5 mm thickness; FoV (Field of View) = 24 × 24; 256 × 256; 1 NEX (Number of Excitations).

VBM group analysis was carried out on SMP2 (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, University College London, London, UK, http://www.fil.ion.ucl.ac.uk.spm) running in Matlab (Mathworks, Natick, MA, USA). We followed a standard VBM procedure [27]. Briefly, after having reoriented all the data, they were normalized to a standardized template [4]. The spatially normalized images were then segmented into gray matter, white matter and cerebrospinal fluid. We took segmented gray matter images and smoothed them with an isotropic Gaussian kernel of 6 mm owing to the small size of the region of interest.

The hippocampus can be divided into three segments: (1) an anterior part, or head; (2) an intermediate part or body; and (3) a posterior part or tail [12]. For head, body and tail differentiation of the hippocampus we used visual inspection from VBM images. According to Hackert et al. [17], the anterior 35 % of coronal slices included the head with the hippocampal digitations, while the intermediate 45 %

represented the body of the hippocampus, and the remaining 20 % the tail [22]. Taking into account both, boundaries described by Duvernoy's hippocampus atlas and percentages in Hackert, in our MRI study we considered that slices ranging from –40 to –34 in the coronal plane correspond to the tail, from –32 to –18 to the body, and from –16 to –4 to the head (see Fig. 1). The threshold was set for the creation of the figures was 0.05 corrected by False Discovery Rate (FDR).

The statistical significance of the hippocampal gray matter density was analyzed using two sample t-test group comparison. We performed comparisons for all the groups. Differences in gray matter density were assessed by applying FDR with a threshold of corrected $p < 0.05$. Both left and right ROIs (region of interest) of the hippocampus were assessed using WFU-Pickatlas toolbox software for SPM version [26]. Only clusters larger than ten contiguous voxels were considered in the analysis. For the cluster level we also took a threshold of $p < 0.05$ corrected.

For the VBM individual analysis, each PD patient's hippocampus was compared with the mean of the entire control group, searching for individual hippocampal gray matter density differences. We followed the procedures described in Salmond et al. [33]. Finally, to investigate the correlation between gray matter loss in hippocampus and memory, we performed SPM2 correlation analysis for each group. A corrected threshold of $p < 0.05$ was used for every analysis.

Results

Demographical and clinical results

Gender and disease evolution did not show significant differences among groups. Differences in UPDRS III score and Hoehn and Yahr stages achieved statistical significance only between PDD and PD-VH. Depression scores were also significantly higher in the PD+VH group compared to those in PD-VH patients. Demographic and clinical characteristics of the sample are shown in Table 1.

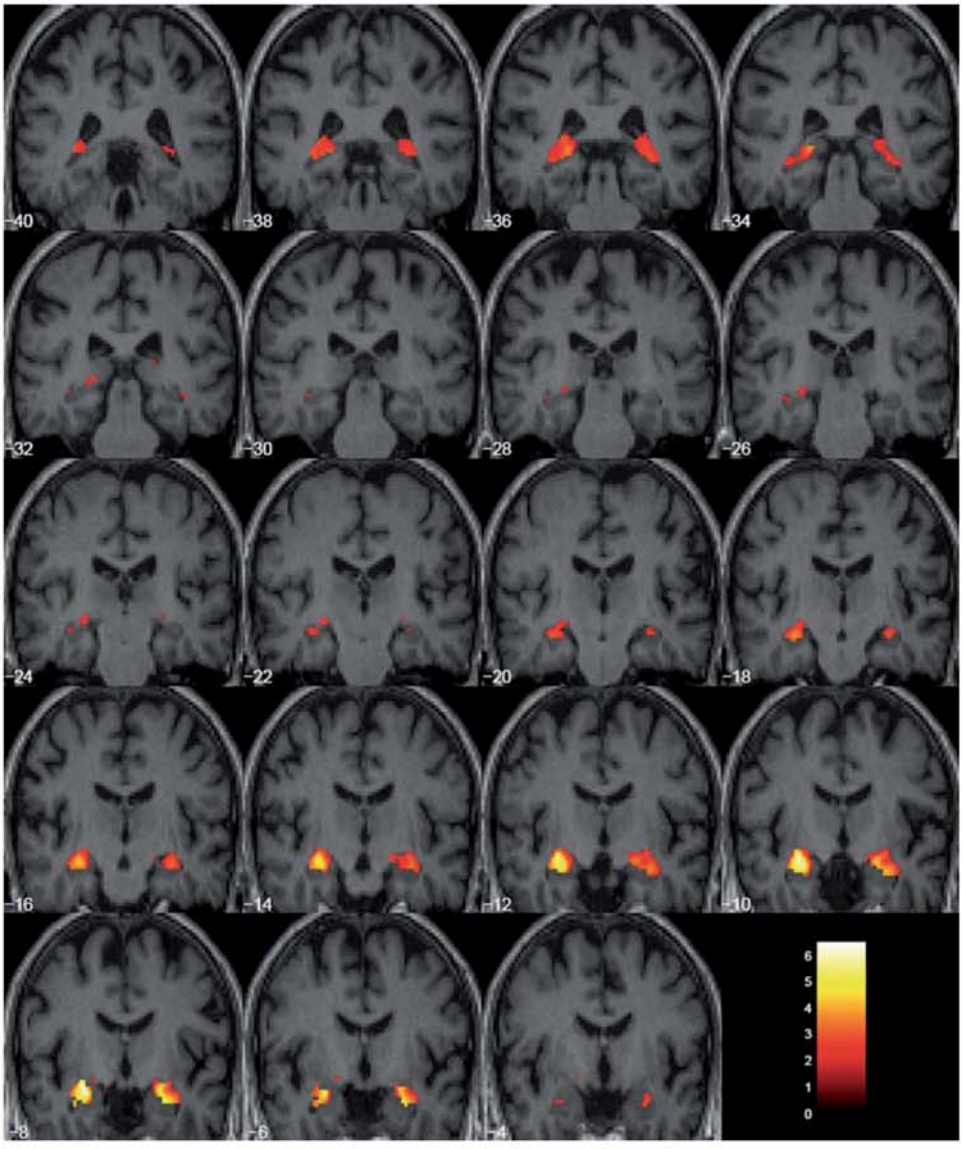

Fig. 1 Hippocampal gray matter loss in demented PD patients. Colored bars indicate significant t values. Yellow colors are more significant than the red ones. Clusters of gray matter density differences are observed in the head and the tail

Neuropsychological results

Significant differences were found among all the groups in global cognitive functions (MMSE) and in learning and forgetting variables from the RAVLT test (Table 2). In contrast, the variable recognition from the RAVLT test was only significant when comparing PDD and controls.

VBM results

Group analysis

Analyzing the three groups of patients together, we observed hippocampal gray matter reductions compared to controls. The most striking differences in hippocampal gray matter densities were seen when comparing

Table 1 Demographical and clinical characteristics of the sample

	Controls N = 56	PDD N = 9	PD + VH N = 16	PD – VH N = 19	F/χ^2 test	p
Age (yr)	73 ± 6.7	69.8 ± 9.5	73.5 ± 5.1	72.5 ± 5.8	0.69*	0.566
Gender (men/women)	28/28	5/4	5/11	8/11	2.20**	0.530
Education (yr)	7.9 ± 4.6	8.6 ± 6.1	7.6 ± 3.9	7.8 ± 3.3	0.11*	0.957
Disease evolution (yr)	–	13.1 ± 5.4	12.9 ± 5.9	10.9 ± 4.2	0.84*	0.438
Hamilton depression	–	4.3 ± 4.4	7.4 ± 4.4	3.6 ± 2.8	12.36**	0.006[a]
Hoehn & Yahr	–	3.8 ± 1.0	3.2 ± 1.1	2.5 ± 0.7	7.13*	0.002[b]
UPDRS III	–	42.8 ± 17.4	29.7 ± 12.8	24.7 ± 14.3	4.83**	0.013[b]

* *F* test; ** χ^2 test
[a] Significant differences between PD – VH and PD + VH
[b] Significant differences between PDD and PD – VH

Table 2 Neuropsychological performance

	Controls N = 56	PDD N = 9	PD + VH N = 16	PD – VH N = 19	F/χ^2 test	p
MMSE	28.7 ± 3.1	15.7 ± 5.4	26.0 ± 2.1	28.2 ± 1.7	33.58*	0.0001[a, b, c, d, e]
Learning	41.9 ± 7.2	18.0 ± 11.4	26.5 ± 7.4	39.3 ± 6.5	27.17*	0.0001[a, b, c, d, e]
Forgetting	9.3 ± 2.4	1.1 ± 1.3	4.9 ± 1.9	8.2 ± 2.1	38.60*	0.0001[a, b, c, d, e]
Recognition	13.6 ± 1.5	11 ± 1.4	12.4 ± 1.8	12.9 ± 2.2	3.36*	0.026[d]

* *F* test; ** χ^2 test
[a] Significant differences between PDD and PD + VH
[b] Significant differences between PDD and PD – VH
[c] Significant differences between PD – VH and PD + VH
[d] Significant differences between Controls and PDD
[e] Significant differences between Controls and PD + VH

Table 3 Group comparisons

Structure	Talairach Coordinate*			cluster dimension	T score	p corrected
	x	y	z			
The 3 groups of patients together vs controls						
Left anterior hippocampus	–28	–7	–11	198	5.87	0.017
Left posterior hippocampus	–28	–37	2	157	4.80	0.035
Right anterior hippocampus	30	–8	–11	171	3.74	0.027
PDD patients vs controls:						
Left hippocampus	–28	–10	–13	445	6.49	< 0.001
Right anterior hippocampus	24	–8	–15	260	4.89	< 0.001
Right posterior hippocampus	30	–35	–3	102	2.90	0.036
PD + VH vs controls:						
Right anterior hippocampus	30	–8	–11	42	3.75	0.010
Left anterior hippocampus	–28	–8	–11	29	5.02	0.003

Each reported anatomical location has a significance $p < 0.05$ corrected for multiple comparisons.
Cluster size denotes the extent of the cluster of significant voxels in mm^3.
* Talairach coordinate refers to the location of the most statistically significant voxel in the cluster.
The anterior or posterior predominance was determined according the maxima.

PDD with controls. Comparison of hippocampal gray matter density between PD+VH and controls showed significant differences only in the head of hippocampus (Table 3). Neither comparisons between PD-VH and control group nor comparisons between PD patients groups showed statistical significant differences.

In Figs. 1 and , 2 we depicted the coronal slices covering all the hippocampus. In Fig. 1, we can observe that gray matter loss in PDD involved the entire hippocampus, but the t value of the hippocampal head had higher significance than the tail, and the hippocampal body was relatively spared. Fig. 2 illustrates the hippocampal gray matter reduction of PD+VH that involved only the head.

Individual analysis

Comparing the VBM results of each patient to the mean of gray matter density of the control group, we observed significant individual hippocampal reductions in 7 out of 9 (78 %) PDD patients, 5 of the 16 (31 %) PD+VH patients and 5 of the 19 (26 %) PD-VH patients. These proportions of hippocampal atrophy in each group were statistically significant ($\chi^2 = 7.40$; $p < 0.025$). From those 17 patients who had regional gray matter loss, 15 (88 %) had anterior predominance. Only 2 from 17 patients showed posterior predominance.

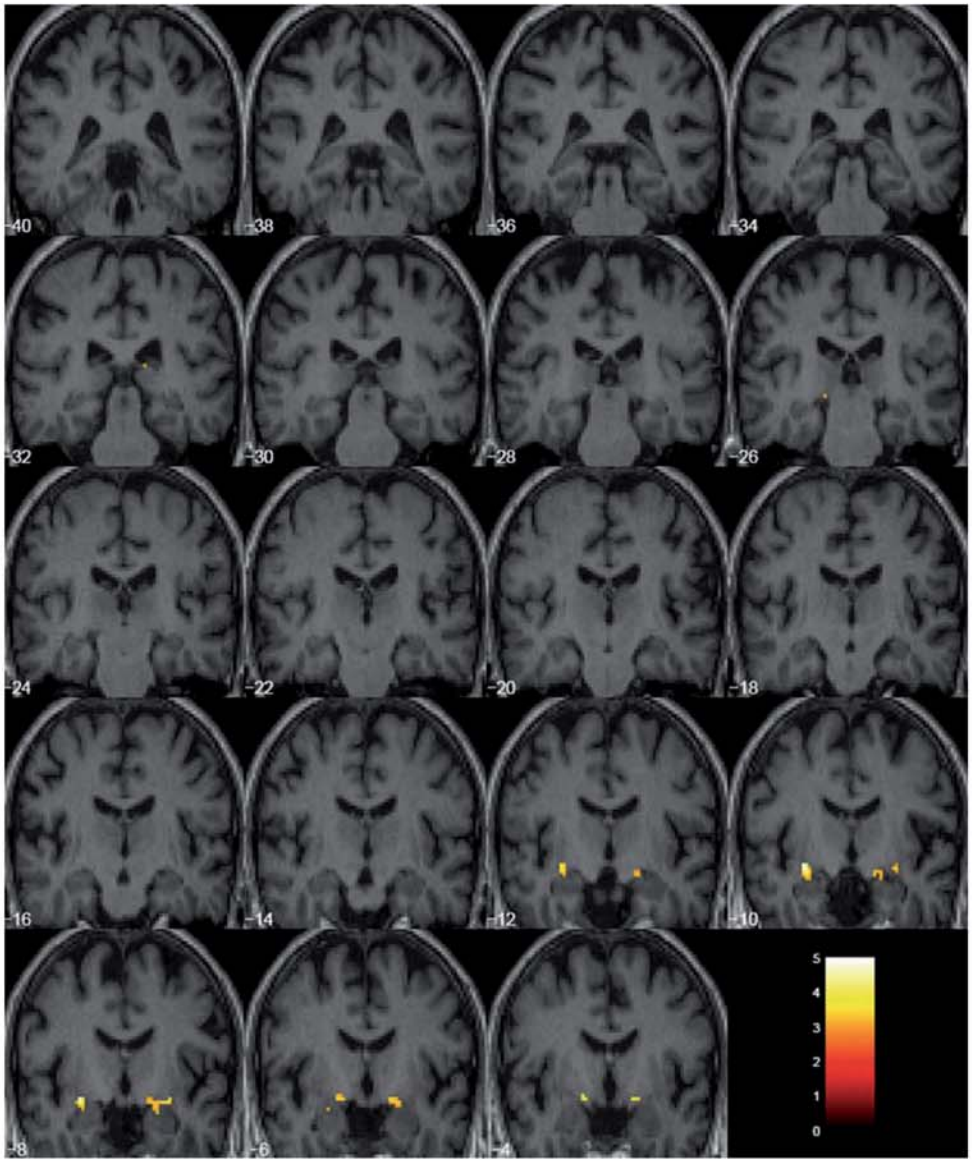

Fig. 2 Hippocampal gray matter loss in non-demented PD patients with visual hallucinations. Colored bars indicate significant t values. Yellow colors are more significant than the red ones. Clusters of gray matter density differences are observed only in the head

Relationship between hippocampal reductions and memory impairment

When all patients from the three patients groups studied (n = 44) were considered together a significant correlation was found between the gray matter density loss in the anterior part of hippocampus bilaterally and learning scores. This correlation was higher when only PD+VH patients were considered in the correlation analysis. Results from both correlation analyses with VBM are described in Table 4. No significant correlations were found when considering PDD and PD-VH groups independently. Regarding clinical scales, no correlation was found between any of them and the hippocampus.

The relationship between memory and hippocampus was also found after comparing verbal learning performance between patients with individual hippocampal loss and patients without hippocampal gray matter reductions. These subgroups were considered according to the results from VBM individual analysis. The scores for the first group were 22.53±11.98 and for the second one 33.63±10.07 ($t = 3.308$; $P < 0.002$). Different learning scores between those individuals who had hippocampal gray matter reductions and those who did not were also found excluding patients who had dementia. The scores for the first group were 28.20±11.48 and for the second group 35.52±7.70 ($t = -2.199$; $p < 0.035$)

Discussion

The results from this VBM study show that hippocampal atrophy is present in PD. PD patients with dementia have gray matter loss involving all the hippocampus; patients with visual hallucinations but not demented have hippocampal gray matter loss only in the anterior regions.

By focusing in the regional distribution of the density changes, we observed in PDD patients diffuse hippocampal atrophy, involving the entire hippocampal axis, but with prominent involvement in both anterior and posterior regions and relative sparing of the central region. The highest statistical significance of difference in hippocampal gray matter loss between PDD patients and controls was seen in the anterior region. This pattern of VBM changes is similar to that described in the hippocampus of patients with Alzheimer's disease [16, 23].

In the PD+VH group we observed regional gray matter loss only in the anterior part of hippocampus. Some studies have pointed out that PDD patients have higher densities of LBs and LNs in the CA-2-3 fields of the hippocampus [10]. According the MRI three-dimensional reconstructions from Frisoni et al. [15], the CA2 and CA3 fields are located on the dorsal surface of the hippocampal formation in a strip stretching from medial to lateral between the most posterior head region and the most anterior tail region. We expected to find gray matter loss in these hippocampal regions in PD+VH patients, because they are at risk of dementia. However, we found hippocampal gray matter loss in the head of this structure, which mainly corresponds with the CA1 field. Our findings agree with those reported in a recent study that showed head predominance of hippocampal atrophy in demented and aged PD patients [6] and with the data from normal subjects demonstrating that the hippocampal head is particularly susceptible to age-related changes [28] and it is especially vulnerable to degenerative changes such as Alzeimer's disease even at the early stages [16, 36]. Furthermore, Apostolava et al. [3] recently reported that smaller hippocampus and specifically CA1 and subicular involvement are associated with increased risk for conversion from Mild Cognitive Impairment to Alzheimer's disease.

Our neuropsychological data showed a more pronounced memory impairment in PD+VH compared to PD-VH, these results agree with previous findings in transversal and longitudinal studies [29, 30]. The neuropsychological data reported here together with the MRI results reinforces the idea that the presence of hallucinations is indicative of cognitive impairment and evolution towards dementia.

Contrarily to previous reports, in this study we did not find significant differences between PD-VH and

Table 4 Correlations between gray matter loss in hippocampus and learning scores

Structure	Talairach Coordinate			cluster dimension	r score	p corrected
	x	y	z			
Correlations in the three group of patients together						
Right anterior hippocampus	31	−10	−16	159	0.54	0.003
Left anterior hippocampus	−28	−10	−15	101	0.65	0.015
Correlations in PD + VH patients						
Right anterior hippocampus	28	−12	−13	139	0.83	< 0.001
Left anterior hippocampus	−24	−16	−14	105	0.77	< 0.001

Each reported anatomical location has a significance $p < 0.05$ corrected for multiple comparisons.
Cluster size denotes the extent of the cluster of significant voxels in mm^3.
* Talairach coordinate refers to the location of the most statistically significant voxel in the cluster.

controls. This could be due to the level of the statistical significance applied in this study and also to the type of MRI analysis used. We used a VBM study that measures gray matter density of hippocampus and takes a level of significance of corrected $p < 0.05$, a more strict level than other studies performed with VBM that used a non-corrected $p < 0.001$ [31, 34]. In a previous VBM study of our group [32] comparing the whole brain of PD-VH with controls, we observed gray matter reductions in several cortical gray-matter regions but not for the hippocampus. On the other hand, previous MRI studies that reported hippocampal atrophy in non-demented PD patients were performed using manual ROI's volumetric analysis [9, 22, 35]. It is possible that manual volumetric measures would be more sensitive than VBM procedures to subtle degeneration.

Individual analyses of cases showed that 78 % of PDD patients, 31 % of PD+VH and 26 % of PD-VH had gray matter density loss in the hippocampus. The regional pattern of atrophy was of anterior predominance in almost all patients, suggesting that the hippocampal atrophy starts in this region and later extends to the posterior part.

Correlation analyses with SPM showed that the gray matter loss in the anterior part of hippocampus correlated with the verbal learning scores when scores from all patients from the three groups studied were considered together. This correlation was also found when only the PD+VH group was assessed. Moreover, those PD patients that showed in the individual analysis gray matter loss in the anterior part of hippocampus scored significantly lower in verbal learning compared to those who did not have anterior hippocampal reduction. This relationship between hippocampal head atrophy and verbal memory performance is in agreement with previous studies in non-demented elderly. In a sample of 511 normal subjects aged 60–90 Hackert et al. [18] found significant correlations between the head of the hippocampus and verbal memory but not for the body and tail. In a Bouchard et al. study [6], the left hippocampus head correlated with delayed recall, but not with recognition scores. In our work, we also found that the hippocampal head correlated with a measure of recall (learning scores) but not with recognition. On the other hand, the fact that hippocampal head gray matter loss did not correlate with UPDRS score indicates that hippocampal head atrophy related only to memory impairment and not to the overall disease severity.

As a limitation in our study we must mention that the ROI's procedure from the SPM2 program is less precise than other manual methods because it involves the anatomical imprecision inherent of voxel-based morphometry procedures. Nevertheless, our results agree with those reported by Bouchard et al. (2007) using the manual ROI's approach.

In conclusion, widespread hippocampal gray matter loss in PDD patients and atrophy limited to the head of hippocampus in PD+VH suggest that the neurodegenerative process in the hippocampus starts in the anterior part of this structure. Longitudinal studies are needed to verify if this pattern of hippocampal head atrophy later spreads to the tail in PD+VH patients.

Conflict of interest The authors declare no conflict of interest.

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References

1. Aarsland D, Andersen K, Larsen JP, Lolk A, Kragh-Sorensen P (2003) Prevalence and characteristics of dementia in Parkinson disease: an 8-year prospective study. Arch Neurol 60:387–392
2. American Psychiatric Association (2003) Manual diagnóstico y estadístico de los trastornos mentales, Cuarta edición revisada: DSM-IV-TR. Masson SA, Barcelona
3. Apostolova L, Dutton RA, Dinov ID, Hayashi KM, Toga AW, Cummings JL, Thompson PM (2006) Conversión of mild cognitive imapirment to Alzheimer's disease predicted by hippocampal atrophy maps. Arch Neurol 63:693–699
4. Ashburner J, Friston KJ (2000) Voxel-based morphometry – the methods. Neuroimage 11:805–821
5. Beyer MK, Janvin CC, Larsen JP, Aarsland D (2007) An MRI study of patients with Parkinson's disease with mild cognitive impairment using voxel-based morphometry. J Neurol Neurosurg Psychiatry 78:254–259
6. Bouchard TP, Malykhin N, Martin WRW, Hanstock CC, Emery DJ , Fisher NJ , Camicioli RM (2007) Age and dementia-associated atrophy predominates in the hippocampal head and amygdale in Parkinson's disease. Neurobiol Aging 29:1027–1039
7. Braak H, Del Tredici K, Rüb U, de Vos RAI, Ernst NH, Steur J, Braak E (2003) Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24:197–211
8. Burn DJ (2004) Cortical Lewy body disease. J Neurol Neurosurg Psychiatry 75:175–178
9. Camicioli R, Moore MM, Kinney A, Corbridge E, Glassberg K, Kaye JA (2003) Parkinson's disease is associated with hippocampal athophy. Mov Disord 18:784–790
10. Churchyard A, Lees AJ (1997) The relationship between dementia and direct involvement of the hippocampus and amygdala in Parkinson's disease. Neurology 49:1570–1576

11. Daniel SE, Lees AJ (1993) Parkinson's Disease Society Brain Bank, London: overview and research. J Neural Transm 39(Suppl):165–172
12. Duvernoy HM (1998) The human hippocampus: An atlas of applied anatomy. Berlin: Springer-Verlag
13. Fahn S, Elton RL, and members of the UPDRS development committee (1987) Unified Idiopathic Parkinson's disease Rating Scale. In: Fahn S, Marsden CD, Calne D, Goldstein M (eds) Recents developments in Parkinson's disease (Vol. 2). Florham Park, New Jersey, Mac Millan Healthcare Information; USA, pp 153–164
14. Folstein MF, Folstein SE, MCHugh PR (1975) "Mini.mental State". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12:189–198
15. Frisoni GB, Sabattoli F, Lee AD, Dutton RA, Toga AW, Thompson PM (2006) In vivo neuropathology of the hippocampal formation in AD. A radial mapping MR-based study. Neuroimage 32: 104–110
16. Frisoni GB, Testa C, Zorzan A, Sabattoli F, Beltramello A, Soininen H, Laakso MP (2002) Detection of grey matter loss in mild Alzheimer's disease with voxel based-morphometry. J Neurol Neurosurg Psychiatry 73:657–664
17. Galvin JE, Pollack J, Morris JC (2006) Clinical phenotype of Parkinson disease in dementia. Neurology 67: 1605–1611
18. Hackert VH, den Heijer T, Oudkerk M, Koudstaal PJ, Hofman A, Breteler MB (2002) Hippocampal head size associated with memory performance in nondemented elderly. Neuroimage 17:1365–1372
19. Hamilton M (1996) A rating scale for depression. J Neurol Neurosurg Psychiatry 23:56–62
20. Hughes TA, Ross HF, Musa S, Bhattacherjee S, Nathan RN, Mindham RHS, Spokes EGS (2000) A 10-year study of the incidence of and factors predicting dementia in Parkinson's disease. Neurology 54:1596–1603
21. Jankovic J, McDermott M, Carter J, Gauthier S, Goetz C, Golbe L, Huber S, Koller W, Olanow C, Shoulson I, Stern M, Tanner C, Weiner W and the Parkinson Study Group (1990) Variable expression of Parkinson's disease: A base-line analysis of the DATATOP cohort. Neurology 40:1529–1534
22. Junque C, Ramirez-Ruiz B, Tolosa E, Summerfield C, Martí MJ, Pastor P, Gómez-Ansón B, Mercader (2005) Amygdalar and hippocampal MRI volumetric reductions in Parkinson's disease with dementia. Mov Disord 20:540–544
23. Laakso MP, Frisoni GB, Könönen M, Mikkonen M, Beltramello A, Geroldi C, Bianchetti A, Trabucchi M, Soininen H, Aronen HJ (2000) Hippocampus and entorhinal cortex in frontotemproal dementia and Alzheimer's disease: a morphometric MRI study. Biol Psychiatry 47:1056–1063
24. Laakso MP, Partanen K, Riekkinen PJ, Lehtovirta M, Helkala EL, Hallikainen M, Hanninen T, Vainio P, Soininen H (1996) Hippocampal volumes in Alzheimer's disease, Parkinson's disease with and without dementia, and in vascular dementia: An MRI study. Neurology 46:678–681
25. Lezak MD, Howieson DB, Loring DW, Hannay HJ, Fischer JS (2004) Neuropsychological assessment. 4th ed. Oxford University Press Inc, New York
26. Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH (2003) An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage 19: 1233–1239
27. Mechelli A, Price CJ, Friston KJ, Ashburner J (2005) Voxel-based morphometry of the human brain: Methods and applications. Current Medical Imaging Reviews 1:105–113
28. Pruessner JC, Collins DL, Pruessner M, Evans AC (2001) Age and gender predict volume decline in the anterior and posterior hippocampus in early adulthood. J Neurosci 21:194–200
29. Ramírez-Ruiz B, Junque C, Martí MJ, Valldeoriola F, Tolosa E (2007) Cognitive changes in Parkinson's disease patients with visual hallucinations. Dement Geriatr Cogn Disord 23: 281–288
30. .Ramírez-Ruiz B, Junqué C, Martí MJ, Valldeoriola F, Tolosa E (2006) Neuropsychological deficits in Parkinson's disease patients with visual hallucinations. Mov Disord 21:1483–1487
31. Ramírez-Ruiz B, Marti MJ, Tolosa E, Bartrés-Faz D, Summerfield D, Salgado-Pineda P, Gómez-Ansón B, Junqué C (2005) Longitudinal evaluation of cerebral morphological changes in Parkinson's disease with and without dementia. J Neurol 252:1345–135
32. Ramírez-Ruiz B,Martí MJ, Tolosa E, Giménez M, Bargalló N, Valldeoriola F, Junqué C (2007) Cerebral atrophy in Parkinson's disease patients with visual hallucinations. Eur J Neurol 14:750–756
33. Saldmond CH, de Haan M, Friston KJ, Gadian DG, Vargha-Khadem F (2003) Investigating individual differences in brain abnormalities in autism. Phil Trans R Soc London B 358:405–413
34. Summerfield C, Junqué C, Tolosa E, Salgado-Pineda P, Gómez-Ansón B, Martí MJ, Pastor P, Ramírez-Ruíz B, Mercader J (2005) Structural Brain Changes in Parkinson Disease with Dementia. Arch Neurol 62:281–285
35. Tam CW, Burton EJ, McKeith IG, Burn DJ, O'Brien JT (2005) Temporal lobe atrophy on MRI in Parkinson's disease with dementia: A comparison with Alzheimer's disease and dementia with Lewy bodies. Neurology 64:861–865
36. Wang L, Swank JS, Glick IE, Mokhtar HG, Miller MI, Morris JC, Csernansky JG (2003) Changes in hippocampal volume and shape across time distinguish dementia of the Alzheimer type from healthy aging. Neuroimage 20: 667–682

PAPER II

Differential progression of brain atrophy in Parkinson disease with and without visual hallucinations

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Naroa Ibarretxe-Bilbao (PhD)[1,2,3]; Blanca Ramirez-Ruiz (PhD)[2,3]; Carme Junque (PhD)[1,2,3]; Maria-Jose Marti (MD, PhD)[1,2,4]; Francesc Valldeoriola (MD, PhD)[1,2,4]; Nuria Bargallo (MD, PhD)[2,5]; Silvia Juanes (BSc)[1]; Eduardo Tolosa (MD, PhD)[1,2,4]

[1]*Centro de Investigación en Red de Enfermedades Neurodegenerativas (CIBERNED).*
[2]*Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), Barcelona, Spain.*
[3]*Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Spain.*
[4]*Parkinson Disease and Movement Disorders Unit, Neurology Service, Institut Clínic de Neurociències (ICN), Hospital Clínic de Barcelona, Spain.*
[5]*Centre de Diagnòstic per la Imatge Clínic (CDIC), Hospital Clínic de Barcelona, Spain.*

Corresponding author:

Prof. Carme Junque

Department of Psychiatry and Clinical Psychobiology. University of Barcelona

Casanova 143 (08036) Barcelona, Spain

Phone: (+34) 93 402 45 70 // Fax: (+34) 93 403 52 94 // E-mail: cjunque@ub.edu

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ABSTRACT

Objective: To determine the course of cognitive deficits and the regional progression of brain atrophy in patients with Parkinson's disease (PD) with and without visual hallucinations (VH).

Methods: We performed MRI and neuropsychological assessment at entry to the study and at follow-up (mean ± SD= 29.91 ± 5.74 months) in a sample of initially non demented 12 PD patients with VH, 14 PD patients without VH and 12 healthy controls (HC). Gray matter changes over time were assessed by means of voxel-based morphometry (VBM) and cognitive changes by an extensive neuropsychological battery.

Results: At follow-up, 75% of patients with VH developed dementia. The greatest decline was observed in verbal memory, semantic fluency, language comprehension and visuoperceptive functions. None of the patients without VH met criteria for dementia and did not show worsening in cognitive functions over time. Patients with VH showed widespread limbic, paralimbic and neocortical gray matter loss, whereas in the PD without VH group gray matter loss was restricted to a small region in frontal cortex and cerebellum. We also found significant correlations between the changes in several cognitive functions and gray matter loss over time in PD patients with VH.

Conclusion: The presence of VH in PD determines a different cognitive outcome and a different pattern of progressive brain atrophy. PD patients with VH, unlike PD without VH, frequently develop dementia and show a widespread atrophy involving limbic, paralimbic and neocortical areas.

INTRODUCTION

Visual hallucinations (VH) have been reported to occur in 50% of patients with Parkinson's disease (PD).[1] Generally, VH occur during the second half of the disease's course[1] and have a persistent and progressive nature.[2] Although VH can be exacerbated by dopaminergic treatment, several studies did not find a direct relationship between antiparkinsonian agents and presence of VH.[1,2] The presence of VH has been associated with Lewy Body (LB) pathology. While VH are considered characteristic feature of PD and Dementia with Lewy Bodies, they are rarely reported in other parkinsonian disorders such as Corticobasal Degeneration or Supranuclear Palsy.[1]

Cross-sectional studies have reported that non-demented PD patients with VH present greater neuropsychological impairment compared to those without VH in domains such as verbal[3-5] and visual memory,[6] language comprehension,[4] and visuospatial[5] and visuoperceptive functions.[4, 6] Frontal dysfunction has also been described in PD with VH including deficits in verbal fluency,[3, 4, 7, 8] sustained attention,[9] and inhibition.[7, 8] Moreover, longitudinal studies have shown the presence of VH as a significant predictor of dementia in PD[10-12] and it is associated with a more rapid general cognitive decline assessed by MMSE.[13,14] In a previous longitudinal study, we reported that 45% of the PD patients with VH developed dementia after 1 year follow-up and showed a significant progressive decline in visual memory and visuoperceptive functions.[15] The combination of degraded visual information from the environment and failing visual memory together with deficits in executive functions [7,8] have been pointed out as important causal factors for the occurrence of VH.[16]

The brain mechanisms underlying VH in PD are not completely understood. Structural and functional abnormalities within the primary visual system and visual association areas, including ventral and dorsal pathways, have been reported in PD with VH.[16] We previously reported that non-demented PD patients with VH showed areas of gray matter reduction in parieto-occipital areas in comparison with non-hallucinating PD patients.[17] Functional imaging studies also reported perfusion abnormalities in occipital, temporal and parietal areas.[18-21] In addition to the dysfunction in posterior areas, increased frontal activation was reported in fMRI studies in response to simple [22]

and complex[23] visual stimuli. The involvement of the frontal lobe in VH has also been confirmed by PET studies showing a pattern of frontal hypermetabolism in patients with PD and VH.[24] Neuropathological studies showed the association between VH and the presence of high densities of LBs in medial temporal areas.[25] Interestingly, we have recently reported hippocampal head reductions in PD with VH in comparison with healthy controls.[26]

The involvement of all these areas (visual associative, frontal and medial temporal areas) is indicative of brain abnormalities in PD patients with VH already when they are not demented. In PD patients with dementia the pattern of brain dysfunction involves these and other areas and it is more severe and extended. Specifically, VBM studies have revealed that PD patients with dementia present basal ganglia,[27, 28] limbic,[27, 29] paralimbic,[27] and widespread neocortical atrophy;[28, 29] and functional imaging studies have reported an extensive hypoperfusion in anterior in addition to posterior brain regions.[30]

As previously mentioned, if patients with VH are at high risk of developing dementia, [10, 11] it is probable that the brain atrophy in these patients will progressively affect all the areas described to be involved in dementia in PD including limbic, paralimbic and neocortical areas. To test this hypothesis longitudinal MRI studies are needed but so far there are only two longitudinal studies using VBM in PD. The first one showed limbic and temporo-occipital areas of gray matter reduction after 25 months follow-up[31] while the other study did not show any areas of gray matter loss in PD patients after a follow-up period of 1.4 years.[32] These studies did not differentiate between patients with and without hallucinations. The present study used VBM to assess progression of regional brain atrophy and cognitive deficits over time in patients with and without VH. We expected that, unlike patients without VH, hallucinating PD patients will show a pattern of progressive cortical atrophy parallel to the development of dementia.

METHODS

Participants

Patients were recruited from an outpatient movement disorders clinic (Parkinson's disease and Movement Disorders Unit, Department of Neurology, Hospital Clinic, Barcelona) in collaboration with the Department of Psychiatry and Psychobiology (University of Barcelona). The participants were part of a previously studied sample[4, 15, 17, 26] and they were invited by telephone for a follow-up assessment.

Twelve PD patients with VH, 14 PD patients without VH and 12 controls participated in the follow-up assessment. The average follow-up period was 29.91 months (S.D= 5.74; range= 26-32). The inclusion criterion for patients with VH was the presence of well-formed VH. Patients with only minor forms consisting of a sensation of presence, a sideways passages or illusions were not included in the study.

All participants included in the present study had neuropsychological and MRI evaluation both at base-line and follow-up. At base-line 18 patients with VH and 20 without VH participated in the study and at follow-up there was an attrition of 33,3% and 30% in each group respectively. Two participants from the original PD sample with VH died. In another four cases, the next of kin decided not to give the consent for participation due to severe deterioration of the patients' condition. From the original PD without VH group three subjects declined to participate. In three others cases, brain MRI could not be obtained due to severe motor impairment.

The base-line and follow-up examinations included assessment of:

1) Hallucinations: To evaluate the phenomenology of hallucinations we used a structured interview designed at our hospital which comprised items covering modality (visual, auditory, tactile and olfactory), content (animals, people, objects) and temporal aspects (time of the day, frequency and duration). The severity of the VH was rated using the Spanish version of the neuropsychiatric inventory (NPI) (subscale hallucinations). The VH in the PD sample consisted of well-formed images of people, faces or animals. Hallucinations occurred only in the visual modality while patients were alert and had their eyes open. None of the VH patients experienced auditory hallucinations. Insight

into the hallucinatory nature of the phenomenon was maintained in 58.3% of the patients. Associated delusions were present in 33.3% of the patients. These delusions were primarily paranoid in type and involved elementary misbelieves concerning infidelity or theft.

2) Dementia: The diagnosis of dementia was based on an interview with the patient and the caregiver using the Diagnostic and Statistical Manual of Mental Disorders, Revised, Fourth Edition (DSM IV-TR), and the Movement Disorder Society's diagnostic criteria for Parkinson Disease Dementia.[33] The algorithm for the diagnosis of dementia in PD (level I) requires: i) a diagnosis of PD, ii) the development of PD prior to the onset of dementia iii) MMSE below 26; iv) presence of cognitive deficits severe enough to impact daily living, v) impairment in more than one cognitive domain including attention, executive function, visuo-constructive ability or memory.[33]

3) Clinical and demographical variables: Age, education, duration of illness and medication were recorded. Illness severity was staged according to Hoehn and Yahr and motor subscale from the Unified Parkinson's Disease Rating Scale (UPDRS). Mood was assessed using the Hamilton Depression Rating Scale (HDRS). All patients were treated with levodopa alone or a combination of levodopa and dopamine-agonist (pramipexole, ropinirole or pergolide). In order to take into account the amount of all dopaminergic drugs taken, we calculated a levodopa-equivalent dose for each patient. Four (33.3%) PD patients with VH were taking atypical antipsychotics (quetiapine and clozapine). None of the patients was treated with anticholinesterases. All patients were highly l-dopa responsive. Both base-line and follow-up clinical and cognitive examinations were performed in the on phase.

4) Neuropsychological functions: The evaluation included assessment of general cognitive ability with Information and Similarities subtest from WAIS-III, verbal memory with Rey's Auditory Verbal Learning Test (RAVLT); visual memory by means of the Warrington Recognition Memory for Faces; visuoperceptive functions with the Visual Form Discrimination Test and Benton Facial Recognition Test; frontal functions with phonetic and semantic fluencies; language comprehension with the Token Test and Boston Naming Test for naming. The neuropsychological battery was administered by a trained neuropsychologist (B.R-R), and completed in a single session lasting one to two hours. The

neuropsychologist in charge of the assessment was the same at base-line and follow-up examinations but she was not blinded to the presence of VH.

The study was approved by the institutional ethics committee. Written informed consent was obtained from the patients or their caregiver in the case of patients with overt cognitive impairment, and from healthy controls after full explanation of the procedures involved in the study.

Neuropsychological and clinical statistical analysis

Statistical analysis was carried out using SPSS 14.0. Multivariate analysis of variance was performed on demographic, clinical and neuropsychological variables to examine the differences between groups at base-line. Post-hoc pair-wise Tukey's test was performed where appropriate. The analysis for changes in clinical and neuropsychological variables between baseline and follow-up was performed using the General linear mixed model (GLM) for repeated measures to test whether these variables differed across time in the groups. Two-factor (3x2) ANOVA was performed for each of the variables. Factors were: group (PD with VH, PD without VH and controls) and time (base-line and follow-up) and we obtained effect of time (F_T), effect of group (F_G) and interaction between group and time (F_{GxT}) for each of the variables. In addition, to adjust for base-line performance in comparing rate of decline between groups we calculated a percentage of loss for each of the cognitive variables with significant interaction between time and group using the following formula:

[(score at base-line) – (score at follow-up) / (score at base-line)] x 100

MRI acquisition and Voxel-based morphometry analysis

All scans were obtained from a 1.5T GE Nvi/Cvi 8.4 machine (GE, Milwaukee, WI, USA). The imaging protocol included an axial 3D IR Prep SPGR (Inversion Recovery Prepared Spoiled Gradient-echo) sequence of the entire brain and the following parameters: TR (Repetition Time)= 17; TE (Echo Time)= 5; TI (Inversion Time)= 300; 1.5 mm thickness; FoV (Field of View)= 24x24; 256x256; 1 NEX (Number of Excitations). No hardware or software upgrades were made over the

period of assessment since longitudinal techniques are very sensitive to any change in image acquisition.

VBM analysis[34] was carried out on SMP5 (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, University College London, London, UK, http:// www.fil.ion.ucl.ac.uk.spm) running in Matlab 7.0 (Mathworks, Natick, Massachusetts, USA). Firstly, images were reoriented according to the anterior-posterior commissure and were then segmented into gray matter, white matter, and cerebrospinal fluid using the unified model that integrates segmentation and normalization. The spatial normalization involves registering each of the images onto the SPM T1 template, whereas the segmentation step uses a priori probability maps to segment different tissues. A separate 'modulation' step was used to control for deformations from the spatial normalization step and the gray matter modulated images obtained were smoothed with an 8-mm full-width at half-maximum isotropic Gaussian kernel. The smoothed gray matter images were analyzed in a factorial design (3x2) ANOVA. MMSE score at base-line was also introduced as a covariate in an ANCOVA model.

Our main interest was the comparison between base-line versus follow-up for each of the groups, specifically for the progression of volume loss in PD patients with and without VH over time.

We also carried out correlation analyses in SPM5 between the progression of cerebral atrophy (image at base–line minus image at follow-up) and the decline in neuropsychological tests (score at base-line minus score at follow-up). The correlation analyses were performed with those neuropsychological variables that showed a significant decline over time.

All results were thresholded at a cluster and voxel level of $p < 0.05$ corrected for multiple comparisons by False Discovery Rate (FDR) which controls the expected proportion of the rejected hypothesis that are falsely rejected.

RESULTS

Neuropsychological and clinical variables

There were no differences in age, gender, years of education and years of disease duration between groups (see table 1) but groups were not matched for global cognitive function and the MMSE scores were significantly lower in those with VH.

Table 1. Demographic and clinical characteristics of the sample at base-line.

	Controls (n=12)	PD without VH (n=14)	PD with VH (n=12)	F^a, t^b, χ^c	p
Age	70.7 ± 7.2	71.1 ± 5.7	73.3 ± 5.9	0.597[a]	0.556
Gender (male/female)	4/8	5/9	3/9	0.188[c]	0.665
Education (years)	8.1 ± 4.7	8.3 ± 4.1	7.5 ± 4.1	0.113[a]	0.894
Disease evolution (years)	-	11.9 ± 4.3	12.1 ± 5.7	0.040[b]	0.968
Disease evolution before VH (years)	-	-	9.9 ± 5.7	-	-

Values are mean ± SD. PD= Parkinson's disease, VH= visual hallucinations.
[a] One factor ANOVA, [b] t-test, [c] chi square

Neuropsychological and clinical data both at base-line and follow-up are presented in table A in supplemental material. None of the patients in either group met criteria for dementia at base-line examination but the PD patients with VH were already more cognitively impaired than PD without VH. Specifically, at base-line, patients with VH showed impairment in verbal memory and facial recognition in comparison with PD without VH.

Two and a half years after the base-line evaluation, 9 out of 12 PD with VH presented dementia whereas none of the PD without VH patients met criteria for dementia. The neuropsychological functions showing a significant interaction between group and time are presented in figure 1. In PD with VH group we observed a 6.5 point decline in the MMSE (F_G= 13.8, $p < 0.001$; F_T= 19.6, $p < 0.001$; F_{GxT}= 9.2; $p < 0.001$). In the specific cognitive domains, PD with VH group showed a faster and steeper cognitive decline than PD without VH and controls in verbal learning (F_G= 12.7, p <

0.001; F_T= 0.4, p < 0.5; F_{GxT}= 12.7, p < 0.001), delayed recall (F_G= 34.7, p < 0.001; F_T= 0.8, p < 0.4; F_{GxT}= 6.5; p < 0.004), semantic fluency (F_G= 11.4, p < 0.001; F_T= 13.6, p < 0.001, F_{GXT}= 3.6; p < 0.04), language comprehension (F_G= 10.9; p < 0.001; F_T= 2.5; p < 0.13; F_{GxT}= 6.5; p < 0.004) and visuoperceptive function (facial recognition) (F_G= 20.3; p < 0.001; F_T= 16.7; p < 0.001; F_{GxT}= 4.7; p < 0.016). When adjusting for base-line performance the percentage of loss in each of these neuropsychological variables remained statistically significant (see table 2).

Table 2. Percentage of loss in each of the neuropsychological variables with significant interaction between time and group

	Controls (n=12)	PD without VH (n=14)	PD with VH (n=12)	F	p
Learning	-20.2 ± 17.2	-4.3 ± 18.9	25.1 ± 29.9	11.935	< 0.001[a,b]
Delayed recall	-19.9 ± 24.1	-13.1 ± 23.1	46.0 ± 41.4	16.040	< 0.001[a,b]
Semantic fluency	7.2 ± 27.4	3.6 ± 28.1	40.8 ± 30.2	7.379	0.002[a,b]
Language comprehension	-2.8 ± 6.9	-1.8 ± 12.1	13.9 ± 18.6	6.742	0.004[a,b]
Facial recognition	0.8 ± 8.1	4.8 ± 6.7	13.1 ± 11.2	4.836	0.015[a,b]
MMSE	4.8 ± 6.9	0.6 ± 6.2	25.5 ± 25.5	9.321	0.001[a,b]

PD= Parkinson's disease, VH= visual hallucinations, MMSE= Mini-Mental State Examination. Values are mean ± S.D of the percentage of loss in each of the variables calculated with the following formula: (score at base-line) – (score at follow-up) / (score at base-line). The F and p values refer to the MANOVA to check differences in percentage of cognitive decline between groups. Post-hoc analyses were performed with Tukey's test: [a] significant differences between controls and PD with VH; [b] significant differences between PD with and without VH

Regarding the clinical scales, PD patients with VH were at a more advanced stage of the disease at base-line (see table 2). At follow-up, patients from both groups showed a significant motor deterioration measured using the UPDRS-III motor scale (F_G= 3.1; p < 0.097; F_T= 17.5; p < 0.001; F_{GxT}= 2.1; p < 0.164) and a progression of the illness rated by means of the Hoehn and Yahr scale (F_G= 6.5; p < 0.018; F_T= 9.1; p < 0.007; F_{GxT}= 0.4; p < 0.527) with no significant interactions between time and group. L-dopa dosage was only increased from base-line to follow-up in PD without VH group and not in the VH sample in an attempt to reduce the VH.

Voxel-based morphometry results

From base-line to follow-up, PD with VH patients showed gray matter loss in the bilateral parietal cortex (including precuneus and supramarginal gyrus), bilateral insula, bilateral superior and inferior temporal gyrus, bilateral superior and inferior frontal gyrus, bilateral anterior (ventral and dorsal) and left dorsal posterior cingulate gyrus, bilateral thalamus and limbic areas (nucleus accumbens, amygdala, and hippocampus) (see figure 2 and table 3). In the PD without VH group only small clusters of gray matter loss were observed in right frontal areas including primary motor, pre-motor, supplementary motor areas, and the anterior and posterior areas of the cerebellum (see table 4 and figure 2). Healthy controls did not show any clusters of significant gray matter loss.

Table 3. Gray matter reductions from base-line to follow-up in PD with VH with MMSE score at base-line as a covariate.

Region	**Cluster size (voxels)**	**Talairach coordinates (x, y, z)**	**t value**
-Left insula (BA13) -Left thalamus, hippocampus and and amygdala	2814	-42, -6, 0 -38, 3, -12	6.82 6.59
-Right superior temporal gyrus (BA38) -Right insula, hippocampus, amygdala and thalamus. -Right inferior and superior frontal gyrus	4415	32, 7, -19 42, 0, -5 20, 57, -15	6.06 5.59 5.43
-Left supramarginal gyrus (BA40) -Left superior temporal gyrus (BA41) -Left frontal premotor cortex (BA6)	423	-61, -24, 21 -57, -17, 10 -55, -3, 7	4.67 4.21 4.05
-Left frontal pole (BA10) -Left frontal superior gyrus -Left frontal inferior gyrus	305	-32, 49, 12 -24, 60, -10 -36, 54, -14	4.65 4.36 4.10
-Left anterior cingulate gyrus (BA24) -Left posterior cingulate gyrus (BA31) -Left orbitofrontal cortex (BA11)	318	-2, 41, 2 -2, 30, -13 -3, 40, -20	4.65 4.56 3.95
-Left precuneus	269	-4, -9, 45	4.32
-Right anterior cingulate -Right posterior cingulate -Right precuneus	186	2, -27, 40 4, -43, 39 2, -43, 32	4.47 4.42 3.89

Voxel and cluster level $p < 0.05$ corrected by FDR; coordinates correspond to the voxel of maximum significance.

Table 4. Gray matter reductions from base-line to follow-up in PD without VH with MMSE score at base-line as a covariate.

Region	Cluster size (voxels)	Talairach coordinates (x, y, z)	t value
-Right primary motor cortex (BA4) -Right premotor and supplementary motor cortex (BA6)	340	22, -24, 55 40, -7, 61	5.59 5.48
-Left posterior cerebellum (inferior semi-lunar lobule) -Left posterior cerebellum (vermis)	1118	-8, -68, -37 -4, -73, -30	5.25 4.79

Voxel and cluster level p <0.05 corrected by FDR.
Coordinates correspond to the voxel of maximum significance.

Correlation analyses between cognitive decline and progression of atrophy

We looked for correlations between gray matter loss over time and those cognitive functions that showed a significant decline in PD patients with VH. The decline in learning was correlated with hippocampal head atrophy and delayed recall worsening was related to gray matter loss in left prefrontal cortex. The decline in semantic fluency was related to progressive atrophy in the thalamus and language comprehension was related to atrophy in the amygdala (see table 5). The correlations between the maxima of each of the significant clusters and cognitive decline are plotted in figure A (supplemental material).

Table 5. Correlations between gray matter loss over time and cognitive decline in PD patients with VH.

	Talairach coordinates (x, y, z)	cluster size (voxels)	*Cluster threshold*	r
Learning				
Left hippocampus	-20, -14, -9	11	0.042	0.88
Delayed recall				
Left prefrontal cortex	-14, 51, 18	212	< 0.001	0.95
Semantic fluency				
Left thalamus	-2, -18, -6	281	< 0.001	0.95
Language comprehension				
Left amygdala	-26, -3, -15	53	0.048	0.89

MNI coordinates refer to the location of the maxima. Cluster size denotes the extent of the significant cluster in voxels. Cluster threshold *P* values are corrected for multiple comparisons using FDR. The correlation coefficient (r) is calculated with the maxima of the significant cluster.

DISCUSSION

The present study provides evidence of progression to dementia associated with distinct brain atrophy in PD patients with VH. These patients presented progressive and extensive gray matter loss involving limbic, paralimbic and neocortical areas whereas PD patients without VH only showed small clusters of progressive atrophy in frontal areas and cerebellum.

The cognitive domains that showed a significant decline over time in PD patients with VH compared to patients without hallucinations were verbal memory, semantic fluency, language comprehension and visuoperceptive functions. In our cross-sectional study[4] we already reported impairment in these cognitive domains. Interestingly, semantic fluency[35] and verbal memory[36] have previously been reported to predict dementia in PD. After one year of follow-up, nearly half of these patients met criteria for dementia.[15] In the present study, 2 years and 6 months after the initial evaluation, 75% of patients with VH developed dementia.

The cognitive decline in PD patients with VH was accompanied of progressive brain atrophy involving limbic, paralimbic and neocortical areas. Gray matter reductions were extensive and bilaterally symmetric, involving both anterior and posterior cortical regions. This pattern of grey matter atrophy is comparable to that reported in PD with dementia. Specifically, cross-sectional VBM studies found that PD patients with dementia present basal ganglia,[28, 37] limbic,[27, 29] paralimbic[37] and widespread neocortical atrophy.[28, 29] Functional imaging studies showed extensive hypoperfusion billateraly involving posterior but also anterior regions.[30] In our previous cross-sectional VBM study we found that non-demented PD patients with VH showed atrophy of the visual association areas in comparison with non-hallucinating PD patients.[17] Functional imaging studies have also reported perfusion abnormalities in visual association areas in non-demented hallucinating patients.[18-21] These findings could suggest that in PD patients with VH there is an initial involvement of posterior neocortical areas extending to all associative neocortical areas when dementia is developed.

Neuropathological studies in PD propose limbic and/or neocortical Lewy bodies as the main substrate of dementia in PD.[38] The cause of VH in PD, even if for many years was thought to be a

complication of antiparkinsonian treatment, is now thought to be nerve-cell loss and Lewy-body pathology in the ventral-temporal regions of the brain.[1, 25] We recently reported hippocampal head reductions in PD with VH in comparison with healthy controls.[26] In this longitudinal study we found that the atrophy of medial temporal areas increases from base-line to follow-up suggesting that both VH and dementia may form part of the same degenerative process being VH the first expression and dementia the later. In contrast, patients without VH did not show a significant decline in the cognitive domains explored, and the gray matter loss only affected to small regions in frontal areas and cerebellum.

In our study both PD with and without VH groups had the same age and disease duration although PD patients with VH showed a more advanced stage of the illness and a lower cognitive status. These characteristics, together with the severity of motor symptoms, have been consistently associated with the presence of dementia in PD patients.[39] The remarkable 6.5 point decline in the MMSE of PD patients with VH was even greater to that reported in demented patients.[13] This could suggest that PD patients with VH were already in the starting point of a more general cognitive decline which later leaded to dementia. However, the different pattern of brain atrophy in PD patients with and without VH can not be explained by differences in general cognitive function at base-line since the grey matter loss in specific brain areas remained significant after MMSE was introduced as a covariate in the VBM analysis.

We found that the progressive atrophy in specific brain areas was associated with a decline in several cognitive domains but not with the MMSE. The correlation analyses revealed that the higher the decline in verbal memory the higher the atrophy in medial temporal and frontal areas. Specifically, the learning decline was related to hippocampal head atrophy and the worsening in free delayed recall performance was related to progressive prefrontal cortex. We previously reported correlation between hippocampal head atrophy and learning in PD [26] but this new finding gives further evidence of progressive hippocampal atrophy as neuroanatomical substrate for learning decline in PD with VH. On the other hand, delayed recall was related to gray matter atrophy in prefrontal cortex, showing that both encoding and retrieval deficits are responsible for memory dysfunction in PD. The decline in

semantic fluency showed a correlation with progressive thalamic atrophy which is in agreement with the fact that vascular lesions in the thalamus impair verbal fluency.[40] Finally, language comprehension also showed a great decline over time and was related to the progressive atrophy of medial temporal structures, specifically, the amygdala. These results supported the evidence that not only the dopamine deficiency affecting cortico-striatal-cortico information exchange in PD has an impact on cognitive dysfunction, but that structural gray matter changes may account for it.

One of the methodological limitations of the present work was that the neuropsychologist involved in the cognitive assessment at baseline and follow-up was not blind to the presence of VH, which might have had and influence on the neuropsychological results but unlikely on the neuroimaging ones. Another limitation was that our neuropsychological battery was designed to evaluate mainly temporal lobe functions. However, frontal dysfunction has been found to play an important role in the presence of VH.[3-5, 7, 8] In our study, we only used verbal fluencies to assess executive dysfunction but they have been shown to be index of the progressive deterioration of executive functions in PD.[41] In addition, following the diagnostic procedures recommended by the Movement Disorder Society Task Force, we considered demented patients only those PD patients with a MMSE below 26. We appreciate that this is a strict criterion because patients with MMSE score above this might also present dementia. Finally, the small number of patients in each group and the attrition of 33,3% in the VH group and 30% in the non VH sample is another methodological limitation of the present study.

In conclusion, this 2 years and a half follow-up of a cohort of PD patients shows that, unlike PD patients without VH, PD patients with VH frequently developed dementia which is associated with a significant gray matter loss in limbic, paralimbic and neocortical areas. Our present work gives a new insight into the study of progression of brain atrophy in PD showing a different pattern of regional atrophy in PD patients with and without VH.

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REFERENCES

[1] Williams DR, Lees AJ. Visual hallucinations in the diagnosis of idiopathic Parkinson's disease: a retrospective autopsy study. *Lancet Neurol* 2005;**4**(10):605-610.

[2] Goetz CG, Leurgans S, Pappert EJ*, et al*. Prospective longitudinal assessment of hallucinations in Parkinson's disease. *Neurology* 2001;**57**(11):2078-2082.

[3] Grossi D, Trojano L, Pellecchia MT*, et al*. Frontal dysfunction contributes to the genesis of hallucinations in non-demented Parkinsonian patients. *Int J Geriatr Psychiatry* 2005;**20**(7):668-673.

[4] Ramirez-Ruiz B, Junque C, Marti MJ*, et al*. Neuropsychological deficits in Parkinson's disease patients with visual hallucinations. *Mov Disord* 2006;**21**(9):1483-1487.

[5] Sinforiani E, Zangaglia R, Manni R*, et al*. REM sleep behavior disorder, hallucinations, and cognitive impairment in Parkinson's disease. *Mov Disord* 2006;**21**(4):462-466.

[6] Barnes J, Boubert L, Harris J*, et al*. Reality monitoring and visual hallucinations in Parkinson's disease. *Neuropsychologia* 2003;**41**(5):565-574.

[7] Barnes J, Boubert L. Executive functions are impaired in patients with Parkinson's disease with visual hallucinations. *J Neurol Neurosurg Psychiatry* 2008;**79**(2):190-192.

[8] Imamura K, Wada-Isoe K, Kitayama M*, et al*. Executive dysfunction in non-demented Parkinson's disease patients with hallucinations. *Acta Neurol Scand* 2008;**117**(4):255-259.

[9] Meppelink AM, Koerts J, Borg M*, et al*. Visual object recognition and attention in Parkinson's disease patients with visual hallucinations. *Mov Disord* 2008;**23**(13):1906-1912.

[10] Aarsland D, Andersen K, Larsen JP*, et al*. Prevalence and characteristics of dementia in Parkinson disease: an 8-year prospective study. *Arch Neurol* 2003;**60**(3):387-392.

[11] Galvin JE, Pollack J, Morris JC. Clinical phenotype of Parkinson disease dementia. *Neurology* 2006;**67**(9):1605-1611.

[12] Santangelo G, Trojano L, Vitale C*, et al*. A neuropsychological longitudinal study in Parkinson's patients with and without hallucinations. *Mov Disord* 2007;**22**(16):2418-2425.

[13] Aarsland D, Andersen K, Larsen JP*, et al*. The rate of cognitive decline in Parkinson disease. *Arch Neurol* 2004;**61**(12):1906-1911.

[14] Sinforiani E, Pacchetti C, Zangaglia R*, et al*. REM behavior disorder, hallucinations and cognitive impairment in Parkinson's disease: a two-year follow up. *Mov Disord* 2008;**23**(10):1441-1445.

[15] Ramirez-Ruiz B, Junque C, Marti MJ*, et al*. Cognitive changes in Parkinson's disease patients with visual hallucinations. *Dement Geriatr Cogn Disord* 2007;**23**(5):281-288.

[16] Diederich NJ, Fenelon G, Stebbins G*, et al*. Hallucinations in Parkinson disease. *Nat Rev Neurol* 2009;**5**(6):331-342.

[17] Ramirez-Ruiz B, Marti MJ, Tolosa E*, et al*. Cerebral atrophy in Parkinson's disease patients with visual hallucinations. *Eur J Neurol* 2007;**14**(7):750-756.

[18] Okada K, Suyama N, Oguro H*, et al*. Medication-induced hallucination and cerebral blood flow in Parkinson's disease. *J Neurol* 1999;**246**(5):365-368.

[19] Oishi N, Udaka F, Kameyama M*, et al*. Regional cerebral blood flow in Parkinson disease with nonpsychotic visual hallucinations. *Neurology* 2005;**65**(11):1708-1715.

[20] Matsui H, Nishinaka K, Oda M*, et al*. Hypoperfusion of the visual pathway in parkinsonian patients with visual hallucinations. *Mov Disord* 2006;**21**(12):2140-2144.

[21] Boecker H, Ceballos-Baumann AO, Volk D*, et al*. Metabolic alterations in patients with Parkinson disease and visual hallucinations. *Arch Neurol* 2007;**64**(7):984-988.

[22] Stebbins GT, Goetz CG, Carrillo MC*, et al*. Altered cortical visual processing in PD with hallucinations: an fMRI study. *Neurology* 2004;**63**(8):1409-1416.

[23] Ramirez-Ruiz B, Marti MJ, Tolosa E*, et al*. Brain response to complex visual stimuli in Parkinson's patients with hallucinations: a functional magnetic resonance imaging study. *Mov Disord* 2008;**23**(16):2335-2343.

[24] Nagano-Saito A, Washimi Y, Arahata Y*, et al*. Visual hallucination in Parkinson's disease with FDG PET. *Mov Disord* 2004;**19**(7):801-806.

[25] Harding AJ, Broe GA, Halliday GM. Visual hallucinations in Lewy body disease relate to Lewy bodies in the temporal lobe. *Brain* 2002;**125**(2):391-403.

[26] Ibarretxe-Bilbao N, Ramirez-Ruiz B, Tolosa E*, et al*. Hippocampal head atrophy predominance in Parkinson's disease with hallucinations and with dementia. *J Neurol* 2008;**255**(9):1324-1331.

[27] Summerfield C, Junque C, Tolosa E*, et al*. Structural brain changes in Parkinson disease with dementia: a voxel-based morphometry study. *Arch Neurol* 2005;**62**(2):281-285.

[28] Burton EJ, McKeith IG, Burn DJ*, et al*. Cerebral atrophy in Parkinson's disease with and without dementia: a comparison with Alzheimer's disease, dementia with Lewy bodies and controls. *Brain* 2004;**127**(4):791-800.

[29] Beyer MK, Larsen JP, Aarsland D. Gray matter atrophy in Parkinson disease with dementia and dementia with Lewy bodies. *Neurology* 2007;**69**(8):747-754.

[30] Wallin A, Ekberg S, Lind K*, et al*. Posterior cortical brain dysfunction in cognitively impaired patients with Parkinson's disease--a rCBF scintigraphy study. *Acta Neurol Scand* 2007;**116**(6):347-354.

[31] Ramirez-Ruiz B, Marti MJ, Tolosa E*, et al*. Longitudinal evaluation of cerebral morphological changes in Parkinson's disease with and without dementia. *J Neurol* 2005;**252**(11):1345-1352.

[32] Brenneis C, Egger K, Scherfler C*, et al*. Progression of brain atrophy in multiple system atrophy. A longitudinal VBM study. *J Neurol* 2007;**254**(2):191-196.

[33] Dubois B, Burn D, Goetz C*, et al*. Diagnostic procedures for Parkinson's disease dementia: recommendations from the movement disorder society task force. *Mov Disord* 2007;**22**(16):2314-2324.

[34] Ashburner J, Friston KJ. Voxel-based morphometry--the methods. *Neuroimage* 2000;**11**(6 Pt 1):805-821.

[35] Williams-Gray CH, Foltynie T, Brayne CE*, et al*. Evolution of cognitive dysfunction in an incident Parkinson's disease cohort. *Brain* 2007;**130**(Pt 7):1787-1798.

[36] Levy G, Jacobs DM, Tang MX*, et al*. Memory and executive function impairment predict dementia in Parkinson's disease. *Mov Disord* 2002;**17**(6):1221-1226.

[37] Summerfield C, Junque C, Tolosa E*, et al*. Structural brain changes in Parkinson disease with dementia: a voxel-based morphometry study. *Arch Neurol* 2005;**62**(2):281-285.

[38] Aarsland D, Perry R, Brown A*, et al*. Neuropathology of dementia in Parkinson's disease: a prospective, community-based study. *Ann Neurol* 2005;**58**(5):773-776.

[39] Aarsland D, Kurz MW. The epidemiology of dementia associated with Parkinson disease. *J Neurol Sci* 2009 (in press)

[40] Annoni JM, Khateb A, Gramigna S*, et al*. Chronic cognitive impairment following laterothalamic infarcts: a study of 9 cases. *Arch Neurol* 2003;**60**(10):1439-1443.

[41] Azuma T, Cruz RF, Bayles KA*, et al*. A longitudinal study of neuropsychological change in individuals with Parkinson's disease. *Int J Geriatr Psychiatry* 2003;**18**(12):1115-1120.

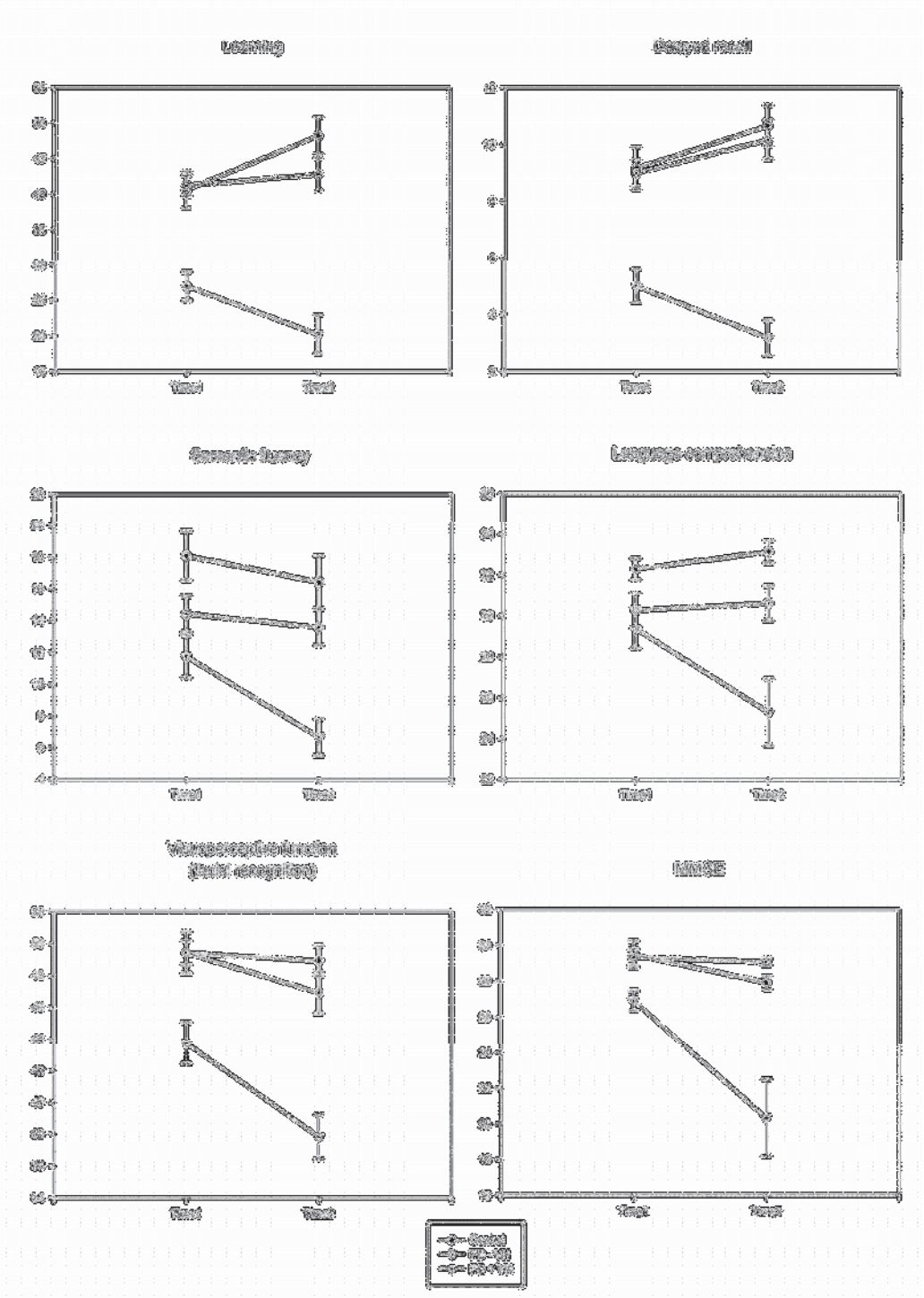

Figure 1. Plots showing the significant interactions between changes over time and group in the cognitive functions assessed. Only PD patients with VH showed cognitive decline over time. Values are mean± standard error

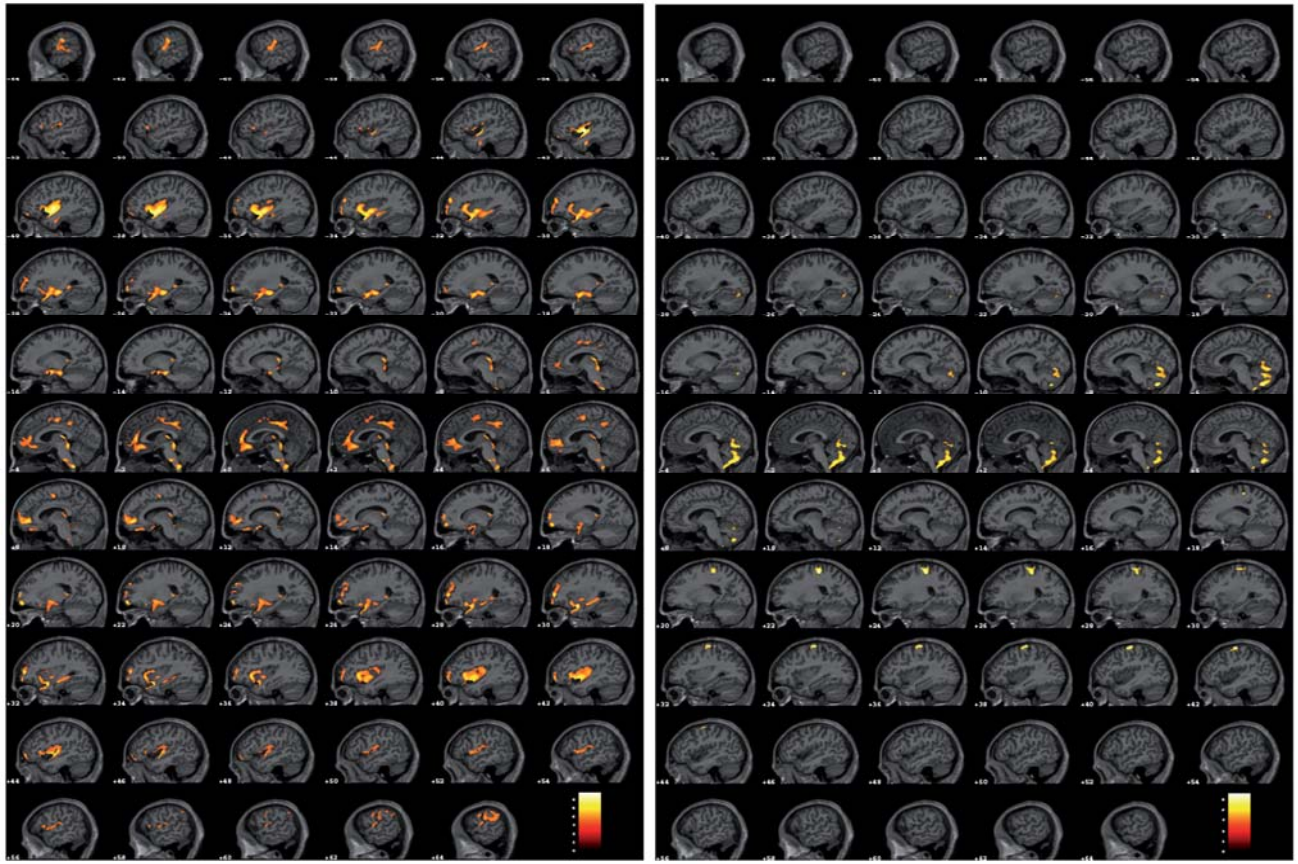

Figure 2. Bilateral sagittal display of areas showing progressive gray matter loss when comparing base-line versus follow-up MRI images assessed by VBM and introducing the MMSE score at base-line as a covariate (results are $p < 0.05$ corrected by FDR). In the left side of the figure we can see an extensive gray matter loss in PD with visual hallucinations, in the right side we can observe that PD patients without hallucination only showed gray matter loss in the cerebellum and motor cortical areas.

Suplemmental material, paper II

Table A. Clinical and neuropsychological data at base-line and at follow-up

		PD withVH (n=12)		PD without VH (n=14)		Controls (n=12)		F*	p-value*
		Base-line	Follow-up	Base-line	Follow-up	Base-line	Follow-up		
General cognitive function	Similarities	13.0±4.7	9.9±6.1	13.4±6.9	14.8±5.8	15.1±5.8	15.7±5.8	0.449	0.642
	Information	11.7±7.6	11.2±7.2	14.2±6.1	13.7±6.5	12.8±6.3	12.9±6.5	1.083	0.351
Verbal memory	RAVLT Learning	27.0±6.7	20.2±8.9	41.2±4.9	42.8±7.8	40.7±8.9	48.2±8.9	14.913	< 0.001[b, c]
	RAVLT Delayed recall	5.0±2.0	3.2±2.1	9.0±1.6	10.2±2.4	9.2±2.5	10.7±2.4	15.556	< 0.001[b, c]
	RAVLT Recognition	12.7±1.2	12.2±2.1	13.7±1.5	13.9±1.7	13.7±1.5	14.2±1.6	2.014	0.150
Visual memory	WRMF	30.9±5.4	28.6±3.9	33.4±7.2	35.3±5.4	41.3±5.5	41.3±7.0	8.145	0.001[a, c]
Frontal function	Phonetic fluency	8.5±5.6	6.3±4.5	10.0±3.5	11.5±5.4	13.0±6.6	11.4±6.0	1.809	0.180
	Semantic fluency	11.7±4.6	6.7±4.0	14.4±3.9	13.6±3.8	18.1±5.4	16.4±5.7	5.074	0.012[c]
Visuo-perceptive function	VFD	27.8±3.1	23.7±5.3	29.7±2.1	29.1±1.9	30.3±2.0	29.2±4.7	3.077	0.060
	BFR	43.7±4.6	37.8±4.82	49.42±4.4	46.9±4.5	49.4±2.6	48.9±3.2	7.546	0.002[b, c]
Language	Token Test	29.4±3.4	25.3±5.7	30.3±2.9	30.6±2.9	32.3±1.8	33.1±1.9	2.788	0.077
	BNT	48.8±6.4	43.7±10.2	52.5±5.0	51.0±7.0	53.6±4.3	53.6±4.0	2.345	0.112
Clinical scales	UPDRS	27.2±12.1	45.2±21.9	22.2±13.4	31.1±13.0	----------	----------	0.893	0.355
	H&Y	3.1±1.1	3.8±1.3	2.3±0.5	2.7±1.0	----------	----------	4.970	0.036[b]

	HDRS	7.0±4.6	8.7±6.2	3.3±3.1	4.7±5.4	3.6±3.5	3.7±4.7	5.240	0.032
	MMSE	26.9±1.9	20.3±7.5	29.3±1.6	29.1±0.7	29.5±2.6	27.9±1.3	11.079	0.003[b, c]
Medication	LEDD	846.4±381.4	821.8±191.5	879.3±580.7	1063.7±314.1	----------	----------	0.025	0.875

RAVLT: Rey's auditory verbal learning test; WRMF: Warrington's recognition memory for faces test; VFD: Visual form Discrimination; BFR: Benton Facial Recognition test; BNT: Boston Naming test; UPDRS: Unified Parkinson's disease rating scale; HDRS: Hamilton Depression Rating Scale; MMSE: Mini-mental state examination; LEDD: Levodopa equivalent daily dose.

*The F and p values refer to the MANOVA to check differences in clinical and neuropsychological variables between groups at base-line. Post-hoc analyses were performed with Tukey's test:

[a] significant differences between controls and PD without VH

[b] significant differences between PD with and without VH

[c] significant differences between controls and PD with VH

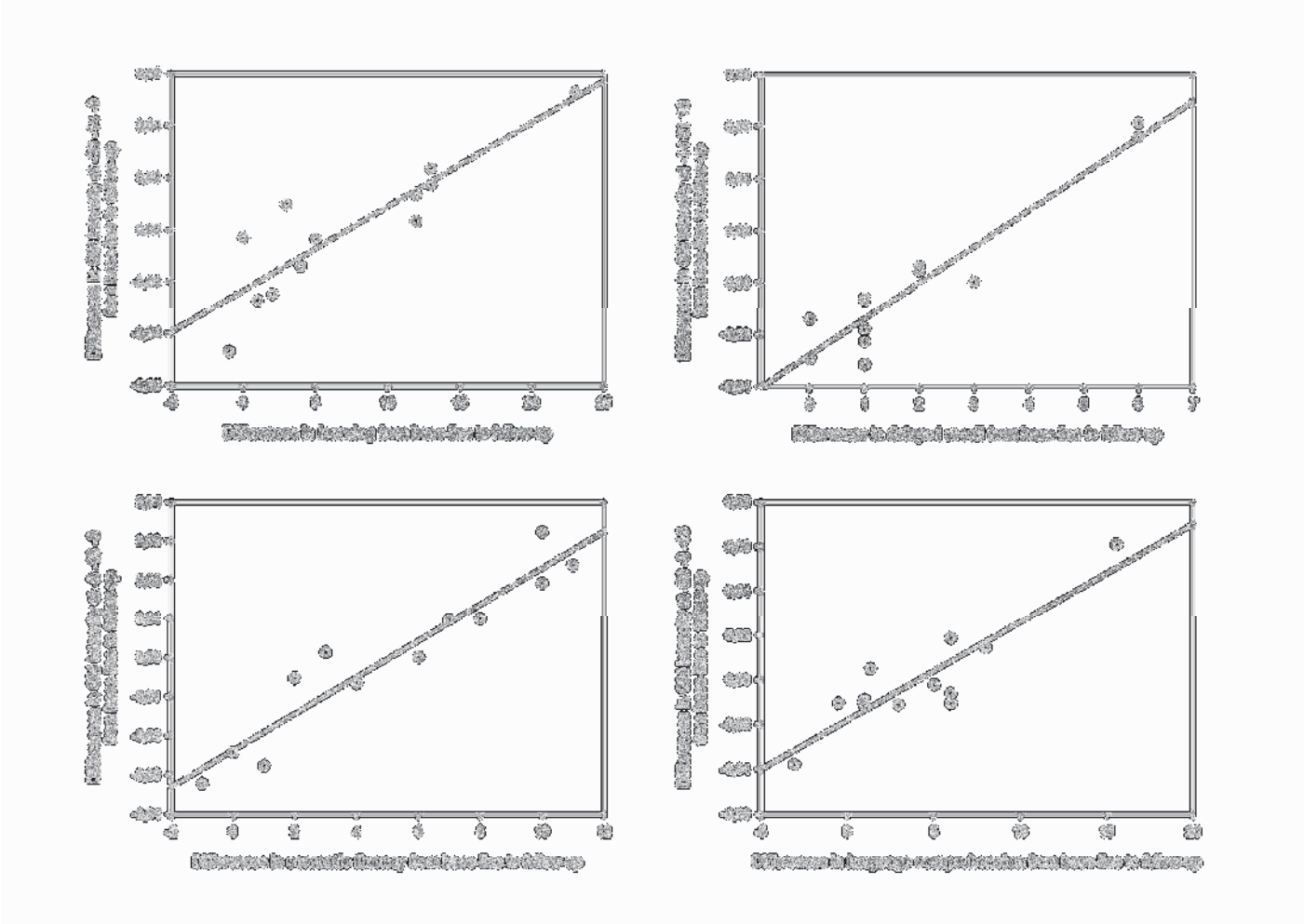

Figure A (supplemental material). Regression plots showing the relationship between gray matter loss over time and cognitive decline in PD patients with VH. The gray matter intensity refers to the voxel of the maxima of the correlation analyses with SPM5.

VBM results with ROI of the hippocampus at follow-up:

PD patients with VH presented the same pattern of diffuse hippocampal atrophy as the pattern of atrophy previously reported in PD patients with dementia (paper I). The atrophy involved the entire hippocampal axis, but with prominent involvement in both posterior and anterior regions and relative sparing of the central región (see figure A). PD patients without VH did not show hippocampal gray matter loss compared to controls at follow-up.

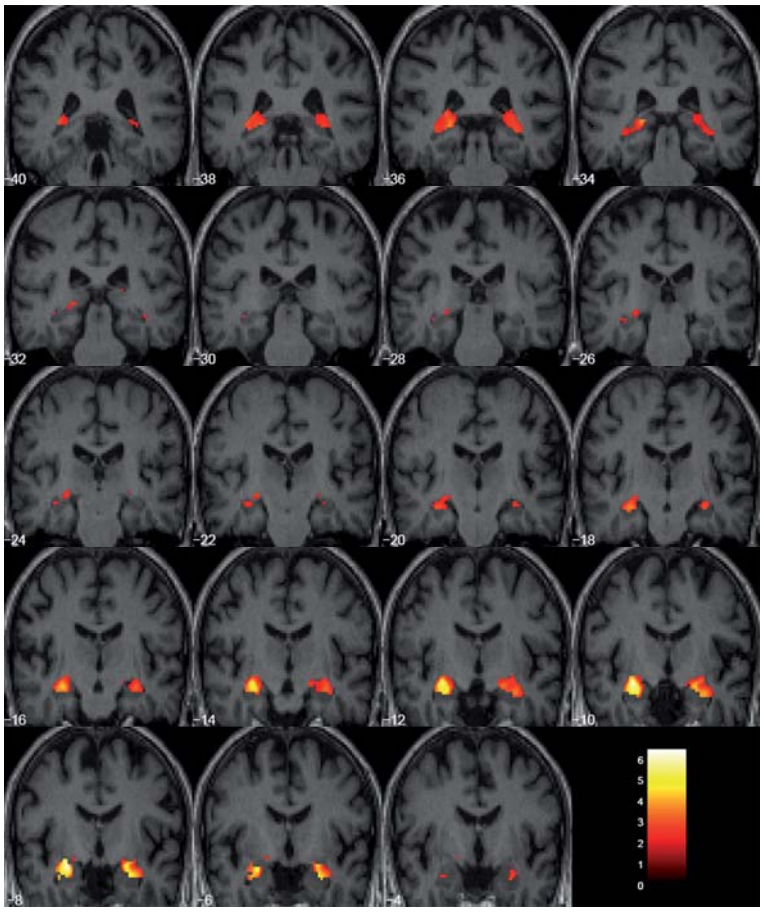

Figure B. Hippocampal gray matter loss of PD patients with VH in comparison with healthy controls at follow-up.

PAPER III

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Neuroanatomical correlates of impaired decision-making and facial emotion recognition in early Parkinson's disease

Naroa Ibarretxe-Bilbao,[1,2,3] Carme Junque,[1,2,3] Eduardo Tolosa,[1,2,4] Maria-Jose Marti,[1,2,4] Francesc Valldeoriola,[1,2,4] Nuria Bargallo[2,5] and Mojtaba Zarei[6]

[1]Centro de Investigation en Red de Enfermedades Neurodegenerativas (CIBERNED), Hospital Clinic de Barcelona, Barcelona, Spain

[2]Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), Barcelona, Spain

[3]Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Barcelona, Spain

[4]Movement Disorders Unit, Neurology Service, Institut Clinic de Neurociencies (ICN), Hospital Clinic de Barcelona, Barcelona, Spain

[5]Centre de Diagnostic per la Imatge Hospital Clinic de Barcelona (CDIC), Hospital Clinic de Barcelona, Barcelona, Spain

[6]Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB), University of Oxford, Oxford, UK

Keywords: amygdala, gambling task, magnetic resonance imaging, orbitofrontal cortex, voxel-based morphometry

Abstract

Decision-making and recognition of emotions are often impaired in patients with Parkinson's disease (PD). The orbitofrontal cortex (OFC) and the amygdala are critical structures subserving these functions. This study was designed to test whether there are any structural changes in these areas that might explain the impairment of decision-making and recognition of facial emotions in early PD. We used the Iowa Gambling Task (IGT) and the Ekman 60 faces test which are sensitive to the integrity of OFC and amygdala dysfunctions in 24 early PD patients and 24 controls. High-resolution structural magnetic resonance images (MRI) were also obtained. Group analysis using voxel-based morphometry (VBM) showed significant and corrected ($P < 0.05$ FEW-small volume correction) gray matter (GM) loss in the right amygdala and bilaterally in the OFC in PD patients. Volumetric analyses were also performed but did not yield significant differences between groups. Left lateral GM volume in OFC showed a slight correlation with the IGT, and bilateral OFC GM was strongly correlated with Ekman test performance in PD patients. We conclude that: (i) impairment in decision-making and recognition of facial emotions occurs at the early stages of PD, (ii) these neuropsychological deficits are accompanied by degeneration of OFC and amygdala, and (iii) bilateral OFC reductions are associated with impaired recognition of emotions, and GM volume loss in left lateral OFC is related to decision-making impairment in PD.

Introduction

Neuropsychological studies using the gambling task have shown impairment of decision-making in early (Perretta *et al.*, 2005; Kobayakawa *et al.*, 2008) and advanced PD (Mimura *et al.*, 2006; Pagonabarraga *et al.*, 2007). In addition, several studies have reported impairment of recognition of facial expressions of emotions in PD. A general impairment in the recognition of all emotions (Yip *et al.*, 2003, 2003) or specific impairment in some types of emotion have been described (Kan *et al.*, 2002; Sprengelmeyer *et al.*, 2003; Suzuki *et al.*, 2006; Lawrence *et al.*, 2007; Ariatti *et al.*, 2008; Clark *et al.*, 2008). However, some authors failed to detect differences between patients and controls (Adolphs *et al.*, 1998; Pell & Leonard, 2005).

Correspondence: C. Junque, [3]Departament de Psiquiatria i Psicobiologia Clinica, as above.
E-mail: cjunque@ub.edu

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The orbitofrontal cortex (OFC) has been identified as a crucial structure in decision-making (Fellows & Farah, 2005; Denburg *et al.*, 2007; Wallis, 2007). Decision-making performance has been mostly assessed by the Iowa Gambling Task (IGT) (Bechara *et al.*, 1999); lesion studies in the OFC have reported impaired performance on this task (Bechara, 2004). The OFC is also a crucial structure in the recognition of facial expressions of emotions (Adolphs, 2002a,b). PET studies have reported activation in orbital regions when recognizing facial expressions of emotions (Dolan *et al.*, 1996; Blair *et al.*, 1999; Nakamura *et al.*, 1999), and bilateral or unilateral lesions in the OFC may impair emotional face expression identification (Hornak *et al.*, 2003; Heberlein *et al.*, 2008); however, lesions elsewhere in the frontal cortex (dorsal or lateral) do not impair this function (Heberlein *et al.*, 2008). The OFC is associated with medial temporal limbic structures that are critical for the processing of internal states such as affect and motivation which in turn affect the decision-making process (Miller & Cohen, 2001). The amygdala is involved in the process of decision-making (Bechara *et al.*, 1999) as well as in recognition of

facial expressions of emotions (Adolphs *et al.*, 1995, 1999; Adolphs, 2002a,b; Sato *et al.*, 2002), mainly in the recognition of fear (Calder *et al.*, 1996).

Brain pathology in PD is characterized by the development of Lewy neurites (LNs) and Lewy bodies (LBs), and evolves according to a predictable ascendant topographical sequence affecting limbic areas early in the disease course (Braak *et al.*, 2003). The amygdala is considered to be one of the key structures of the limbic system and the OFC is the limbic portion of the frontal association cortex (Porrino *et al.*, 1981). The patients in our study are in the early stages of the disease and we think that structural changes assessed by MRI may already be detected in the OFC and amygdala of these patients.

In this study, we used voxel-based morphometry (VBM) to examine the neuroanatomical correlates of emotion recognition and decision-making performance in PD. Previous studies with VBM reported associations between cognitive performance and anatomical MRI data in PD. One study reported bilateral temporal lobe and left precentral gyrus atrophy in PD patients with mild cognitive impairment compared to PD patients without cognitive impairment (Beyer *et al.*, 2007). In addition, Nagano-Saito *et al.* (2005); found that scores on Raven Coloured Progressive Matrices (RCPM) correlated positively with GM density in the dorsolateral prefrontal cortex and the parahippocampal gyrus, and our group recently reported a correlation between GM density in the head of hippocampus and verbal learning scores assessed by Rey's Auditory Verbal Learning Test (RAVLT) (Ibarretxe-Bilbao *et al.*, 2008). The present study also uses the correlation approach to identify the structural bases of neuropsychological dysfunctions in early PD.

Specifically, this study was designed to examine two questions: (i) does impairment in decision-making and recognition of facial expression of emotions occur at the early stages of PD? And, if so, (ii) are these impairments associated with possible degeneration of OFC and amygdala detectable by T1W MRI images? Finding answers to these questions may broaden our knowledge of the non-motor deficits associated with PD and improve our understanding of the structural changes underlying these deficits.

Materials and methods

Subjects

This study was approved by the institutional ethics committee. All subjects provided written informed consent specifically to participate in this study.

Twenty-four patients with PD and 24 healthy controls (HC) took part in this study. Patients were recruited from the Parkinson's Disease Movement Disorders Unit, Neurology Service, Hospital Clinic, Barcelona. HC were recruited from friends and spouses of patients and matched by age, gender and years of education.

All patients fulfilled the UK PD Society Brain Bank (PDSBB) diagnostic criteria for PD (Daniel & Lees, 1993). Other inclusion criteria for patients were: (i) age 40–65 years; (ii) Hoehn and Yahr stage < II; (iii) disease duration < 5 years; and (iv) absence of motor fluctuations. Exclusion criteria for all subjects were: (i) the presence of dementia that was diagnosed by a neurologist according to the Movement Disorder Society diagnostic criteria for Parkinson disease dementia (Dubois *et al.*, 2007); (ii) the presence of other neurological or psychiatric disorders such as depression that was evaluated by means of the Beck's Depression Inventory (BDI-II); and (iii) the presence of visual hallucinations assessed by the Neuropsychiatric Inventory Questionnaire (NPI-Q).

Three patients were taking no medication and 21 were on anti-Parkinsonian treatment at the time of investigation: MAO-B inhibitor (n = 7), L-dopa monotherapy (n = 5), dopamine agonist monotherapy (n = 3) or a combination of L-dopa and dopamine agonist (n = 6). All patients were symptomatically stable and no patient was asked to change her/his medication for this study. None of the patients were receiving psychoactive medication at the moment of the study because all psychiatric characteristics such as depression and the presence of visual hallucinations were considered exclusion criteria. Clinical and sociodemographical aspects of the sample are summarized in Table 1.

The demographic data show no differences in age, gender proportion and years of education between groups (Table 1), suggesting that the PD and HC groups were well matched. In addition, Mini-Mental State Examination (MMSE) showed no difference between groups. There were no significant differences between PD and HC in depression and NPI-Q scores. Table 1 also shows that the motor deficit in the PD group was mild according to the Hoehn and Yahr staging (stage I: 10 patients; stage II: 14 patients) and the Unified Parkinson's Disease Rating Scale motor section.

Neuropsychological assessment

We selected two tasks that have been shown to be sensitive to OFC dysfunctions: the Iowa Gambling Task (IGT) for assessing decision-making, and the Ekman test for recognition of facial expressions of emotions. In addition, Conners' Continuous Performance Test II (CPT II), a test that measures sustained attention, was included because sustained attention may influence performance on other neuropsychological tests and because this test has been reported to be sensitive to frontal dysfunctions (Cabeza & Nyberg, 2000). Vocabulary subtest and backward digit span from the Wechsler Adults Intelligence Scale (WAIS-III) were also included as a measure of premorbid intelligence quotient (IQ) and working memory respectively (Lezak *et al.*, 2004) since these functions have been related to performance in emotion recognition (Assogna *et al.*, 2008; Mathersul *et al.*, 2009) and gambling tasks (Dunn *et al.*, 2006).

Decision-making

Decision-making was assessed by the computerized version of the IGT (Bechara *et al.*, 1999). The task consists of four decks of cards, A, B, C, and D; subjects have to choose between decks that yield high

TABLE 1. Demographic and clinical characteristics of the sample

	HC (n = 24)	PD (n = 24)
Age	57.58 ± 8.9	56.13 ± 8.5
Gender (male/female)	16/8	16/8
Education (years)	13 ± 3.8	10.96 ± 5.4
MMSE	29.83 ± 0.4	29.63 ± 0.5
BDI-II	4.46 ± 5.1	6.75 ± 4.8
NPI	1.38 ± 1.7	3.88 ± 7.1
Disease evolution (years)	–	3.06 ± 1.6
Predominance (left/right)	–	15/9
UPDRSIII	–	14.67 ± 3.5
H&Y	–	1.73 ± 0.4
Total LEDD	–	299.58 ± 321.1

Values are mean ± SD. HC, Healthy controls; PD, Parkinson's disease; MMSE, Mini-Mental State Examination; BDI-II, Beck's Depression Inventory; NPI, Neuropsychiatry Inventory; UPDRS III, Unified Parkinson's Disease Rating Scale (motor section); H&Y, Hoehn and Yahr staging; LEDD, levodopa equivalent daily dose.

immediate gain but larger future loss (A and B) and decks that yield low immediate gain but smaller future loss (C and D). These reward/punishment schedules are pre-programmed and known to the examiner, but not to the subject. The scores consisted of the numbers of advantageous choices (C ± D) minus disadvantageous choices (A ± B) for each of the 5 blocks of 20 cards and for the total of the 100 cards.

Recognition of facial expressions of emotions

We selected the Ekman 60 Faces test to assess the abilities for recognition of facial emotion expressions. A series of pictures of faces (six female, four male) from the Ekman and Friesen series of Pictures of Facial Affect (Ekman & Friesen, 1976) are presented. For each face, subjects must decide whether the expression corresponds to anger, disgust, fear, happiness, sadness or surprise. The Spanish words that we used for the basic emotions were enfado (anger), asco (disgust), miedo (fear), alegria (happiness), tristeza (sadness) and surprise (sorpresa). The faces were each shown for 5 s, but the subject could take as long as wished to decide on the emotion. There are ten facial expressions of each emotion, leading to a score out of a maximum of 60 for overall performance, or scores out of 10 for recognition of each of the six basic emotions.

Statistical analysis

Analyses were carried out using the Statistical Package for Social Sciences V14.0 (SPSS Inc, Chicago, IL, USA). Group differences in demographic, clinical, cognitive and behavioral characteristics test were analyzed with independent two-tailed t tests for normally distributed variables, the Mann–Whitney test for non-normally distributed, and the chi-squared test for categorical variables. Repeated measures analyses of variance (ANOVAs) were used to assess the differences between PD and HC in IGT performance across blocks (within-subject factor: IGT performance across five blocks; between-subject factor: group) and recognition of specific emotions (within-subject factor: emotions; between-subject factor: group). In the case of non-sphericity, a Greenhouse-Geisser correction was applied to the degrees of freedom. Associations between the neuropsychological test and the demographic, clinical and cognitive variables were analyzed with Spearman's rank correlations and P values were corrected for multiple comparisons using Bonferroni's test.

Image acquisition and analysis

Images were acquired using a TIM TRIO 3T scanner (Siemens, Germany). A set of high-resolution 3-dimensional T1-weighted images was acquired with a MPRAGE sequence in sagittal orientation (TR/TE = 2300/2.98 ms; TI = 900 ms; 256 × 256 matrix, 1 mm isotropic voxel).

Voxel-based morphometry analysis

Structural data were analyzed with FSL-VBM (Douaud *et al.*, 2007), a voxel-based morphometry style analysis (Ashburner & Friston, 2000) was carried out with FSL software (Smith *et al.*, 2004). First, non-brain tissue from structural images was extracted. Next, tissue-type segmentation was carried out and the resulting gray matter (GM) partial volume images were aligned to MNI152 standard space using the affine registration. The resulting images were averaged to create a study-specific template, to which the native GM images were then non-linearly re-registered. The registered partial volume images were then modulated (to correct for local expansion or contraction) by dividing by the Jacobian of the warp field. The modulated segmented images were then smoothed with an isotropic Gaussian kernel with a sigma of 3.5 mm (8 mm FWHM).

A permutation-based non-parametric inference method within the framework of the general linear model (Nichols & Holmes, 2002) was used to investigate the changes in the distribution of GM between groups and to correlate the neuropsychological performance with the GM loss in PD and HC. Total GM volume was calculated from segmented images and introduced as covariate in correlation analyses. To look specifically at the gradients of GM loss within the amygdala and OFC that were of particular interest in this study we performed region of interest (ROI)-based analyses of these areas. The threshold was set at $P < 0.05$, Family Wise Error (FWE) correction for multiple comparisons in small volumes [small volume correction (svc)] (Worsley *et al.*, 1996). ROI of the amygdala was 642 voxels in size and it was defined using the Harvard-Oxford probabilistic subcortical atlas (http://www.fmrib.ox.ac.uk/fsl/fslview/atlas-descriptions.html) thresholded at 70%. The OFC was 14863 voxels in size and it was defined following the parcellation proposed by Lacerda *et al.* (2003) and included six major anatomical subregions (from medial to lateral): gyrus rectus (BA 14), medial orbital gyrus (BAs 11 and 13), anterior orbital gyrus (BA 11), posterior orbital gyrus (BA 13), lateral orbital gyrus (BA 12). Brodmann maps were extracted from the MRIcron and then were registered into the standard MNI152 T1 2 mm template available in FSL atlas tools. Correlation analyses focused mainly on the relationship between OFC and amygdala volume and neuropsychological tasks.

The statistical threshold was set at $P < 0.05$ Family Wise Error (FWE) corrected for multiple comparisons within the ROI. We then extracted the mean GM values from anatomically defined OFC ROIs and plotted the relationship with Ekman and IGT tests. The r values were also calculated from the mean GM of OFC ROI, and not from the maxima, to avoid the non independence error (Poldrack & Mumford, 2009).

Volumetric analysis

In addition to VBM analysis, volumetric analysis of the OFC, amygdala and primary olfactory cortex were performed. First, native images were segmented into GM, WM and CSF. Then, the standard masks of the structures were normalized to the gray matter native space of each subject and finally the volumes were obtained using matlab (spm_volumes extraction option). We obtained raw volumes in mm^3 of the right and left sides of each of the measured structures.

Results

Neuropsychological results

Table 2 shows the difference in performance between PD and HC. Patients obtained significantly worse scores in the gambling task and in the total score of recognition of facial expressions of emotions but not in the measure of sustained attention (detectability) and the reaction time measured by the CPT. There were no significant differences in the vocabulary scores between groups. However, reverse digit span was significantly shorter in PD patients than in the HC group.

There was a correlation between IGT and Ekman total score ($r = 0.41$, $P = 0.02$), but neither test correlated with other neuropsychological, clinical or demographical data except for a significant correlation between IGT and reverse digit span (for Spearman rank order correlation coefficients see Table 3).

TABLE 2. Neuropsychological differences between PD and HC

	HC (n = 24)	PD (n = 24)	P	P_{corr}
IGT (CD minus AB)	36.50 ± 22.7	5.16 ± 30.7	0.001	0.004
Ekman total	51 ± 4.5	42.5 ± 10.5	0.001	0.004
CPT II (d')	0.84 ± 0.4	0.82 ± 0.3	0.880	0.999
CPT II (Hit RT)	420.88 ± 61.5	456.97 ± 58.4	0.043	0.197
Vocabulary	46.67 ± 7.0	41.25 ± 12.1	0.067	0.293
Backward digit span	7.21 ± 1.7	5.38 ± 1.9	0.001	0.004

Values are mean ± SD. HC, Healthy controls; PD, Parkinson's disease patients; P corr, values corrected by Bonferroni; IGT, Iowa Gambling Task; CPT II, Conners' Continuous Performance Test II; (d'), detectability; Hit RT, Hit reaction time.

Decision-making

A repeated measures ANOVA was conducted on the performance of PD patients and HC across IGT blocks. This revealed a significant effect of group [$F_{1,46}$ = 14.56, P = 0.001], a significant effect of IGT block [$F_{2,44,\ 112,19}$ = 28.49, P = 0.001] and a significant group × IGT block interaction [$F_{2,44,\ 112,19}$ = 7.55, P = 0.001]. PD patients and HC performed in a similar way in the first and second blocks (first 40 cards). From the third block onwards, PD patients selected less advantageous decks than HC and the differences in performance between groups become more pronounced as the task advanced (see Fig. 1).

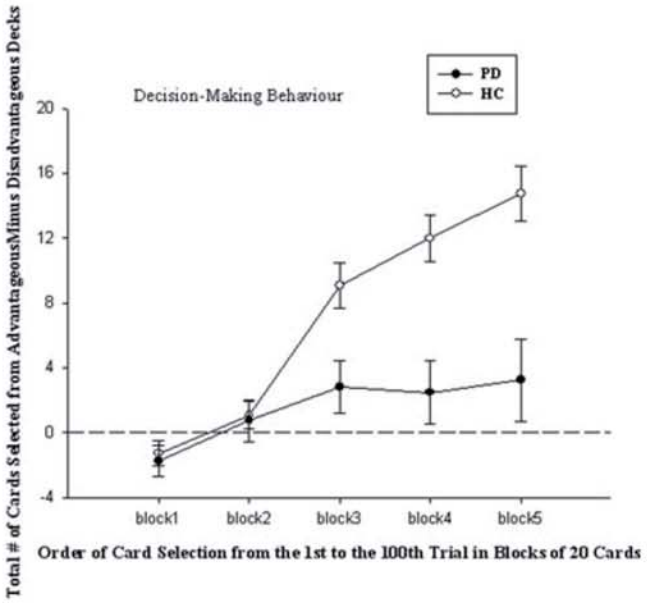

FIG. 1. Gambling task performance in PD and HC across blocks: PD performance on gambling task was significantly worse than HC. Values are expressed as total number of cards (mean ± SEM) selected from advantageous minus disadvantageous decks from the 1st to the 100th trial in five blocks of 20 cards.

Recognition of facial expression of emotions

The repeated measures ANOVA in the performance of facial recognition of basic emotions showed a significant effect of group [$F_{1,46}$ = 19.58, P = 0.001], a significant effect of emotion [$F_{4,22,\ 194,26}$ = 29.27, P = 0.001] and a significant group × emotion interaction [$F_{4,22,\ 194,26}$ = 2.48, P = 0.042]. PD patients obtained lower scores than HC in all emotions except happiness (see Fig. 2). Performance on recognition of happiness was very similar between PD and HC and a ceiling-effect was observed for this emotion (see Fig. 2)

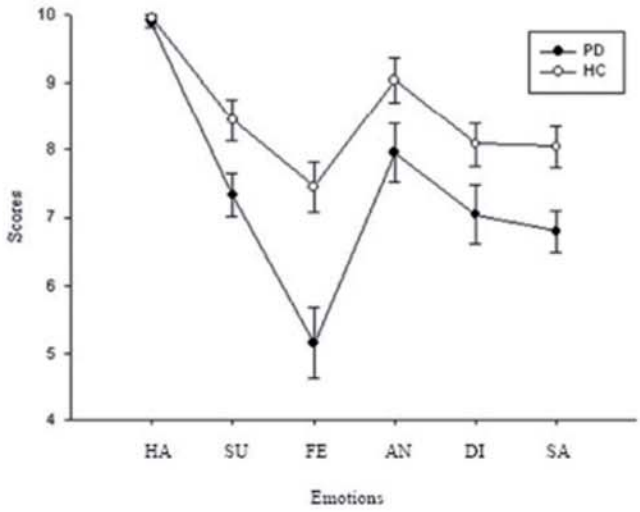

FIG. 2. Mean ± SEM of recognition of facial expressions for each of the six basic emotions in PD and HC. HA, happiness; SU, surprise; FE, fear; AN, anger; DI, disgust; SA, sadness.

Voxel-based morphometry (VBM) results

Group comparisons

ROI analysis showed that PD patients had significant volume loss in the right amygdala and bilateral OFC in comparison with HC (see Table 4 and Fig. 3). Close inspection of GM loss in amygdala shows that degeneration affects the medial and basal aspect of this structure. OFC shows a gradient of degeneration that is worse in the left ventrolateral area; in the right part the differences are more dorsally and medially located. We also performed a comparison between groups using a ROI of the primary olfactory cortex (piriform and entorhinal cortex) because, according to the Braak stages, these areas are believed to be affected earlier than OFC. However, we did not find significant results at $P < 0.05$ corrected for multiple comparisons.

TABLE 3. Correlation matrix between decision-making and recognition of emotions and other neuropsychological tasks, clinical and sociodemographical variables

	Age	Education	Vocabulary	NPI	Digit span	BDI	CPT (d')	HitRT	H&Y	UPDRS	LEED
IGT	−0.27	0.25	0.26	−0.12	0.48**	−0.19	0.07	−0.22	0.29	0.19	−0.09
Ekman	−0.28	0.26	0.22	−0.10	−0.29*	−0.20	0.19	−0.13	−0.34	−0.24	−0.17

Values correspond to Spearman rank order correlation coefficients. *$P < 0.05$; **$P < 0.01$. IGT, Iowa Gambling Task (total score); Ekman, Ekman 60 faces test (total score); CPT, Continuous Performance Test; (d'), detectability; Hit RT, Hit reaction time in the CPT; BDI, Beck's Depression Inventory; NPI, Neuropsychiatric Inventory; UPDRS III, Unified Parkinson's Disease Rating Scale (motor section); H&Y, Hoehn and Yahr staging; LEDD, levodopa equivalent daily dose.

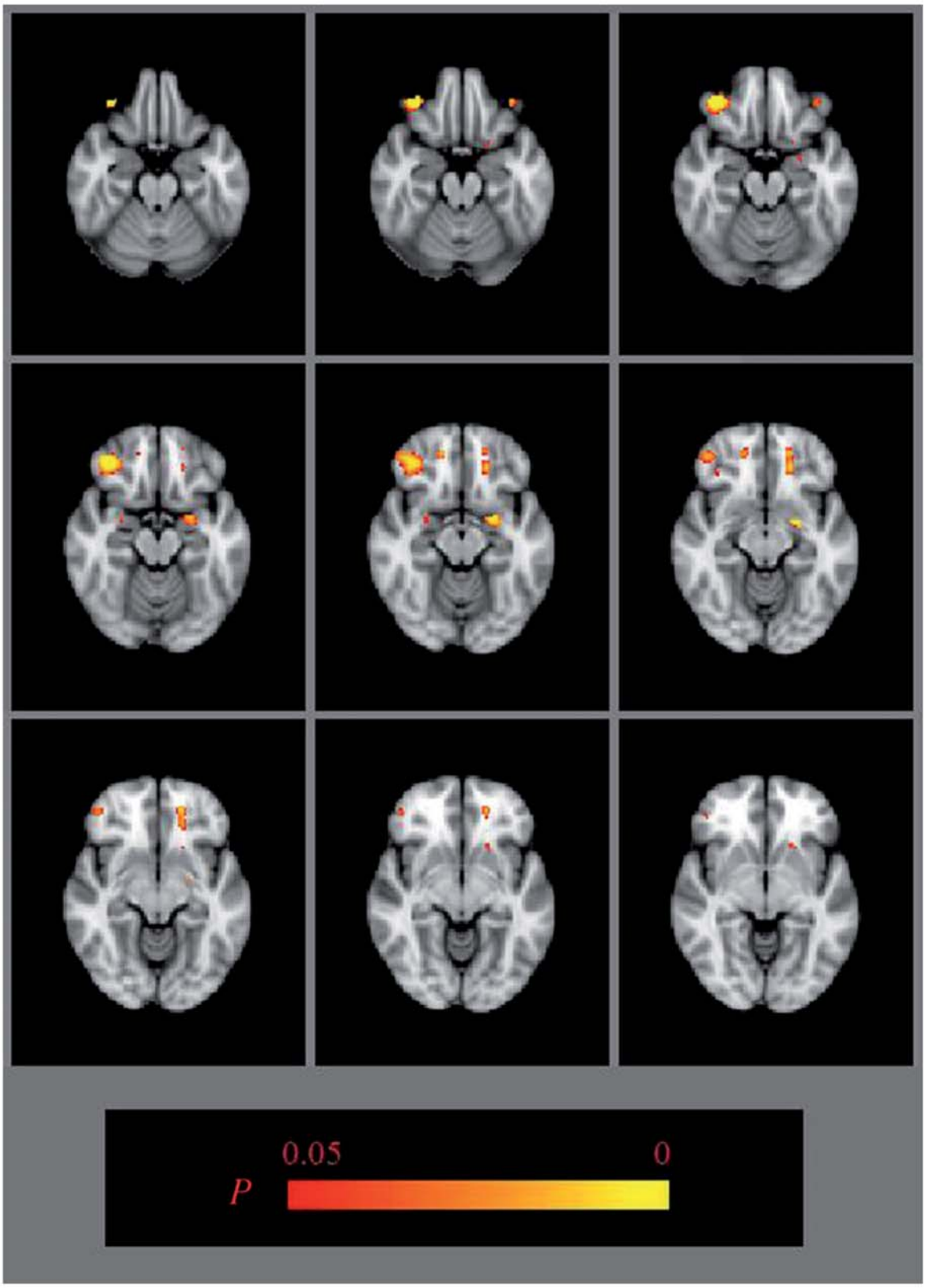

FIG. 3. ROI analysis on amygdala and OFC contrasting HC > PD showed significant loss in the right amygdala and bilateral OFC. Close inspection of GM loss in amygdala shows that degeneration occurs in the medial and basal aspect of this structure. OFC shows a gradient of degeneration that is worse in the left ventrolateral area and in the right part is dorsomedially located. The threshold was set at $P < 0.05$, Family Wise Error (FEW) corrected for multiple comparisons using small volume correction (svc).

TABLE 5. Correlations between OFC volume and neuropsychological tests in PD after adjusting for total gray matter volume

Structure	MNI	Cluster size (mm^3)	Cluster threshold	r	P
Correlation with Ekman					
Bilateral OFC	−30, 52, 22	36192	0.001	0.58	0.003
Correlation with IGT					
Left OFC (BA47)	−48, 22, 12	600	0.021	0.49	0.011

Cluster size denotes the extent of the cluster of significant voxels in cubic millimetres. MNI coordinates refer to the location of the maxima and the r values are not calculated from this maxima but from the mean GM of an anatomically defined OFC ROI to avoid the non independence error. Cluster threshold corrected for P values are corrected for multiple comparisons using FWE.

lesions of the OFC to support this theory (Bechara, 2004). However, there is an alternative interpretation of the failure of OFC patients to perform well on the IGT: the fact that decks A and B, which turn out to be bad in the longer term, initially appear very good raises the possibility that the poor performance of these patients on the IGT is based on a reversal learning deficit (Maia & McClelland, 2004; Fellows & Farah, 2005; Dunn *et al.*, 2006). In our study, PD and HC groups presented similar performance on the IGT until trial 40 (block 3), but the control group continued to improve afterwards, whereas the PD group did not. PD patients failed to shift their choices to decks C and D when the initially high-gain decks A and B began to lose large sums of money. IGT total scores in our PD sample correlated with GM volume in OFC. However, the correlation was observed in the lateral part of the orbitofrontal cortex - not in the ventromedial region, as the previous findings of Bechara *et al.* would lead us to expect. Our correlational results partially agree with functional MRI studies in healthy subjects that showed that activation in lateral OFC (BA47) was positively associated with gambling task performance (Lawrence *et al.,* 2009). In addition, lateral OFC is related to the evaluation of punishers (O'Doherty *et al.,* 2001) which may lead to a change in ongoing behavior (Kringelbach & Rolls, 2004).

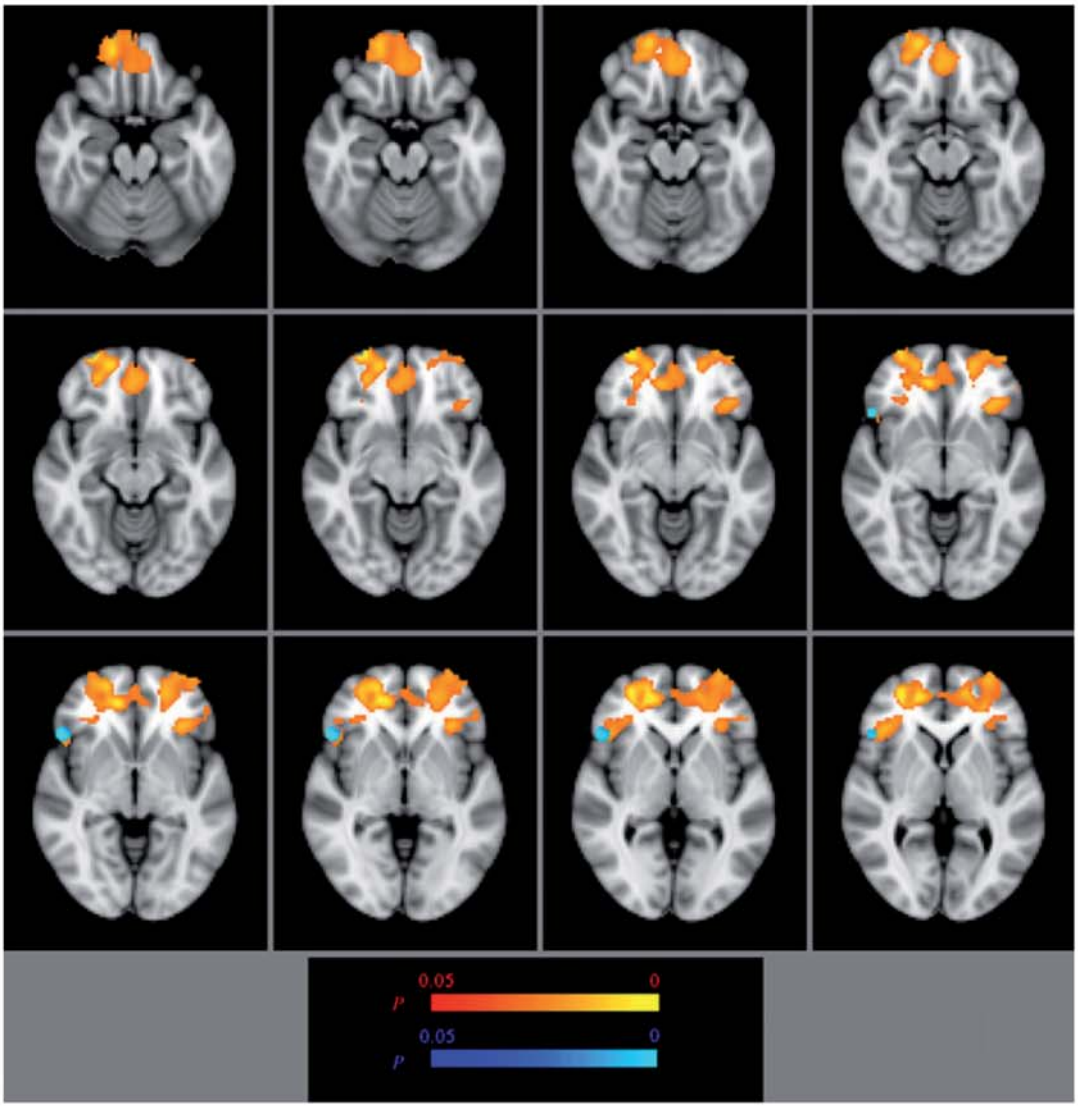

FIG. 4. Left lateral OFC GM volume is correlated with performance on the IGT (lower calibration bar, see blue in on-line graphic) and widespread bilateral OFC GM volume with Ekman total score (upper bar, yellow in on-line graphic) in PD patients. The threshold was set at $P < 0.05$, Family Wise Error (FEW) corrected for multiple comparisons within the ROI of the OFC.

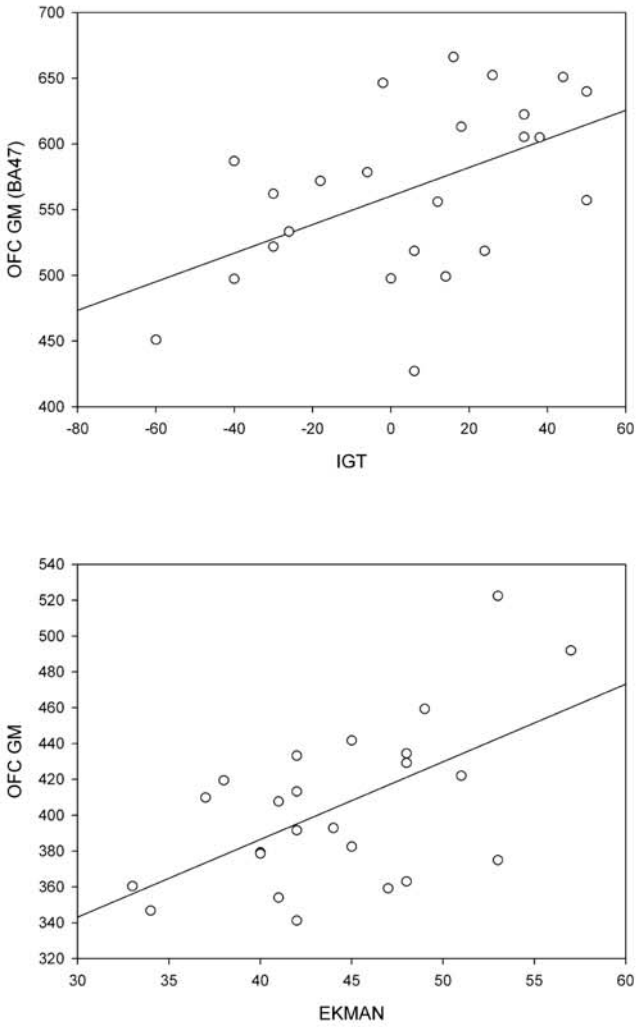

FIG. 5. In the top part, scatterplot showing the relationship between lateral OFC (BA47) mean GM and IGT total scores in PD patients. In the bottom, the scatterplot of the relationship between the bilateral OFC mean GM and Ekman total scores in PD.

TABLE 6. Volumetric measures in native space of OFC, amygdala and primary olfactory areas (entorhinal cortex and piriform cortex) in PD and HC

Structure		HC (n = 24)	PD (n = 24)
OFC	R	24858.5 ± 3813.4	24660.7 ± 4301.1
	L	25517.9 ± 3815.7	24483.3 ± 4974.3
Primary olfactory cortex	R	5015.0 ± 641.7	4948.5 ± 673.5
	L	4450.6 ± 521.7	4382.8 ± 619.7
Amygdala	R	846.5 ± 437.2	831.2 ± 385.7
	L	790.3 ± 383.5	763.9 ± 362.2
TIV		1645016.2 ± 119628.7	1644593.4 ± 149701.1

Values are volumes (mean ± SD) in mm. HC, Healthy controls; PD, Parkinson's disease; OFC, orbitofrontal cortex; TIV, total intracraneal volume; R, right; L, left.

Patients with amygdala lesions have also been reported to be impaired in IGT (Bechara *et al.*, 1999). However, we found no correlation between gambling score and GM loss in amygdala in PD patients. This lack of correlation is in line with recent work in non-human primates, suggesting that OFC and amygdala make distinct contributions to the decision-making process based on the findings that monkeys with amygdala lesions are able to flexibly change stimulus-reward associations (Rudebeck & Murray, 2008).

We found impairment of recognition of facial expressions of emotions in early PD patients. The existing neuropsychological literature on facial emotion recognition in PD is quite mixed. Some studies did not find emotion recognition deficits (Adolphs *et al.*, 1998; Pell & Leonard, 2005) whereas the studies that reported impairment in this ability are divided into those that reported emotion-specific impairment (Kan *et al.*, 2002; Sprengelmeyer *et al.*, 2003; Suzuki *et al.*, 2006; Lawrence *et al.*, 2007; Ariatti *et al.*, 2008; Clark *et al.*, 2008) and those that claimed a broad impaired recognition of facial expressions of emotions in PD (Yip *et al.*, 2003; Dujardin *et al.*, 2004). Our PD patients scored significantly lower in the recognition of all six basic emotions except in happiness. These findings are in line with studies reporting a broad impairment of facial emotion recognition, even though those studies were carried out in patients at a more advanced stage of the disease (Yip *et al.*, 2003; Dujardin *et al.*, 2004).

The inconsistency of the results across studies in recognition of facial emotion expression in PD has been attributed to factors such as emotion assessment, perception deficit, cognitive impairment, behavioral symptoms, illness severity and anti-Parkinsonian therapy (Assogna *et al.*, 2008). However, we did not find any correlation between Ekman recognition scores and clinical or demographical variables. Another problem that has been reported relates to the conventional methods (that is, forced-choice labeling task of prototypical facial expressions) that have been used to assess recognition of emotions because of the differential difficulty levels across emotions (Suzuki *et al.*, 2006). Indeed, we found a ceiling effect in the case of recognition of happiness and the greatest differences were found in the recognition of fear, which is known to be the most difficult emotion to recognize. In the light of all these results, we are not in a position to claim that early PD impairs the recognition of one specific emotion above others, but the disease definitely influences the general recognition of emotions. Actually, total scores on the Ekman test correlated with bilateral OFC GM volume in PD patients but not in HC. The lack of correlation in the control group could be driven by a ceiling effect on the task. The OFC is a crucial structure in the recognition of facial expressions of emotions (Adolphs, 2002a,b). PET studies have reported activation in orbital regions when recognizing facial expressions of emotions (Dolan *et al.*, 1996; Blair *et al.*, 1999; Nakamura *et al.*, 1999) and lesions in the OFC can impair emotional face expression identification (Hornak *et al.*, 2003; Heberlein *et al.*, 2008). Right rather than left hemisphere lesions are related to impaired recognition of emotions (Adolphs *et al.*, 1996). In our study, the maxima of the correlation between OFC GM volume and Ekman test corresponded to the left hemisphere. However, there was an extensive correlation with both hemispheres including both lateral and medial aspects of the OFC. Evidence from behavioral and lesion studies do suggest that different structures are activated by different emotions (Posamentier & Abdi, 2003). The best established role is that of the amygdala in the recognition of fear, supported by lesion (Calder *et al.*, 1996; Adolphs, 2002a,b; Sato *et al.*, 2002) and functional studies (Morris *et al.*, 1996; Whalen *et al.*, 1998) but we did not find a correlation between amygdalar volume and recognition of fear.

In our sample, performance on decision-making and recognition of emotions was independent of sustained attention and vocabulary scores, but not independent of reverse digit span. A role of working memory in the performance of the IGT has been suggested previously (Dunn *et al.*, 2006) but these two functions are known to depend on

different structures: the gambling task is related more to the orbitofrontal cortex and working memory to the dorsolateral prefrontal cortex (Bechara *et al.*, 1998).

VBM identified regional differences between HC and PD. However, volumetric analysis did not yield significant differences between the two groups. The discrepancy between the two techniques may reflect a greater sensitivity of VBM to subtle changes in early stages of the disease. On the other hand, the normalization stage within the VBM analysis, which is required to ensure that the same brain regions can be compared between subjects, transforms the shape of the brain image and may distort the abnormal tissue and artificially inflate the atrophied areas (Mechelli *et al.*, 2005). The volumetric approach does not present this problem and takes into consideration the anatomical brain variability across subjects. In this study, the VBM analysis was performed using a non-linear registration method that estimates an exact match between brain structures. In addition, an exhaustive and careful visual inspection was carried out after normalization and segmentation, ensuring that the areas of atrophy we found are unlikely to be systematic shape differences attributable to misregistration from the spatial normalization step.

In conclusion, we found evidence of OFC structural and functional deficits in the early stages of PD. Structurally, patients have bilateral gray matter reductions in this region. Functionally, we found impairments in the recognition of facial emotions and in learning the rules of a cognitive task according to the contingency of reward. We also found correlations between structure and function, suggesting that the neuropsychological deficits reflect the underlying gray matter loss. These results are in agreement with recent literature suggesting that cognitive deficits in PD have a structural basis in addition to the neurochemical ones.

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Abbreviations

ANOVA, Analysis of Variance; BDI, Beck Depression Inventory; BA, Brodmann area; CPT, Conner's Continuous performance Test; fMRI, functional magnetic resonance imaging; GM, gray matter; HC, healthy controls; IGT, Iowa Gambling Task; IQ, intelligence quotient; MRI, magnetic resonance imaging; OFC, orbitofrontal cortex; PD, Parkinson's disease; PET, positron emission tomography; RAVLT, Rey's Auditory Verbal Learning test; ROI, region of interest; svc, small volume correction; UPDRS III, Unified Parkinson Disease Rating Scale, motor section; VBM, Voxel-based morphometry; WAIS, Wechsler Adults Intelligence Scale.

References

Adolphs, R. (2002a) Recognizing emotion from facial expressions: psychological and neurological mechanisms. *Behav. Cogn. Neurosci. Rev.*, **1**, 21–62.

Adolphs, R. (2002b) Neural systems for recognizing emotion. *Curr. Opin. Neurobiol.*, **12**, 169–177.

Adolphs, R., Tranel, D., Damasio, H. & Damasio, A.R. (1995) Fear and the human amygdala. *J. Neurosci.*, **15**, 5879–5891.

Adolphs, R., Damasio, H., Tranel, D. & Damasio, A.R. (1996) Cortical systems for the recognition of emotion in facial expressions. *J. Neurosci.*, **16**, 7678–7687.

Adolphs, R., Schul, R. & Tranel, D. (1998) Intact recognition of facial emotion in parkinson's disease. *Neuropsychology*, **12**, 253–258.

Adolphs, R., Tranel, D., Hamann, S., Young, A.W., Calder, A.J., Phelps, E.A., Anderson, A., Lee, G.P. & Damasio, A.R. (1999) Recognition of facial emotion in nine individuals with bilateral amygdala damage. *Neuropsychologia*, **37**, 1111–1117.

Ariatti, A., Benuzzi, F. & Nichelli, P. (2008) Recognition of emotions from visual and prosodic cues in parkinson's disease. *Neurol. Sci.*, **29**, 219–227.

Ashburner, J. & Friston, K.J. (2000) Voxel-based morphometry – the methods. *Neuroimage*, **11**, 805–821.

Assogna, F., Pontieri, F.E., Caltagirone, C. & Spalletta, G. (2008) The recognition of facial emotion expressions in parkinson's disease. *Eur. Neuropsychopharmacol.*, **18**, 835–848.

Bechara, A. (2004) The role of emotion in decision-making: evidence from neurological patients with orbitofrontal damage. *Brain Cogn.*, **55**, 30–40.

Bechara, A., Damasio, H., Tranel, D. & Anderson, S.W. (1998) Dissociation of working memory from decision making within the human prefrontal cortex. *J. Neurosci.*, **18**, 428–437.

Bechara, A., Damasio, H., Damasio, A.R. & Lee, G.P. (1999) Different contributions of the human amygdala and ventromedial prefrontal cortex to decision-making. *J. Neurosci.*, **19**, 5473–5481.

Beyer, M.K., Janvin, C.C., Larsen, J.P. & Aarsland, D. (2007) A magnetic resonance imaging study of patients with parkinson's disease with mild cognitive impairment and dementia using voxel-based morphometry. *J. Neurol. Neurosurg. Psychiatry*, **78**, 254–259.

Blair, R.J., Morris, J.S., Frith, C.D., Perrett, D.I. & Dolan, R.J. (1999) Dissociable neural responses to facial expressions of sadness and anger. *Brain*, **122** (Pt 5), 883–893.

Braak, H., Del Tredici, K., Rub, U., de Vos, R.A., Jansen Steur, E.N. & Braak, E. (2003) Staging of brain pathology related to sporadic parkinson's disease. *Neurobiol. Aging*, **24**, 197–211.

Burton, E.J., McKeith, I.G., Burn, D.J., Williams, E.D. & O'Brein, J.T. (2004) Cerebral atrophy in Parkinson's disease with and without dementia: a comparison with Aizheimer's disease, dementia with Lewy bodies and controls. *Brain*, **127**, 791–800.

Cabeza, R. & Nyberg, L. (2000) Imaging cognition II: an empirical review of 275 PET and fMRI studies. *J. Cogn. Neurosci.*, **12**, 1–47.

Calder, A.J., Young, A.W., Rowland, D., Perrett, D.I., Hodges, J.R. & Etcoff, N.L. (1996) Facial emotion recognition after bilateral amygdala damage: differentially severe impairment of fear. *Cogn. Neuropsychol.*, **13**, 699–745.

Clark, U.S., Neargarder, S. & Cronin-Golomb, A. (2008) Specific impairments in the recognition of emotional facial expressions in parkinson's disease. *Neuropsychologia*, **46**, 2300–2309.

Czernecki, V., Pillon, B., Houeto, J.L., Pochon, J.B., Levy, R. & Dubois, B. (2002) Motivation, reward, and parkinson's disease: influence of dopatherapy. *Neuropsychologia*, **40**, 2257–2267.

Daniel, S.E. & Lees, A.J. (1993) Parkinson's disease society brain bank, london: overview and research. *J. Neural Transm. Suppl.*, **39**, 165–172.

Denburg, N.L., Cole, C.A., Hernandez, M., Yamada, T.H., Tranel, D., Bechara, A. & Wallace, R.B. (2007) The orbitofrontal cortex, real-world decision making, and normal aging. *Ann. N Y Acad. Sci.*, **1121**, 480–498.

Dolan, R.J., Fletcher, P., Morris, J., Kapur, N., Deakin, J.F. & Frith, C.D. (1996) Neural activation during covert processing of positive emotional facial expressions. *Neuroimage*, **4**, 194–200.

Douaud, G., Smith, S., Jenkinson, M., Behrens, T., Johansen-Berg, H., Vickers, J., James, S., Voets, N., Watkins, K., Matthews, P.M. & James, A. (2007) Anatomically related grey and white matter abnormalities in adolescent-onset schizophrenia. *Brain*, **130**, 2375–2386.

Dubois, B., Burn, D., Goetz, C., Aarsland, D., Brown, R.G., Broe, G.A., Dickson, D., Duyckaerts, C., Cummings, J., Gauthier, S., Korczyn, A., Lees, A., Levy, R., Litvan, I., Mizuno, Y., McKeith, I.G., Olanow, C.W., Poewe, W., Sampaio, C., Tolosa, E. & Emre, M. (2007) Diagnostic procedures for parkinson's disease dementia: recommendations from the movement disorder society task force. *Mov. Disord.*, **22**, 2314–2324.

Dujardin, K., Blairy, S., Defebvre, L., Duhem, S., Noel, Y., Hess, U. & Destee, A. (2004) Deficits in decoding emotional facial expressions in parkinson's disease. *Neuropsychologia*, **42**, 239–250.

Dunn, B.D., Dalgleish, T. & Lawrence, A.D. (2006) The somatic marker hypothesis: a critical evaluation. *Neurosci. Biobehav. Rev.*, **30**, 239–271.

Ekman, P. & Friesen, W.V. (1976) *Pictures of Facial Affect*. Consulting Psychologists Press, Palo-alto.

Feldmann, A., Illes, Z., Kosztolanyi, P., Illes, E., Mike, A., Kover, F., Balas, I., Kovacs, N. & Nagy, F. (2008) Morphometric changes of gray matter in parkinson's disease with depression: a voxel-based morphometry study. *Mov. Disord.*, **23**, 42–46.

Fellows, L.K. & Farah, M.J. (2005) Different underlying impairments in decision-making following ventromedial and dorsolateral frontal lobe damage in humans. *Cereb. Cortex*, **15**, 58–63.

Supplemental material, paper III

Whole Brain Analysis VBM results

There were not differences between HC and PD when correction for multiple comparisons was applied. However, when uncorrected *P* values were taken into account the areas that showed GM volume loss in PD patients in comparison with HC were: bilateral occipital (cuneus), parietal (precuneus), temporal (right middle temporal gyrus) bilateral occipitotemporal regions (fusiform gyrus), bilateral OFC, right amygdala, and cerebellum. The size of the significant clusters ranged from 200 to 3431 mm^3 (Figure A). In addition, we also tested association between IGT and Ekman total score with GM volume in these areas that yielded significant differences between HC and PD patients in the whole-brain analysis and did not find any significant result.

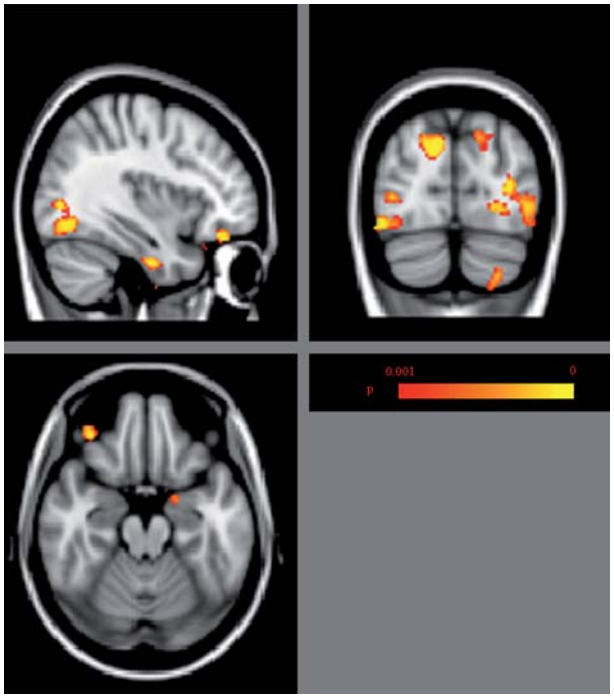

Figure A. Group comparison of whole brain GM contrasting HC > PD showed areas of changes in occipital, occipito-temporal, amygdala and prefrontal cortex. Colour coding relates to the uncorrected p value (*P* = 0.001) with smallest P being brightest.

PAPER IV

Olfactory impairment in early Parkinson's disease is related to fractional anisotropy reduction in central olfactory areas. A voxel-based diffusion tensor imaging (DTI) study

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ABSTRACT

Objective: Olfactory dysfunction is known to be previous to motor signs in Parkinson disease (PD). Diffusion tensor imaging (DTI) studies in PD have reported fractional anisotropy (FA) reductions even at the early stages of the disease. We aimed to investigate the relationship between olfactory dysfunction and white matter (WM) FA in a group of early PD patients.

Methods: Forty-eight subjects (24 with early stage PD and 24 healthy controls matched by age, gender and years of education) participated in this study. DTI was acquired at a 3 Tesla scanner and odor identification was assessed using the University of Pennsylvania Smell Identification Test (UPSIT). Whole brain and central olfactory ROI voxelwise group comparisons were performed using tract-based spatial statistics (TBSS). In addition, correlation analyses with UPSIT scores were carried out.

Results: PD patients presented lower scores in the UPSIT in comparison with healthy controls and presented FA reduction in the corpus callosum, WM adjacent to right posterior cingulated and parieto-occipital cortex, left WM adjacent to fusiform gyrus, bilateral superior longitudinal fasciculus, left WM adjacent to temporal pole, right WM adjacent to gyrus rectus, left thalamus and fornix, and brainstem. Only FA reductions in the right gyrus rectus showed significant correlation with olfactory dysfunction. When focusing on central olfactory areas and dividing the groups according to different degrees of olfactory dysfunction, PD patients (both hyposmic and anosmic groups) had lower FA values than controls in the WM adjacent to gyrus rectus. In addition, patients with anosmia had reduced FA in WM surrounding primary olfactory areas, specifically in the WM adjacent to the enthorrinal cortex.

Conclusions: PD patients at early stages of the disease have micostructural changes in the central olfactory system associated with olfactory dysfunction.

1. Introduction

Patients with PD present marked deficits in odor identification (Doty, et al. 1988, Doty, et al. 1992b, Hawkes, et al. 1997, Stern, et al. 1994, Tissingh, et al. 2001) and these deficits appear early in the disease course (Doty, et al. 1992b, Tissingh, et al. 2001). Olfactory dysfunction precedes classic motor symptoms in PD serving as a preclinical marker and predictor of PD (Berendse, et al. 2001, Ponsen, et al. 2004, Ross, et al. 2008) and is independent of the magnitude of motor impairment, cognitive status, medication, disease duration, and disease severity (Doty, et al. 1988); however, it is not a concomitant element of all parkinsonian syndromes. For example, patients with vascular parkinsonism (Katzenschlager, et al. 2004), progressive supranuclear paralisis PSP (Doty, et al. 1993) or MPTP-induced parkinsonism (Doty, et al. 1992a) do not show olfactory dysfunction.

Olfactory information is transmitted from peripheral olfactory structures (the olfactory epithelium) to more central structures including olfactory bulb that through olfactory tracts and without thalamic relay connects with primary olfactory cortex which involves the piriform cortex, the anterior cortical nucleus of the amygdala, and the rostral enthorrinal cortex. The orbitofrontal cortex (OFC) which represents the main neocortical target of primary olfactory cortex is also involved in odor identification (Price, 2004). Deficits in odor detection may be the consequence of peripheral defects in the olfactory pathway, whereas deficits in "higher order" olfactory functions, such as odor recognition memory and identification may result from involvement of more central olfactory structures (Doty, 2009).

Neuropathological studies have found synuclein pathology across the central olfactory system (Silveira-Moriyama, et al. 2009) and olfaction related structures (i.e., olfactory bulb) are known to be the first sites to get affected in the course of the disease by Lewy pathology (Braak, et al. 2003). It was proposed that a toxic agent that enters the brain via nasal, with anterograde progression into the temporal lobe may cause the disorder (Hawkes, et al. 2007). The normal sense of smell in intravenously administered MPTP-parkinsonism induced patients and the identification of pesticide exposure as a risk factor for PD provoking and almost 80% greater risk of parkinsonism (Tanner, et al. 2009) gives further evidence of an environmental agent entering the brain via olfactory pathways as responsible for the smell dysfunction observed in idiopathic PD.

However, neuroimaging studies have failed to relate consistently olfactory deficits to cerebral changes in PD. One SPECT study reported no correlation between dopamine uptake and the olfactory measures (Lehrner, et al. 1995). However, another study found significant correlation between dopamine transporter uptake in the striatum, mostly putamen, and UPSIT scores in early PD patients (Siderowf, et al. 2005). A functional MRI study suggested that neuronal activity in the hippocampus and amygdala was reduced in PD when receiving olfactory stimulation inside the scanner (Westermann, et al. 2008). Using voxel-wise analysis of diffusion weighted imaging (DWI) increased diffusivity in the olfactory tract of early PD patients was reported (Scherfler, et al. 2006) and a recent study using DTI reported a correlation between diffusion indices in the cerebellum and thresholds of olfactory identification (Zhang, et al. 2009).

DTI allows for in vivo measure of macroscopic axonal organization in nervous system tissues (Mori et al., 2006) and it has been shown to be sensitive to white matter (WM) damage in

neurodegenerative diseases even in normal appearing white matter (Roosendaal, et al. 2009). DTI allows obtaining fractional anisotropy (FA) values which provide information on the degree of diffusion directionality of the fibers and ranges from 0 (isotropic diffusion) to 1 (anisotropic diffusion) being sensitive to the presence and integrity of WM fibers (Assaf and Pasternak. 2008).

In our study, we obtained diffusion tensor imaging (DTI) and olfactory identification scores in a group of early PD patients and healthy controls to assess if there is a relationship between anisotropy measures in central olfactory system and odor identification.

2. Materials and methods

2.1. Participants

The sample of this study was composed of 48 subjects: Twenty-four early PD and 24 healthy controls. Patients were recruited from the Parkinson's disease Movement Disorders Unit, Neurology Service, Hospital Clínic, Barcelona. Healthy controls were recruited from friends and spouses of patients and matched by age, gender and years of education. This study was approved by the institutional ethics committee. All subjects provided written informed consent specifically to participate in this study.

All patients fulfilled the UK PD Society Brain Bank (PDSBB) diagnostic criteria for PD (Daniel and Lees. 1993). The inclusion criteria for early PD group were: i) age 40-65 years; ii) Hoehn and Yahr stage ≤ II; iii) disease duration £5 years; and iv) absence of motor fluctuations. Exclusion criteria for all subjects were i) the presence of dementia that was diagnosed by a

neurologist according to the Movement Disorder Society diagnostic criteria for Parkinson disease dementia (Dubois, et al. 2007) ii) the presence of other neurological or psychiatric disorders such as depression that was evaluated by means of the Beck's Depression Inventory II iii) presence of visual hallucinations assessed by the Neuropsychiatric Inventory Questionnaire (NPI-Q) iv) history of: head trauma, nasal fracture or diagnosis of rhinitis and/or nasal polyps v) a cold two weeks prior and at the moment of the evaluation. Three patients were taking no medication and 21 were on anti-parkinsonian treatment at the time of investigation: MAO-B inhibitor (n=7), L-dopa monotherapy (n=5), dopamine agonist monotherapy (n=3) or combination of L-dopa and dopamine agonist (n=6).

2.2 Assessment of olfactory function

Odor identification was assessed using the University of Pennsylvania Smell Identification Test (UPSIT) (Doty, et al. 1984). The UPSIT is a standardized multiple-choice scratch-and-sniff test consisting of four test booklets with 10 items each. Subjects scratched the impregnated area and were asked to make a selection from one of four possible answers for each item. If the patient could not identify an odor, and the examiner observed that the area was not sufficiently scratched, the examiner either re-scratched the area or suggested that the patient do so until an odor was selected. Related to olfactory function subjects were also asked for: i) history of smoking habit; ii) awareness of smell disorder iii) presence of a cold in the last two weeks.

Following UPSIT manual, scores greater to 33 were considered to reflect normosmia and scores lower or equal to 18 reflected anosmia. Scores between 19 and 32 reflected hyposmia (from 19

to 25: severe microsmia; from 26 to 29: moderate miscrosmia and from 30 to 33: mild microsmia).

2.3. Imaging data acquisition, processing and statistical analysis

DTI was obtained in a sagittal orientation in an anterior-posterior phase direction using a single-shot EPI sequence (TR= 5533 and TE= 88) with diffusion encoding in 30 directions (b values 0 and 1000 s/mm^2). The reconstructed voxel size was 1x1x1 mm^3 and 44 slices were acquired. The acquisition time for the DTI scan was 3 min and 10 s. Immediately after the DTI, a T2-weighted scan was acquired in the same geometry to assess WM lesions due to small vessel ischemic changes.

Voxelwise statistical analysis of the FA data was carried out using TBSS (Tract-Based Spatial Statistics (Smith, et al. 2006), part of FSL (Smith, et al. 2004). First, FA images were created by fitting a tensor model to the raw diffusion data using FDT, and then brain-extracted using BET. All subjects' FA data were then aligned into a common space using the nonlinear registration tool FNIRT, which uses a b-spline representation of the registration warp field. Next, the mean FA image was created and thinned to create a mean FA skeleton which represents the centres of all tracts common to the group. Each subject's aligned FA data was then projected onto this skeleton and the resulting data fed into voxelwise cross-subject statistics. TBSS method improves the analysis by means of 1) carefully tuned non-linear registration, 2) projection onto an alignment – invariant tract representation (the `mean FA skeleton´)

A whole brain comparison without any priori was performed in a voxelwise fashion using FSL's randomise (which combines General Linear Model testing with permutation inference statistics) to identify potential regional areas of FA reduction in PD patients when compared with healthy controls with TBSS ($t > 3$, $p < 0.001$ uncorrected). Mean FA values from each of the clusters with significant differences between groups were calculated and correlation analyses were performed with UPSIT scores.

In addition, we selected areas of white matter surrounding the central olfactory system as region-of-interests (ROI) for TBSS analysis. The selected areas were: i) white matter adjacent to the OFC that corresponds to superficial white matter structures, specifically to inferior frontal blade, ii) the medial temporal lobe white matter including white matter adjacent to amygadala, piriform cortex, enthorrinal cortex (primary olfactory system) iii) the uncinate fasciculus (conexion between frontal and temporal areas) that was obtained from the JHU white matter tractography atlas. ROIs were defined in the MNI standard template of 1x1x1 mm where images were previously aligned to and then were introduced as masks in the statistical analysis of FA comparison between groups. Two WM atlases within the FSL (ICBM-DTI-81 parcellation map and JHU WM tractography atlas) guided the placement of the ROIS and they were further verified using a DTI color map human atlas (Oishi, et al. 2008). Masks were multiplied by white matter's mean FA skeleton to get sure that only areas corresponding to this skeleton were introduced in the analyses. Differences in FA between patients and controls in those ROIs which were related to olfactory function were also analyzed using FSL's randomise and a corrected cluster size significance level of $p > 0.05$ was used to correct for multiple comparisons controlling for FWE.

3. Results

3.1. Olfactory function

UPSIT score (mean ± SD) for PD patients was 21.79 ± 6.31 and for healthy controls 31.17 ± 6.31. PD patients' UPSIT scores were significantly lower compared to healthy controls ($t = 6.22$; $p < 0.001$) and all participants except 5 were unaware of a smell disorder before testing.

From PD group there were 2 patients with normosmia (UPSIT score > 33); 1 patient with mild microsmia (UPSIT score between 30 and 33); 3 patients with moderate microsmia (UPSIT score between 26 and 29); 9 patients with severe microsmia (UPSIT score between 19 and 25); and 9 patients with anosmia (UPSIT score <= 18). In healthy controls the proportion was as follows: 9 with normosmia; 9 with mild microsmia; 5 with moderate microsmia and 1 with severe microsmia. None of the healthy controls presented criteria for anosmia. Because the scores of patients with normosmia (n=2), mild (n=1) and moderate microsmia (n= 3) overlapped with those from healthy controls and they were not a number big enough to make subgroups we exclude d them from the analyses. There were 4 patients and 4 healthy controls with smoking history but since they were an equal number in both groups we did not exclude them from the analyses. Therefore, the analyses were performed in a group of 23 healthy controls and 18 PD, that were latter subdivided for ROI analyses, based on their score in the UPSIT, into PD patients with severe microsmia what we will consider PD patients with hyposmia (UPSIT score between 19 and 25) (n= 9) or PD patients with anosmia (UPSIT score <= 18) (n= 9). Clinical and sociodemographic aspects of the sample are summarized in table 1.

UPSIT scores did not show a significant correlations with clinical variables such as UPDRS-III (r= 0.21; p= 0.39), years of disease evolution (r= -0.21; p= 0.41) or Hoehn and Yahr stage (r= -0.31; p= 0.22) in PD group.

3.2. Whole brain voxel-based FA group comparisons

In table 2 and figure 1 we can see the areas that showed FA reduction in PD patients in comparison with HC as a results of a voxel-based whole brain FA analysis with TBSS (t > 3; 0.001 uncorrected). PD patients presented FA reductions in the caudal and middle corpus callosum, WM adjacent to right posterior cingulated and parieto-occipital cortex, left WM adjacent to fusiform gyrus, bilateral superior longitudinal fasciculus (frontal and temporal part), left WM adjacent to temporal pole, right WM adjacent to gyrus rectus, left thalamus and fornix, and brainstem. There were not differences between groups when correction for multiple comparisons was applied. Control group did not show any corrected or 0.001 uncorrected areas of FA reduction in comparison with PD patients

3.3. FA group comparison using a ROI of the central olfactory areas

Table 3 shows the voxel-based maps reflecting areas with statistically significant FA differences between groups. Both hyposmic and anosmic groups differed from controls in the white matter adjacent to gyrus rectus (BA 14) bilaterally and, in addition, anosmic PD patients differed from controls in the POC bilaterally, specifically in WM adjacent to enthorrinal cortex. In figure 2, we can see the areas of reduced FA in anosmic patients compared to healthy controls. There were not areas showing significant FA differences between PD patients with anosmia and hyposmia. There were not differences in between groups in the uncinate fasciculus.

3.4. Correlation between FA values and olfactory function

We extracted the mean FA value of each of the clusters that showed significant reductions in the PD group when comparing with control group and performed correlation analysis to investigate if there was a significant correlation with UPSIT scores (see table 4). To examine the influence of FA reduction on odor identification we performed a multiple regression analysis (stepwise method) including the mean FA values of each of the clusters that showed significant differences between groups, which indicated the reduction of FA in WM adjacent to gyrus rectus to be the most important variable in determining olfactory function of PD patients ($F = 5.762$, $p < 0.029$). In figure 3 we can see the plot of correlations between FA values in WM adjacent to right gyrus rectus and UPSIT scores for PD patients.

4. Discussion

Our work aimed to investigate the olfactory dysfunction in early PD and its association with WM microstrucutural changes in central olfactory areas. Our early PD patients presented reduced anisotropy assessed by a voxel-wise analysis of FA maps in several brain regions including cortical and subcortical areas. However, only reduction of mean FA value of the WM of the central olfactory areas, specifically the WM adjacent to gyrus rectus, was associated with the olfactory dysfunction in PD patients.

Previous studies have reported impaired odor identification (Doty, et al. 1988, Hawkes, et al. 1997, Stern, et al. 1994, Tissingh, et al. 2001). The PD patients of the present study showed lower UPSIT scores in comparison with sex, age and gender matched healthy controls. Nineteen patients showed anosmia or severe microsmia and only 6 of the patients showed UPSIT scores

ranging from normosmia to mild-moderate microsmia. As previously reported (Doty, et al. 1988), UPSIT scores in PD patients were independent of years of disease evolution, UPDRS motor section and Hoehn and Yahr stage.

Voxel-based analysis of the whole brain showed that PD patients presented reduced FA in the caudal and middle corpus callosum, WM adjacent to right posterior cingulated and parieto-occipital cortex, left WM adjacent to fusiform gyrus, bilateral superior longitudinal fasciculus (frontal and temporal part), left WM adjacent to temporal pole, right WM adjacent to gyrus rectus, left thalamus and fornix, and brainstem. Previous studies using DTI have reported reductions of FA values in the substantia nigra and its proyections in PD (Chan, et al. 2007, Yoshikawa, et al. 2004) even in de novo PD patients (Vaillancourt, et al. 2009). Other areas that have shown decreased FA values in early PD compared to controls are motor areas and the cingulum (Karagulle Kendi, et al. 2008). Recent studies also reported decreased FA or increased MD, localized bilaterally in the cerebellar and orbitofrontal cortex of PD patients (Zhang, et al. 2009); and in the genu of the corpus callosum and superior longitudinal fasciculus (Gattellaro, et al. 2009). PD patients with cognitive deficits, specifically executive dysfunctions, showed reduced FA in the right frontal and left parietal white matter (Matsui, et al. 2007b); and in PD patients with dementia reductions have been reported in bilateral posterior cingulated bundles compared to PD without dementia (Matsui, et al. 2007a).

From all the areas which showed reduced FA in PD patients in comparison with healthy controls the WM adjacent to gyrus rectus was the only area that showed a significant correlation with UPSIT scores in PD patients. When analyzing the differences in FA just focusing on the ROIs from central olfactory areas (primary olfactory cortex and orbitofrontal cortex) and dividing the

PD group into those who presented hyposmia and those with anosmia we demonstrated that both hyposmic and anosmic groups differed from controls in the white matter adjacent to gyrus rectus (BA 14) bilaterally and, in addition, anosmic PD patients differed from controls in the POC bilaterally, specifically in WM adjacent to enthorrinal cortex.

The basis of olfactory dysfunction in PD is unknown, although it could reflect a PD-related vulnerability of the olfactory system to destruction by environmental factors (Hawkes, et al. 2007) or PD-related retrograde degenerative process (Braak, et al., 2003). The olfaction is the only sensory modality that is directly connected into the cerebral hemisphere. Olfactory sensory activity is transferred directly from the olfactory bulb to the primary olfactory cortex without a thalamic relay, and then orbitofrontal cortex receives neocortical olfactory projections form the POC (Price, 2004). It has been proposed that PD and the associated olfactory dysfunction may be caused by the entry of environmental agents or toxins from the nasal cavity into the brain. Indeed, pesticide exposure was reported to be a risk factor for PD provoking and almost 80% greater risk of parkinsonism (Tanner, et al. 2009). In addition, the association of impaired olfaction with incidental LBs suggests that the cause of the deficits maybe linked to the process leading to LB formation (Ponsen, et al. 2004). Olfaction related structures (i.e., olfactory bulb) are known to be the first sites to get affected in the course of the disease by Lewy pathology (Braak, et al. 2003) and neuropathological studies have found synuclein pathology across the central olfactory system (Silveira-Moriyama, et al. 2009).

A decrease in FA value of WM areas may be a sensitive indicator of histological abnormality, even if the values are derived from normal-appearnig tissue by conventional MRI. At present, the pathological interpretation of FA reduction in the brain with neurodegenerative disease is not

clear and can be attributed to a number of physiological factors (Assaf and Pasternak. 2008). However, the murine model of PD have demonstrated that cell loss caused by neurodegenerative disorders is likely to be accompanied by FA decreases in the deteriorated brain regions (Boska, et al. 2007).Therefore, the reduced FA values found in early PD anosmic PD patients could be reflecting a degeneration of the olfactory bulb an in consequence a loss of axonal connections with olfactory related primary central cerebral structures, and also a loss of connections with neocortical structures such as the OFC, which are known to be involved in olfactory identification and therefore can be contributing to the odor identification problem reported in PD. Another possible explanation for FA reduction in our early PD patients could refer to a demyelinating process as a consequence of over expression of synuclein proteins which has been previously associated with demyelinating changes in PD (Galvin, et al. 1999).

In conclusion, we give evidences of abnormal cortical and subcortical structural connectivity in early PD patients and a relationship between olfactory dysfunction and reduced FA anisotropy in central olfactory system.

References

Assaf Y, Pasternak O. Diffusion tensor imaging (DTI)-based white matter mapping in brain research: A review. J Mol Neurosci 2008; 34: 51-61.

Berendse HW, Booij J, Francot CM, Bergmans PL, Hijman R, Stoof JC, et al. Subclinical dopaminergic dysfunction in asymptomatic parkinson's disease patients' relatives with a decreased sense of smell. Ann Neurol 2001; 50: 34-41.

Boska MD, Hasan KM, Kibuule D, Banerjee R, McIntyre E, Nelson JA, et al. Quantitative diffusion tensor imaging detects dopaminergic neuronal degeneration in a murine model of parkinson's disease. Neurobiol Dis 2007; 26: 590-6.

Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic parkinson's disease. Neurobiol Aging 2003; 24: 197-211.

Chan LL, Rumpel H, Yap K, Lee E, Loo HV, Ho GL, et al. Case control study of diffusion tensor imaging in parkinson's disease. J Neurol Neurosurg Psychiatry 2007; 78: 1383-6.

Daniel SE, Lees AJ. Parkinson's disease society brain bank, london: Overview and research. J Neural Transm Suppl 1993; 39: 165-72.

Doty RL. The olfactory system and its disorders. Semin Neurol 2009; 29: 74-81.

Doty RL, Deems DA, Stellar S. Olfactory dysfunction in parkinsonism: A general deficit unrelated to neurologic signs, disease stage, or disease duration. Neurology 1988; 38: 1237-44.

Doty RL, Shaman P, Dann M. Development of the university of pennsylvania smell identification test: A standardized microencapsulated test of olfactory function. Physiol Behav 1984; 32: 489-502.

Doty RL, Singh A, Tetrud J, Langston JW. Lack of major olfactory dysfunction in MPTP-induced parkinsonism. Ann Neurol 1992a; 32: 97-100.

Doty RL, Stern MB, Pfeiffer C, Gollomp SM, Hurtig HI. Bilateral olfactory dysfunction in early stage treated and untreated idiopathic parkinson's disease. J Neurol Neurosurg Psychiatry 1992b; 55: 138-42.

Doty RL, Golbe LI, McKeown DA, Stern MB, Lehrach CM, Crawford D. Olfactory testing differentiates between progressive supranuclear palsy and idiopathic parkinson's disease. Neurology 1993; 43: 962-5.

Dubois B, Burn D, Goetz C, Aarsland D, Brown RG, Broe GA, et al. Diagnostic procedures for parkinson's disease dementia: Recommendations from the movement disorder society task force. Mov Disord 2007; 22: 2314-24.

Galvin JE, Uryu K, Lee VM, Trojanowski JQ. Axon pathology in parkinson's disease and lewy body dementia hippocampus contains alpha-, beta-, and gamma-synuclein. Proc Natl Acad Sci U S A 1999; 96: 13450-5.

Gattellaro G, Minati L, Grisoli M, Mariani C, Carella F, Osio M, et al. White matter involvement in idiopathic parkinson disease: A diffusion tensor imaging study. AJNR Am J Neuroradiol 2009; 30: 1222-6.

Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: A dual-hit hypothesis. Neuropathol Appl Neurobiol 2007; 33: 599-614.

Hawkes CH, Shephard BC, Daniel SE. Olfactory dysfunction in parkinson's disease. J Neurol Neurosurg Psychiatry 1997; 62: 436-46.

Karagulle Kendi AT, Lehericy S, Luciana M, Ugurbil K, Tuite P. Altered diffusion in the frontal lobe in parkinson disease. AJNR Am J Neuroradiol 2008; 29: 501-5.

Katzenschlager R, Zijlmans J, Evans A, Watt H, Lees AJ. Olfactory function distinguishes vascular parkinsonism from parkinson's disease. J Neurol Neurosurg Psychiatry 2004; 75: 1749-52.

Lehrner J, Brucke T, Kryspin-Exner I, Asenbaum S, Podreka I. Impaired olfactory function in parkinson's disease. Lancet 1995; 345: 1054-5.

Matsui H, Nishinaka K, Oda M, Niikawa H, Kubori T, Udaka F. Dementia in parkinson's disease: Diffusion tensor imaging. Acta Neurol Scand 2007a; 116: 177-81.

Matsui H, Nishinaka K, Oda M, Niikawa H, Komatsu K, Kubori T, et al. Wisconsin card sorting test in parkinson's disease: Diffusion tensor imaging. Acta Neurol Scand 2007b; 116: 108-12.

Oishi K, Zilles K, Amunts K, Faria A, Jiang H, Li X, et al. Human brain white matter atlas: Identification and assignment of common anatomical structures in superficial white matter. Neuroimage 2008; 43: 447-57.

Ponsen MM, Stoffers D, Booij J, van Eck-Smit BL, Wolters EC, Berendse HW. Idiopathic hyposmia as a preclinical sign of parkinson's disease. Ann Neurol 2004; 56: 173-81.

Price JL. Olfaction. In: Paxinos G, Mai JK. The human nervous system, 2nd edition. 2004. Elsevier

Roosendaal SD, Geurts JJ, Vrenken H, Hulst HE, Cover KS, Castelijns JA, et al. Regional DTI differences in multiple sclerosis patients. Neuroimage 2009; 44: 1397-403.

Ross GW, Petrovitch H, Abbott RD, Tanner CM, Popper J, Masaki K, et al. Association of olfactory dysfunction with risk for future parkinson's disease. Ann Neurol 2008; 63: 167-73.

Scherfler C, Schocke MF, Seppi K, Esterhammer R, Brenneis C, Jaschke W, et al. Voxel-wise analysis of diffusion weighted imaging reveals disruption of the olfactory tract in parkinson's disease. Brain 2006; 129: 538-42.

Siderowf A, Newberg A, Chou KL, Lloyd M, Colcher A, Hurtig HI, et al. 99mTc]TRODAT-1 SPECT imaging correlates with odor identification in early parkinson disease. Neurology 2005; 64: 1716-20.

Silveira-Moriyama L, Holton JL, Kingsbury A, Ayling H, Petrie A, Sterlacci W, et al. Regional differences in the severity of lewy body pathology across the olfactory cortex. Neurosci Lett 2009; 453: 77-80.

Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, et al. Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. Neuroimage 2006; 31: 1487-505.

Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, et al. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 2004; 23 Suppl 1: S208-19.

Stern MB, Doty RL, Dotti M, Corcoran P, Crawford D, McKeown DA, et al. Olfactory function in parkinson's disease subtypes. Neurology 1994; 44: 266-8.

Tanner CM, Ross GW, Jewell SA, Hauser RA, Jankovic J, Factor SA, et al. Occupation and risk of parkinsonism: A multicenter case-control study. Arch Neurol 2009; 66: 1106-13.

Tissingh G, Berendse HW, Bergmans P, DeWaard R, Drukarch B, Stoof JC, et al. Loss of olfaction in de novo and treated parkinson's disease: Possible implications for early diagnosis. Mov Disord 2001; 16: 41-6.

Vaillancourt DE, Spraker MB, Prodoehl J, Abraham I, Corcos DM, Zhou XJ, et al. High-resolution diffusion tensor imaging in the substantia nigra of de novo parkinson disease. Neurology 2009; 72: 1378-84.

Westermann B, Wattendorf E, Schwerdtfeger U, Husner A, Fuhr P, Gratzl O, et al. Functional imaging of the cerebral olfactory system in patients with parkinson's disease. J Neurol Neurosurg Psychiatry 2008; 79: 19-24.

Yoshikawa K, Nakata Y, Yamada K, Nakagawa M. Early pathological changes in the parkinsonian brain demonstrated by diffusion tensor MRI. J Neurol Neurosurg Psychiatry 2004; 75: 481-4.

Zhang K, Yu C, Zhang Y, Wu X, Zhu C, Chan P, et al. Voxel-based analysis of diffusion tensor indices in the brain in patients with parkinson's disease. Eur J Radiol 2009.

Table 1. Demographic and clinical characteristics of the sample

	Controls (23)	PD with hyposmia (9)	PD with anosmia (9)	F/X^2	p
UPSIT total	31.6 ± 3.2	22.7 ± 2.1	15.5 ± 2.4	111.473	< 0.001*
Age	57.3 ± 8.9	58.0 ± 5.8	57.9 ± 7.9	0.036	0.965
Gender (male/female)	4/2	6/3	8/1	1.479	0.477
Education (years)	13.1 ± 3.9	11.3 ± 6.4	9.2 ± 5.5	2.048	0.143
MMSE	29.8 ± 0.3	29.6 ± 0.5	29.7 ± 0.5	2.031	0.145
Disease evolution (years)		2.7 ± 1.6	3.3 ± 1.6	0.332	0.721
Predominance (left/right)		5/4	7/2	1.000	0.317
UPDRSIII		16.4 ± 3.5	13.8 ± 3.5	2.030	0.156
Hoehn and Yahr		1.8 ± 0.2	1.8 ± 0.3	3.029	0.70
Total LEDD		214.4 ± 347.5	319.4 ± 312.8	0.591	0.563

Group differences in demographical and clinical variables were analyzed with ANOVA for normally distributed variables and the X^2 test for categorical variables.

Values are mean ± SD, HC=Healthy controls; PD=Parkinson's disease, MMSE = Mini-Mental State Examination; BDI-II = Beck's Depression Inventory; NPI = Neuropsychiatric Inventory; UPDRS III=Unified Parkinson's Disease Rating Scale (motor section); LEDD=levodopa equivalent daily dose.

Table 2. Areas of FA reduction in early PD patients in comparison with healthy controls with TBSS analysis.

Region	Cluster size (voxels)	MNI coordinates (x, y, z)	t value*
Caudal corpus callosum and WM adjacent to right posterior cingulate	153	21, -28, 33	3.79
Middle corpus callosum	91	-13, -18, -30	3.31
Right WM adjacent to parieto-occipital cortex	72	27, -73, 24	3.79
Left WM adjacent to fusiform gyrus	39	-38, -49, -13	3.71
Right superior longitudinal fasciculus (frontal)	65	38, -4, 35	3.38
Left superior longitudinal fasciculus (frontal)	31	-39, 7, 17	3.07
Right superior longitudinal fasciculus (temporal)	45	39, -38, 15	3.42
Left superior longitudinal fasciculus (temporal)	30	-42, -35, 3	4.77
Left WM adjacent to temporal pole	64	-44, -2, -24	4.51
Right WM adjacent to gyrus rectus	54	7, 25, -18	3.89
Left thalamus	36	-14, -29, -4	4.18
Left fornix	30	-19, -32, 2	4.77
Brainstem	20	10, -28, -6	3.33

*results are uncorrected at $p < 0.001$

Only clusters bigger or equal to 20 voxels were included in the table.

MNI= Montreal Neurological Institute; WM= white matter

Table 3. Voxel-based differences between groups in WM FA values from DTI images.

	Cluster size	MNI Coordinate	t*
Differences in the white matter adjacent to gyrus rectus			
Anosmic PD < Controls	101	11 31 -17 (R)	2.32
Hyposmic PD < Controls	100	8 36 -21 (R)	1.95
Differences in primary olfactory cortex			
Anosmic PD < Controls	143	-43, -9, -32 (L)	2.99

Cluster size denotes the extent of the cluster of significant voxels.

MNI coordinates refer to the location of the most statistically significant voxel in the cluster.

*Differences are significant at $p < 0.05$ corrected for multiple comparisons by FWE.

Table 4. Mean FA value of regions with reduced FA and correlation with UPSIT in PD

Region	FA value (Mean ± SD)	r value	p
Caudal corpus callosum and WM adjacent to right posterior cingulate	0.5030 ± 0.0297	0.240	0.337
Middle corpus callosum	0.6464 ± 0.0384	0.060	0.812
Right WM adjacent to parieto-occipital cortex	0.5068 ± 0.0301	0.049	0.848
Left WM adjacent to fusiform gyrus	0.3820 ± 0.0338	0.200	0.427
Right superior longitudinal fasciculus (frontal)	0.4635 ± 0.0277	-0.020	0.936
Left superior longitudinal fasciculus (frontal)	0.4358 ± 0.0283	0.055	0.827
Right superior longitudinal fasciculus (temporal)	0.5025 ± 0.0285	0.437	0.070
Left superior longitudinal fasciculus (temporal)	0.5286 ± 0.0217	0.432	0.074
Left WM adjacent to temporal pole	0.3806 ± 0.0263	0.101	0.689
Right WM adjacent to gyrus rectus	0.2349 ± 0.0394	0.515	0.029*
Left thalamus	0.4206 ± 0.0222	0.215	0.391
Left fornix	0.4229 ± 0.0225	0.202	0.421
Brainstem	0.4687 ± 0.0295	0.042	0.870

*significant results at $p < 0.05$

The r value refers to the spearman rank correlation between mean FA value of each of the clusters and UPSIT scores in PD patients.

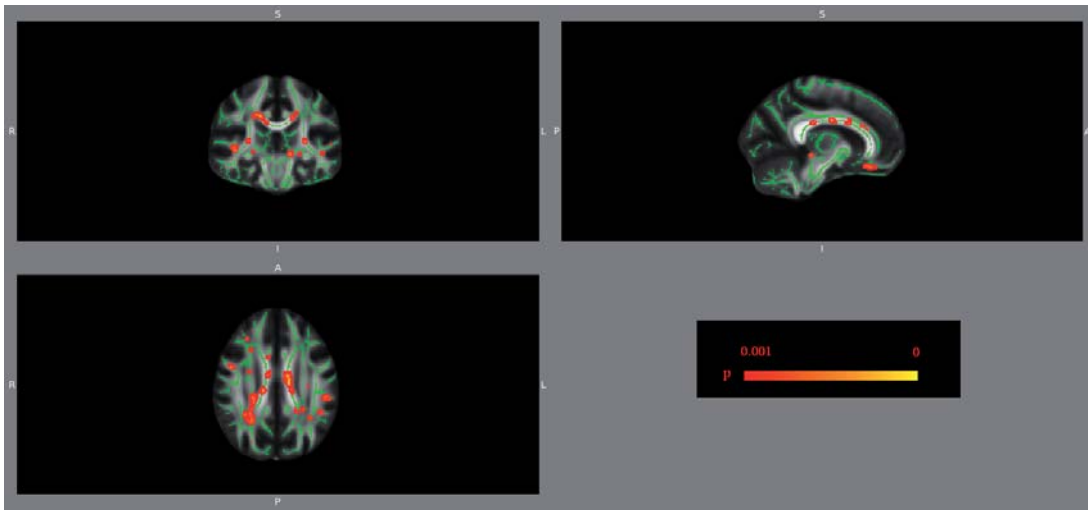

Figure 1. Voxelwise group differences as a result of the exploratory whole brain comparison between early PD patients and healthy controls overlaid on mean FA skeleton (in green). Differences are considered significant at $t > 3$; $p < 0.001$ uncorrected.

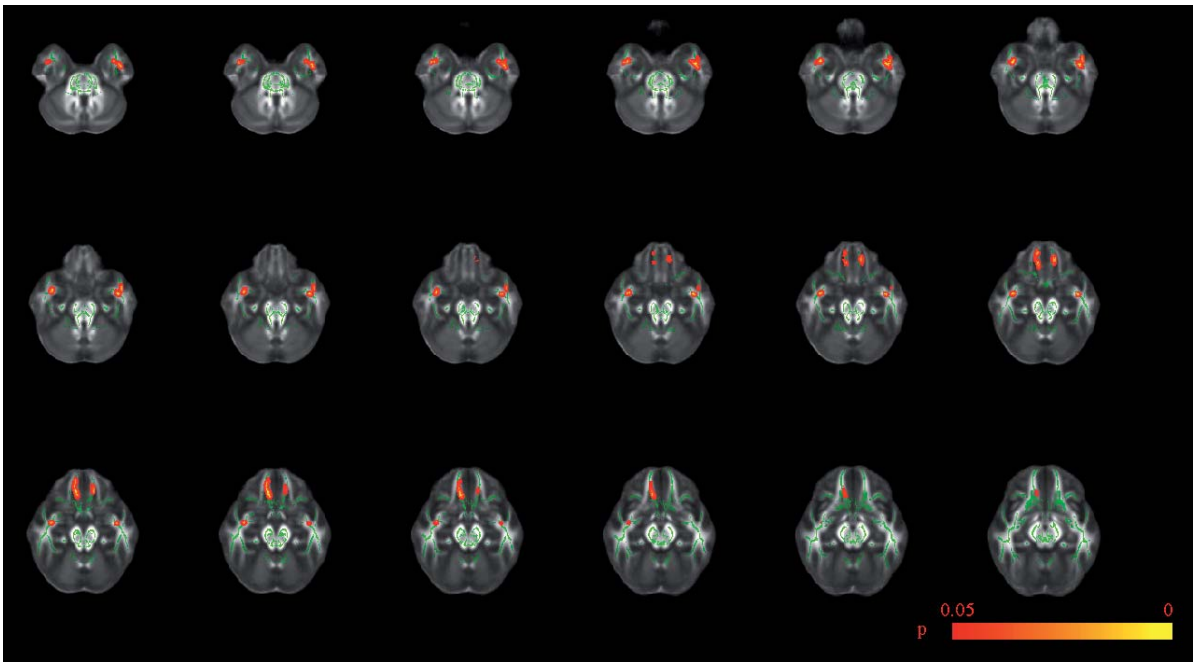

Figure 2. PD patients with anosmia demonstrated significantly lower fractional anisotropy in the WM adjacent to gyrus rectus and to the primary olfactory cortex ($p < 0.05$ corrected by FWE) in comparison with healthy controls. The significant differences correspond to the red-yellow regions overlaid on mean FA skeleton (in green). All images are in radiological convention, i.e.the left hemisphere of the brain corresponds to the right side of the image.

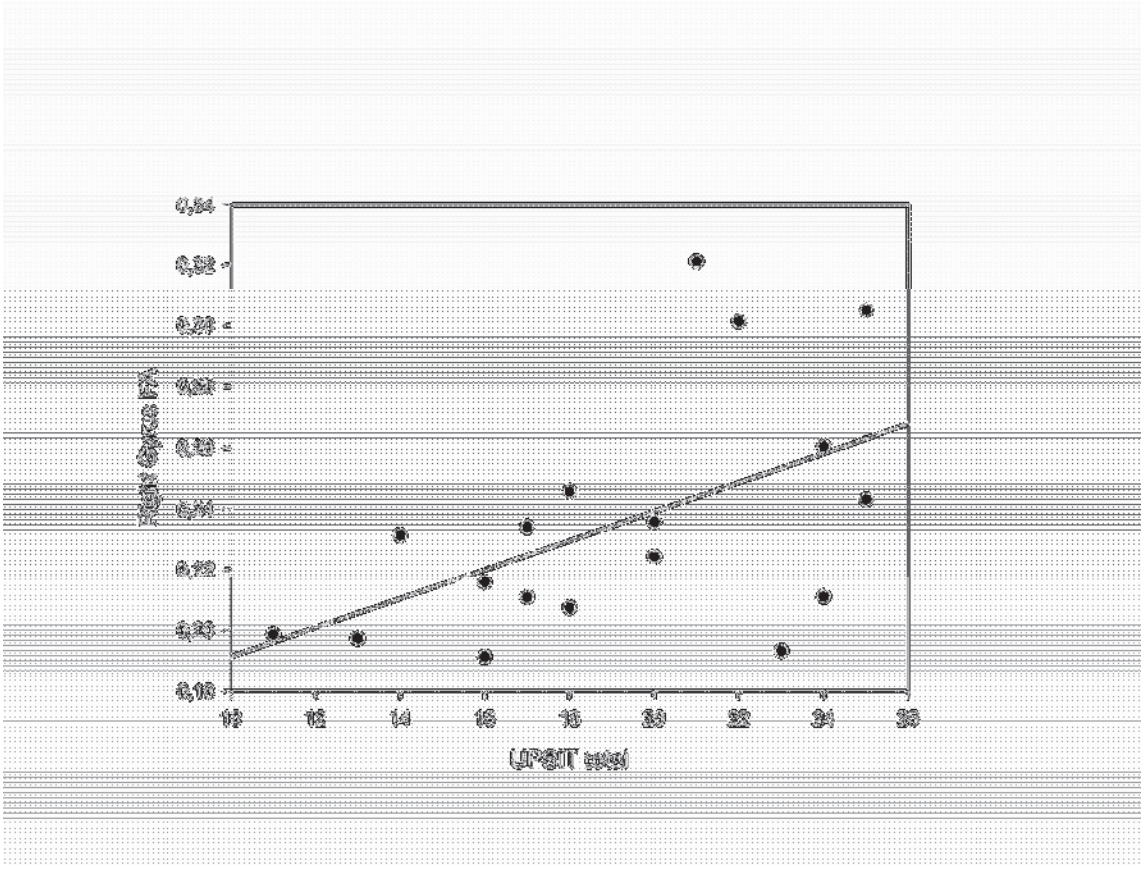

Figure 3. Regression plot between FA values in white matter adjacent to gyrus rectus and UPSIT scores in PD patients.

PAPER V

Early alterations in brain functional networks during memory retrieval task in Parkinson's disease

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ABSTRACT

In this study we used the Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) from FSL software to assess brain networks during an episodic memory retrieval task in a group of 24 healthy controls and 24 early stage PD patients. There were not differences in the amount of words correctly recognized between PD patients and controls but fMRI analyses revealed a reduced activation of specific memory-related networks including frontal pole, lateral OFC, paracingulate, visual and angular gyrus in PD patients. The substantia nigra also showed a reduced activation in PD, but the supramarginal region showed increased activation in PD compared to controls what could be considered a compensatory mechanism to perform the memory task properly. In addition, PD patients showed reduced deactivation of default network areas including temporo-occipital, ventromedial OFC and medial temporal areas while performing the memory task. These data provide functional evidence of the malfunctioning of networks involved in memory and default mode in early PD.

Key words: Parkinson's disease; fMRI; ICA, default mode network, memory.

INTRODUCTION

Understanding the interactions among different brain regions is fundamental to our understanding of brain function (Toro, et al. 2008). The functional connectivity is determined by the coordinated functional activation of different brain regions. fMRI studies in healthy subjects have revealed the existence of functional brain networks. The most established brain networks refer to attention (fronto-parietal) (Fox, et al. 2005), motor (cortico-diencephalo-cerebelar) (Postuma and Dagher. 2006) and default mode (cingulo-parietal) (Greicius, et al. 2003). Specifically, default mode network participates in episodic memory and became known for its task-related deactivations across fMRI studies (Fox, et al. 2005, Fransson. 2005, Greicius, et al. 2003); and a recent study using fMRI found malfunctioning of the default mode network while performing an executive task in PD (van Eimeren, et al. 2009).

PD is a neurodegenerative disease and early stages of neurodegeneration may cause dysfunctions in complex neuronal networks (Palop, et al. 2006). Unfortunately, most functional neuroimaging studies focus on motor symptoms and few on cognitive functions, and so relatively little is known about the brain basis of high-level cognitive dysfunction in PD (Tinaz, et al. 2008). There is extensive evidence of declarative memory deficits in PD (Troster. 2008, Whittington, et al. 2000) and structural MRI studies have related memory dysfunctions to hippocampal gray matter loss (Bouchard, et al. 2008, Camicioli, et al. 2003, Ibarretxe-Bilbao, et al. 2008, Junque, et al. 2005).

Functional magnetic resonance imaging (fMRI) research of cognitive functions in Parkinson's disease has been typically aimed at determining regional differences between patients and controls in discrete and isolate brain regions, commonly by creating a model of the expected BOLD-response and estimating its magnitude using a General Linear Model (GLM) analysis. However in studies of aging and disease, it is better not assuming such simple temporal response model because the measured blood-oxygen-level dependent (BOLD) response within such populations does not necessarily correspond to the standard hemodynamic response function (HRF), where the temporal signal variation may differ substantially between groups (D'Esposito, et al. 2003). Exploratory data analysis techniques such as independent component analysis (ICA) do not depend on these assumptions and are able to detect unknown, yet structured spatiotemporal processes in neuroimaging data (Rombouts,

et al. 2009). Tensorial probabilistic ICA (T-PICA) is a model free technique that can be used for analyzing multiple subjects and groups, extracting signals of interest (components) in the spatial, temporal, and also subject domain of fMRI data (Beckmann and Smith. 2005). The model-free approach has been proved more sensitive to detect group differences than the commonly used model-based analysis (Rombouts, et al. 2009).

In this study we used a typical recognition fMRI paradigm, in which studied and nonstudied items are presented in a mixed sequence and the subject's task is to decide which items are old (studied) and which are new (nonstudied) (Cabeza and Nyberg. 2000), to characterise dynamic brain activity changes in early PD using a model free-technique.

Methods

Subjects

We assessed 24 patients with idiopathic PD and 24 healthy controls (HC). All of them were right-handed. Patients were recruited from the Parkinson's disease Movement Disorders Unit, Neurology Service, Hospital Clinic, Barcelona. HC were recruited from friends and spouses of patients and were matched for age, gender and years of education. The study was approved by the institutional ethics committee. Written informed consent was obtained from the subjects after having fully explained the procedures involved in the study.

All patients fulfilled the UK PD Society Brain Bank (PDSBB) diagnostic criteria for PD (Daniel and Lees. 1993). Other inclusion criteria for patients were: i) age 40-65 years; ii) Hoehn and Yahr stage ≤ II; iii) disease duration ≤5 years; and iv) absence of motor fluctuations. Exclusion criteria for all subjects were: i) the presence of dementia that was diagnosed by a neurologist according to the Movement Disorder Society diagnostic criteria for Parkinson disease dementia (Dubois, et al. 2007); ii) the presence of other neurological or psychiatric disorders such as depression that was evaluated by means of the Beck's Depression Inventory (BDI- II); and iii) the presence of visual hallucinations assessed by the Neuropsychiatric Inventory Questionnaire (NPI-Q).

Three patients were taking no medication and 21 were on anti-Parkinsonian treatment at the time of investigation: MAO-B inhibitor (n=7), L-dopa monotherapy (n=5), dopamine agonist monotherapy (n=3) or a combination of L-dopa and dopamine agonist (n=6). All patients were symptomatically stable and no patient was asked to change her/his medication for this study. None of the patients were receiving psychoactive medication at the moment of the study because all psychotic characteristics such as depression and the presence of visual hallucinations were considered exclusion criteria. Clinical and sociodemographical aspects of the sample are summarized in table 1.

-Insert table 1-

fMRI acquisition

Data acquisition was performed on a 3 Tesla TIM TRIO 3T scanner (Siemens, Germany), using multi-slice gradient-echo EPI sequence [repetition time (TR): 2000 ms; echo time (TE): 30 ms; 36 x 3 mm axial slices providing whole brain coverage.

A T1-weighted structural image was also acquired for each subject images with a notational resolution of 1 x 1 x 1 mm^3 [MPRAGE 3D, TR: 2300 ms, TE: 2.98 ms, inversion time (TI): 900 ms; FOV: 256×256, 1mm isotropic voxel).

Experimental setup and episodic verbal memory retrieval paradigm

Subjects studied a list of words before scanning, and during fMRI scanning the performed a cue-specific episodic memory retrieval operation. Specifically, participants first viewed 35 items (duration 2 sec; intertribal interval (ITI), 1 sec) outside the scanner with instructions to remember the words for a subsequent memory test. The stimuli were words in capital letters from the Lexesp-Corco database (Sebastian-Galles et al., 2000) presented with VisuaStim Digital MRI Compatible High Resolution Stereo 3D glasses (Resonance Technology, Inc.) and Presentation ® version 10.1 (Neurobehavioral Systems) running in Windows XP. The words were 4 to 6 letters in length and of moderate frequency. Half of the words referred to living things and half to nonliving things.

Once the subject was placed in the scan, the task started with an *activation* block which consisted of the presentation of 7 words of which 3 had been previously memorized outside the scan and 4 were not. We used a 20-block design task with alternating *activation* and *control* conditions (10 blocks each). The whole experiment had duration of 400 s (20s per block). In figure 1 there is a schematic representation of the task.

In the *activation* blocks, using their right hands, participants pressed one button on a response box to indicate that the item was studied (target) and none to indicate that it was not previously studied (foil). In total, 70 items (35 studied targets and 35 nonstudied foils) were presented. Responses were recorded using a 2 button MR-compatible response box. They were encouraged to response while the word was on the screen (2 s), and responses beyond this interval were not computed. Subjects responded by indicating whether they remembered having read the word in the list before scanning. In the contrast blocks, participants were presented 7 concatenations of letters (simulating the length of a word) of which 3 were the letter "AAAAAA" and the other 4 were random letters. Again, using their right hands, participants pressed one button on a response box to indicate that the item was "AAAAAA" and none when other concatenations of letters appeared.

fMRI data analysis with MELODIC

Analysis was carried out using Tensorial Independent Component Analysis [Beckmann 2005] as implemented in MELODIC (Multivariate Exploratory Linear Decomposition into Independent Components) Version 3.05, part of FSL (FMRIB's Software Library, http://www.fmrib.ox.ac.uk/fsl/). Specifically, the Multi-subject Tensor-ICA option was selected to obtain a 3D Tensor-ICA decomposition of the data. Tensor-ICA decomposes the data into triplets of time courses, spatial maps and subject modes, which - for each component - characterize the signal variation across the temporal, spatial and subject domain. MELODIC attempts to find components which are highly non-Gaussian relative to the full mixed-effects variance of the residuals.

Estimated components typically fall into 2 classes: components which describe effects common to all or most subjects, and components which describe effects only contained in a small number of subjects. The former will have a non-zero estimated effect size while the latter will have an effect size around 0 for most subjects and only few high non-zero values. These different types of components were identified by looking at the boxplots provided. When using Tensor-ICA the components are ordered in order of decreasing amount of median response amplitude.

The following data pre-processing was applied to the input data: masking of non-brain voxels; voxel-wise de-meaning of the data; normalisation of the voxel-wise variance. Pre-processed data were whitened and projected into a 53-dimensional subspace using probabilistic Principal Component Analysis where the number of dimensions was estimated using the Laplace approximation to the Bayesian evidence of the model order (Beckmann and Smith. 2004, Cox, et al. 2000).

The whitened observations were decomposed into sets of vectors which describe signal variation across the temporal domain (time-courses), the subject domain (PD or control) and across the spatial domain (maps) by optimising for non-Gaussian spatial source distributions using a fixed-point iteration technique (Hyvarinen. 1999).

Estimated Component maps were divided by the standard deviation of the residual noise and thresholded by fitting a mixture model to the histogram of intensity values [Beckmann 2004]. Z (Gaussaniased T/F) statistic images were thresholded using clusters determined by $Z > 4$ and a corrected cluster significance threshold of $p < 0.05$ corrected by multiple comparisons.

RESULTS

Behavioral data

The mean number of correct responses and percentage (words correctly recognized or true recognition), incorrect responses (false recognition), omissions (forgotten words) and mean reactions times for PD patients and healthy controls are summarized in table 2. PD patients and controls did not differ in any of these measures.

TICA results

BOLD fMRI data acquired during task performance were analyzed using MELODIC, a novel model-free multivariate analysis approach. Of 53 independent components (IC) defined by MELODIC, 12 components showed a consistent effect across the group in that their standardized subject scores were not driven by outliers and the mean subject score was significantly different from zero. Components representing patterns with known artifacts such as motion and high frequency noise (Beckmann and Smith. 2005) were identified by visual inspection and excluded from further analysis. These 12 components were divided in those that were common to all the subjects (task related activations and deactivations) (6 components) and those that significantly differed between PD and control groups (another 6 components).

Task related activations and deactivations

Activations are defined as increases in signal during the task as compared with baseline; and deactivations are defined as decreases in signal during the task as compared with baseline. In table 3 we summarized the task-related activations and in table 4 the task-related deactivations. It should be noted that the same component may be composed of task-related activations and deactivations. Below, a detailed description of each of the components is provided

Component 1

In component 1, task-related activations were observed in bilateral paracingulate gyrus, bilateral dorsolateral OFC (BA47/12), bilateral frontal pole (lateral part BA10), bilateral angular gyrus, left precentral gyrus (BA6) and occipital pole; whereas task-related deactivations occurred in precuneus cortex and middle temporal gyrus (temporooccipital cortex).

Component 2

In components 2 when task-related activations occurred in bilateral frontal pole (lateral part); bilateral dorsolateral OFC (BA47/12), and bilateral paracingulate gyrus, bilateral angular gyrus; task-related

deactivations were observed in the right middle temporal cortex (parietooccipital cortex) and bilateral ventromedial cortex including gyrus rectus (BA14).

Component 3

Mainly bilateral visual cortex (V1 and V2) task-related activations were observed. There were no task-related deactivations.

Component 4

In this component, similarly to component 2, task related-activations occurred in bilateral paracingulate gyrus, bilateral dorsolateral OFC (BA47/12), bilateral frontal pole (lateral part BA10) However, task related-deactivations involved bilateral ventromedial cortex bilateral temporal pole and medial temporal areas including left hippocampus and hippocampus, and right precuneus.

Component 5

Only left temporal pole task-related deactivations were observed and no areas of activation.

Component 6

Bilateral paracingulate gyrus and right superior frontal gyrus task-related activations were found but no areas of deactivation.

In summary, bilateral paracingulate cortex, bilateral dorsolateral OFC (BA 47/12), bilateral frontal pole (lateral part of BA10), bilateral angular gyrus, bilateral visual cortex (V1 and V2) and precentral gyrus (BA6) showed time courses that were task-correlated. The areas that showed predominantly deactivating task-related signal change for both groups were bilateral ventromedial prefrontal cortex, precuneus, supramarginal gyrus, temporo-occipital cortex, left hippocampus and left parahippocampal gyrus, and left temporal pole. In figure 2 we can see all the task-related activations and deactivations common to both PD and control groups.

Significantly different components between PD and HC

From those 53 components here we will explain those that fit properly to the task and showed statistically significant differences between PD patients and control group. All the differences between PD and NC have a p value < 0.05 corrected for multiple comparisons. In table 5 we summarized the regions that showed an increased activation in healthy controls compared to PD patients; and in table 6 the areas that showed increased activation in PD patients compared to healthy controls.

Component 7

During task, control group showed more activity in bilateral paracingulate gyrus, dorsolateral OFC (BA 47/12), bilateral frontal pole (lateral part of BA10), bilateral angular gyrus, occipital pole in comparison with PD patients; and PD patients showed increased activity in the precuneus bilaterally, temprooccipital cortex, left supramarginal gyrus in comparison with control group ($p < 0.02$).

Component 8

During task, control group showed increased activity in bilateral paracingulate gyrus and right lateral frontal pole; and PD patients had increased activity in bilateral supramarginal gyrus in comparison with controls ($p < 0.007$).

Component 9

During task, controls showed an increased activity in the inferior frontal gyrus pars triangularis and right frontal pole comparing to PD ($p < 0.04$).

Component 10

During task, PD patients had increased activity in the motor cortices bilaterally including BA4 and BA6, and left precuneus compared to controls ($p < 0.035$).

Component 11

During task, PD patients showed increased activity in the frontal pole and ventromedial prefrontal cortex (BA14) compared to PD ($p < 0.01$).

Component 12

During task, controls showed increased activity in the left substantia nigra (see figure 4) comparing to PD patients ($p < 0.04$) and cerebellum. 15 out of 24 PD patients have a left predominance disease.

In summary, there were areas that showed increased activity in control group in comparison with PD patients, or what it is the same areas that showed a significant signal reduction in PD patients in comparison with normal controls. Those areas are depicted in yellow in figure 3 and involve: bilateral paracingulate gyrus, dorsolateral OFC, lateral frontal pole, occipital pole, angular gyrus, inferior frontal gyrus, substantia nigra and cerebelum. On the other hand, PD patients showed areas of increased activity in comparison with control group, or what is the same control group showed reduced activity in these areas in comparison with PD group. Those areas are depicted in blue in figure 3 and involve bilateral supramarginal gyrus, motor cortex (BA4 and BA6), temporooccipital cortex, precunesus and ventromedial prefrontal cortex (BA14).

DISCUSSION

PD patients perform similar to controls in the recognition verbal memory task but fMRI analyses revealed a reduced activation of specific memory-related networks including frontal pole, lateral OFC, paracingulate, visual and angular gyrus in PD patients. The substantia nigra also showed a reduced activation in PD, but supramarginal areas showed more activation in PD compared to controls what could be considered a compensatory mechanism to perform the memory task properly. In addition, PD patients showed reduced deactivation of default network areas including temporo-occipital, ventromedial OFC and medial temporal areas while performing the memory task.

Regarding the recognition memory task, there were not significant differences between PD patients and healthy controls in the amount of words correctly recognized, number of false recognitions, omissions and mean reaction time in answering. It should be noted that although the differences between groups were not statistically significant, the performance of PD patients was lower. There are discrepancies in the literature concerning the existence of deficits in recognition memory among PD

patients. A recent study suggests that non-demented PD patients suffer from deficits in recognition memory but found no recognition deficits in early-stage PD participants at an easier level of the task suggesting that recognition memory deficits depend on task difficulty and disease severity (Whittington, et al. 2006). Our PD group is at the early stage of the disease (Hoehn and Yahr: I, II) and the task was not difficult what could explain the lack of deficits in these sample.

The episodic memory retrieval task was associated with an activation of the bilateral paracingulate cortex, bilateral dorsolateral OFC (BA 47/12), bilateral frontal pole (lateral part of BA10), bilateral angular gyrus, bilateral visual cortex (V1 and V2) and precentral gyrus (BA6) in comparison with the control task in the whole sample. Functional studies have shown that the most strongly associated regions to retrieval memory are anterior prefrontal cortex (BA10), that has been attributed to the production and maintenance of the mental set of episodic retrieval (Lepage, et al. 2000), and lateral OFC (BA47) (Cabeza and Nyberg. 2000). Other areas that have been consistently related to retrieval memory involve medial temporal areas and parietooccipital areas, anterior cingulate, occipital and cerebelar regions (Cabeza and Nyberg. 2000). In our study occipital and cerebelar areas showed task-related activations. Anterior cingulate did not show activation but the anterior paracingulate did. There are several individual morphological differences in this region that may preclude and exact localization in MRI group studies (Yucel, et al. 2001).

The areas that showed predominantly deactivating task-related signal change for both groups were bilateral ventromedial prefrontal cortex, precuneus, temporo-occipital cortex, left hippocampus and left parahippocampal gyrus, left temporal pole. This set of regions has been described previously as being involved in the default-network (Greicius, et al. 2003, Raichle, et al. 2001). Default mode network activity has been shown to be increased during resting fMRI but it is decreased during task requiring attention (Fox, et al. 2005, Raichle, et al. 2001). An intriguing functional property of the default network is that operates in opposition to other brain systems that are used for focused external attention and sensory processing. When the default network is most active, the external attention system is attenuated and vice versa (Buckner, et al. 2008, Greicius, et al. 2003, Raichle, et al. 2001).

Most of the components presented bilateral activations of the involved structures. A meta-analysis of functional studies in healthy subjects showed that the strength of symmetric interhemispheric coactivations is impressively high. Symmetric coactivations are among the strongest functional correlations that we can observe in the brain and these coactivations are likely mediated by interhemispheric callosal fibers (Toro, et al. 2008).

In most of the areas that showed task-related activations PD patients showed hypoactivation in comparison with healthy controls. PD patients showed hypoactivation in bilateral paracingulate gyrus, dorsolateral OFC, lateral frontal pole, occipital pole, angular gyrus and inferior frontal gyrus. In addition, they showed a hyperactivation of areas that were supposed to be deactivated during the fMRI task. Those areas that showed more activation in the PD group involve the ventromedial prefrontal cortex and temporal areas. PD patients showed less deactivation of these areas in comparison with healthy controls. Recent findings suggest that for the successful performance of a memory or executive task the integrity of a network showing task-related deactivations might be just as relevant as a network of other structures showing activations (Buckner, et al. 2008). A recent study in PD reported that patients with PD showed less deactivation of the posterior areas involved in the default mode network but the comparable deactivation of the medial prefrontal cortex (van Eimeren, et al. 2009). In our work PD patients not only showed less deactivation in posterior temporooccipital areas and medial temporal areas but also in the ventromedial prefrontal cortex. In other neurodegenratives diseases such as AD disruption of the default network has been also reported (Celone, et al. 2006, Rombouts, et al. 2009).

Interestingly, it has been recently demonstrated that in healthy controls functionally correlated brain regions involved in the default network feature defined axonal connections (Greicius, et al. 2009) and functional networks also correlate with the gray matter volumes form the involved structures (Seeley, et al. 2009). Therefore, when talking about disruption of the default network in PD patients we are not just talking about functional changes but about a disruption of the anatomical connections of the underlying structures.

However, even if PD patients did not show an efficient activation of areas related to episodic memory retrieval and did not deactivate the default network areas properly, they were able to perform the task as well as control group and showed hiperactivation of the supramarginal gyrus when performing the task. We think that the activation of these areas is a compensating mechanism for frontal dysfunction early in the course of the disease. Indeed, though episodic memory has long been known to depend on the medial temporal lobe memory system and on prefrontal contributions to retrieval, an emerging body of functional imaging evidence suggests that parietal activations might also contribute to episodic memory retrieval (Wagner, et al. 2005). It could be that PD patients are using this network for compensating for the disruption of the OFC network (Alexander, et al. 1986). Those patients whit unaffected compensatory systems may prove more resilient to functional decline. Even with degraded metabolic pathways the cerebral cortex still works before cerebral functions eventually collapse in patients with dementia (Ferrer. 2009)

Lateral motor cortex (BA4 y BA6) also showed a hyperactivation in patients compared to controls. fMRI studies in PD assessing learning of sequential movements or automatic movements (Sabatini, et al. 2000, Wu and Hallett. 2005) have pointed that PD patients have a cortical reorganization to do the task at the same level than healthy controls. Indeed PET and fMRI studies reported that PD patients recruited parallel motor circuits including lateral and primary motor cortex in order to overcome the functional deficit of the striatocortical motor loops (Sabatini, et al. 2000).

Finally, it is of great interest the hypoactivation in PD of the left substantia nigra. With PET it was found that early parkinsonian patients showed significantly reduced F-dopa uptake or reduction in dopaminergic metabolism in the substantia nigra (Ito, et al. 1999), but with fMRI there are not studies to date showing a reduction of BOLD signal in the substantia nigra.

In conclusion, we give evidences of disruption of the memory networks involving the limbic loop that involves the substantia negra and the OFC; and disruption of the default mode network; but recruitment of other circuits such as circuits involving the primary motor and lateral cortex and supramarginal gyrus in early PD.

References

Alexander GE, DeLong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 1986; 9: 357-81.

Beckmann CF, Smith SM. Tensorial extensions of independent component analysis for multisubject FMRI analysis. Neuroimage 2005; 25: 294-311.

Beckmann CF, Smith SM. Probabilistic independent component analysis for functional magnetic resonance imaging. IEEE Trans Med Imaging 2004; 23: 137-52.

Bouchard TP, Malykhin N, Martin WR, Hanstock CC, Emery DJ, Fisher NJ, et al. Age and dementia-associated atrophy predominates in the hippocampal head and amygdala in parkinson's disease. Neurobiol Aging 2008; 29: 1027-39.

Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: Anatomy, function, and relevance to disease. Ann N Y Acad Sci 2008; 1124: 1-38.

Cabeza R, Nyberg L. Imaging cognition II: An empirical review of 275 PET and fMRI studies. J Cogn Neurosci 2000; 12: 1-47.

Camicioli R, Moore MM, Kinney A, Corbridge E, Glassberg K, Kaye JA. Parkinson's disease is associated with hippocampal atrophy. Mov Disord 2003; 18: 784-90.

Celone KA, Calhoun VD, Dickerson BC, Atri A, Chua EF, Miller SL, et al. Alterations in memory networks in mild cognitive impairment and alzheimer's disease: An independent component analysis. J Neurosci 2006; 26: 10222-31.

Cox IJ, Miller ML, Minka TP, Papathomas TV, Yianilos PN. Correction to "the bayesian image retrieval system, pichunter: Theory, implementation, and psychophysical experiments". IEEE Trans Image Process 2000; 9: 524.

Daniel SE, Lees AJ. Parkinson's disease society brain bank, london: Overview and research. J Neural Transm Suppl 1993; 39: 165-72.

D'Esposito M, Deouell LY, Gazzaley A. Alterations in the BOLD fMRI signal with ageing and disease: A challenge for neuroimaging. Nat Rev Neurosci 2003; 4: 863-72.

Dubois B, Burn D, Goetz C, Aarsland D, Brown RG, Broe GA, et al. Diagnostic procedures for parkinson's disease dementia: Recommendations from the movement disorder society task force. Mov Disord 2007; 22: 2314-24.

Ferrer I. Early involvement of the cerebral cortex in parkinson's disease: Convergence of multiple metabolic defects. Prog Neurobiol 2009; 88: 89-103.

Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc Natl Acad Sci U S A 2005; 102: 9673-8.

Fransson P. Spontaneous low-frequency BOLD signal fluctuations: An fMRI investigation of the resting-state default mode of brain function hypothesis. Hum Brain Mapp 2005; 26: 15-29.

Greicius MD, Supekar K, Menon V, Dougherty RF. Resting-state functional connectivity reflects structural connectivity in the default mode network. Cereb Cortex 2009; 19: 72-8.

Greicius MD, Krasnow B, Reiss AL, Menon V. Functional connectivity in the resting brain: A network analysis of the default mode hypothesis. Proc Natl Acad Sci U S A 2003; 100: 253-8.

Hyvarinen A. Fast and robust fixed-point algorithms for independent component analysis. IEEE Trans Neural Netw 1999; 10: 626-34.

Ibarretxe-Bilbao N, Ramirez-Ruiz B, Tolosa E, Marti MJ, Valldeoriola F, Bargallo N, et al. Hippocampal head atrophy predominance in parkinson's disease with hallucinations and with dementia. J Neurol 2008; 255: 1324-31.

Ito K, Morrish PK, Rakshi JS, Uema T, Ashburner J, Bailey DL, et al. Statistical parametric mapping with 18F-dopa PET shows bilaterally reduced striatal and nigral dopaminergic function in early parkinson's disease. J Neurol Neurosurg Psychiatry 1999; 66: 754-8.

Junque C, Ramirez-Ruiz B, Tolosa E, Summerfield C, Marti MJ, Pastor P, et al. Amygdalar and hippocampal MRI volumetric reductions in parkinson's disease with dementia. Mov Disord 2005; 20: 540-4.

Lepage M, Ghaffar O, Nyberg L, Tulving E. Prefrontal cortex and episodic memory retrieval mode. Proc Natl Acad Sci U S A 2000; 97: 506-11.

Palop JJ, Chin J, Mucke L. A network dysfunction perspective on neurodegenerative diseases. Nature 2006; 443: 768-73.

Postuma RB, Dagher A. Basal ganglia functional connectivity based on a meta-analysis of 126 positron emission tomography and functional magnetic resonance imaging publications. Cereb Cortex 2006; 16: 1508-21.

Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. Proc Natl Acad Sci U S A 2001; 98: 676-82.

Rombouts SA, Damoiseaux JS, Goekoop R, Barkhof F, Scheltens P, Smith SM, et al. Model-free group analysis shows altered BOLD FMRI networks in dementia. Hum Brain Mapp 2009; 30: 256-66.

Sabatini U, Boulanouar K, Fabre N, Martin F, Carel C, Colonnese C, et al. Cortical motor reorganization in akinetic patients with parkinson's disease: A functional MRI study. Brain 2000; 123 (Pt 2): 394-403.

Sebastián-Gallés N, Martí MA, Cuetos F, Carreiras M, (2000). LEXESP: Léxico informatizado del español. Barcelona: Edicions de la Universitat de Barcelona.

Seeley WW, Crawford RK, Zhou J, Miller BL, Greicius MD. Neurodegenerative diseases target large-scale human brain networks. Neuron 2009; 62: 42-52.

Tinaz S, Schendan HE, Stern CE. Fronto-striatal deficit in parkinson's disease during semantic event sequencing. Neurobiol Aging 2008; 29: 397-407.

Toro R, Fox PT, Paus T. Functional coactivation map of the human brain. Cereb Cortex 2008; 18: 2553-9.

Troster AI. Neuropsychological characteristics of dementia with lewy bodies and parkinson's disease with dementia: Differentiation, early detection, and implications for "mild cognitive impairment" and biomarkers. Neuropsychol Rev 2008; 18: 103-19.

van Eimeren T, Monchi O, Ballanger B, Strafella AP. Dysfunction of the default mode network in parkinson disease: A functional magnetic resonance imaging study. Arch Neurol 2009; 66: 877-83.

Wagner AD, Shannon BJ, Kahn I, Buckner RL. Parietal lobe contributions to episodic memory retrieval. Trends Cogn Sci 2005; 9: 445-53.

Whittington CJ, Podd J, Stewart-Williams S. Memory deficits in parkinson's disease. J Clin Exp Neuropsychol 2006; 28: 738-54.

Whittington CJ, Podd J, Kan MM. Recognition memory impairment in parkinson's disease: Power and meta-analyses. Neuropsychology 2000; 14: 233-46.

Wu T, Hallett M. A functional MRI study of automatic movements in patients with parkinson's disease. Brain 2005; 128: 2250-9.

Yucel M, Stuart GW, Maruff P, Velakoulis D, Crowe SF, Savage G, et al. Hemispheric and gender-related differences in the gross morphology of the anterior cingulate/paracingulate cortex in normal volunteers: An MRI morphometric study. Cereb Cortex 2001; 11: 17-25.

Table 1. Demographic and clinical characteristics of the sample.

	HC (n=24)	PD (n=24)
Age	57.58 ± 8.9	56.13 ± 8.5
Gender (male/female)	16/8	16/8
Education (years)	13 ± 3.8	10.96 ± 5.4
MMSE	29.83 ± 0.4	29.63 ± 0.5
BDI-II	4.46 ± 5.1	6.75 ± 4.8
NPI	1.38 ± 1.7	3.58 ± 7.1
Disease evolution (years)	-	3.66 ± 1.6
Predominance (left/right)	-	13/9
UPDRSIII	-	14.67 ± 3.5
H&Y	-	1.75 ± 0.4
Total LEDD	-	299.58 ± 321.1

Table 2. Behavioral data from the cued episodic memory retrieval fMRI task.

	PD (n= 24)	Controls (n= 24)	t	p
Words correctly recognized	53.42 ± 9.97	55.12 ± 5.77	0.726	0.471
Incorrect responses	11.25 ± 8.70	7.21 ± 7.41	1.732	0.090
Omissions	16.58 ± 9.97	14.88 ± 5.77	0.726	0.471
Mean reaction time (msec)	682.53 ± 210.64	627.05 ± 81.41	1.204	0.235

Table 3. Areas showing task-related activations.

	Cluster size	MNI coordinates	Z
Component 1			
Bilateral paracingulate gyrus	247	2, 18, 48	11.40
Right frontal pole (lateral BA10)	180	38, 58, 4	7.77
Left dorsolateral OFC (BA47/12)	118	-34, 22, -4	10.2
Left angular gyrus	97	-38, -58, 44	6.62
Right angular gyrus	90	46, -54, 44	7.03
Right dorsolateral OFC (BA47/12)	89	34, 26, -4	9.16
Left precentral gyrus (BA6)	71	-42, 2, 36	5.76
Left occipital pole	63	-14, -102, 8	5.58
Left frontal pole (lateral BA10)	51	-42, 54, 4	6.82
Component 2			
Left frontal pole (lateral BA10)	352	-38, 54, 4	7.99
Bilateral paracingulate gyrus	96	2, 18, 48	7.31
Right dorsolateral OFC (BA47/12)	31	34, 26, -4	6.32
Left angular gyrus	30	-30, -74, 44	4.77
Right frontal pole (lateral BA10)	23	38, 58, 4	5.26
Right angular gyrus	13	46, -54, 44	4.93
Component 3			
Bilateral visual cortex (V1 and V2)	1287	-6, -90, 0	10
Bilateral paracingulate gyrus	62	2, 18, 48	6.37
Left precentral gyrus (BA6)	38	-50, -2, 44	5.6
Component 4			
Right frontal pole (lateral BA10)	40	42, 58, 4	5.66
Left frontal pole (lateral BA10)	38	-42, 54, 4	5.44
Bilateral paracingulate gyrus	37	2, 22, 44	5.64
Left dorsolateral OFC (BA47/12)	27	-34, 22, -4	5.74
Right dorsolateral OFC (BA47/12)	16	34, 26, -4	5.45
Component 6			
Bilateral paracingulate gyrus and right superior frontal gyrus	103		5.19

Table 4. Areas showing task-related deactivations.

	Cluster size (voxels)	MNI coordinates (x, y, z)	Z
Component 1			
Right middle temporal gyrus (temporooccipital cortex)	122	62, -58, 8	5.79
Left precuneus	25	-2, -58, 48	4.78
Component 2			
Right middle temporal gyrus (temporooccipital cortex)	36	62, -54, 0	4.82
Bilateral ventromedial cortex (BA14)	11	2, 30, -20	4.69
Component 4			
Bilateral ventromedial cortex (BA14)	144	2, 30, -16	7.51
Left temporal pole and medial temporal areas	75	-38, -6, -12	5.69
Right temporal pole	21	38, 18, -36	4.88
Right precuneus	9	-2, -62, 48	4.48
Component 5			
Left temporal pole	103	-46, 18, -40	5.19

Only clusters bigger than 10 contiguous voxels.
All results are significant at $z > 4$; $p < 0.05$ corrected by multiple comparisons FWE.
Coordenates refer to the voxel with the maximum significance.

Table 5. Areas of reduced activation in PD patients compared to controls.

	Cluster size (Voxels)	MNI coordinates	Z
Component 7			
Bilateral paracingulate gyrus	140	2, 22, 44	8.62
Right frontal pole (lateral BA10)	119	38, 54, 4	6.81
Left occipital pole	114	-14, -102, 4	6.6
Left dorsolateral OFC (BA 47/12)	57	-34, 22, -4	8.51
Right dorsolateral OFC (BA 47/12)	44	34, 26, -4	7.09
Left frontal pole (lateral BA10)	29	-42, 54, 4	6.04
Right angular gyrus	28	46, -54, 44	5.23
Right middle frontal gyrus	17	46, 34, 28	5.08
Inferior frontal gyrus, pars opercularis	12	-50, 18, 24	4.64
Right occipital pole	12	18, -102, 8	4.7
Component 8			
Bilateral paracingulate gyrus	38	2, 18, 48	6
Right frontal pole (lateral BA10)	13	38, 54, -4	4.69
Component 9			
Right frontal pole (lateral) and inferior frontal gyrus pars triangularis	348	50, 46, 12	6.93
Component 12			
Bilateral cerebelum	528	26, -86, 36	10.7
Left substantia nigra	8	-6, -22, -12	5.06

Only clusters bigger than 10 contiguous voxels.
All results are significant at $z > 4$; $p < 0.05$ corrected by multiple comparisons FWE.
Coordenates refer to the voxel with the maximum significance.

Table 6. Areas of increased activations in PD patients compared to controls.

	Cluster size (voxels)	MNI x, y, z	Max Z
Component 7			
Right middle temporal gyrus, temporooccipital cortex	373	62, -54, 0	7.23
Left supramarginal gyrus	147	-62, -38, 32	5.84
Bilateral precuneus	75	2, -58, 44	5.88
Component 8			
Left supramarginal gyrus	269	-50, -30, 56	6.78
Right supramarginal gyrus	11	66, -38, -28	4.46
Component 10			
Right precentral gyrus, motor cortices BA4 and BA6	2538	62, 2, 28	7.98
Left precentral gyrus, motor cortices BA4 and BA6	306	-58, -10, 16	6.37
Left precuneus (BA7)	26	-2, -58, 36	4.22
Component 11			
Bilateral ventromedial preforntal cortex	494	-10, 70, 0	8.39

Only clusters bigger than 10 contiguous voxels.
All results are significant at $z > 4$; $p < 0.05$ corrected by multiple comparisons FWE.
Coordenates refer to the voxel with the maximum significance.

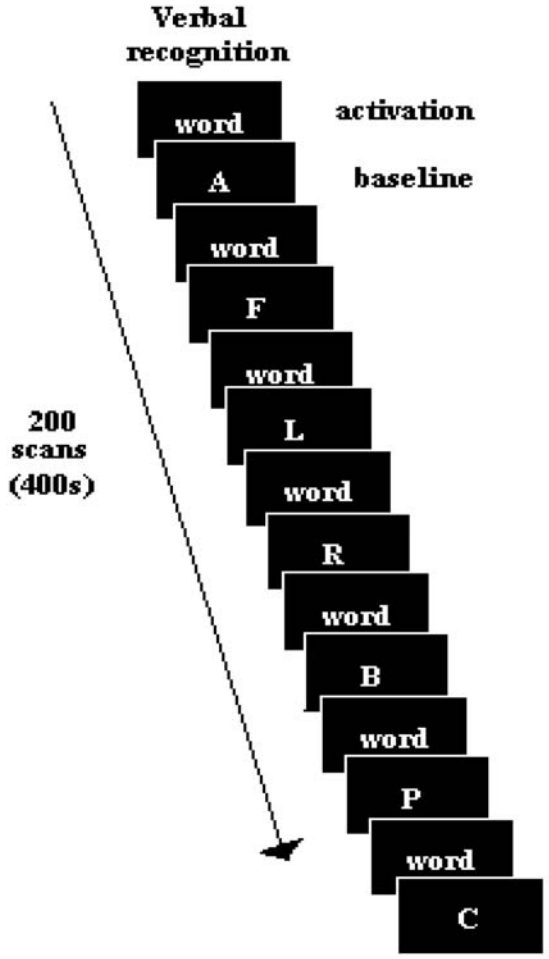

Figure 1. An schematic representation of the recognition memory task.

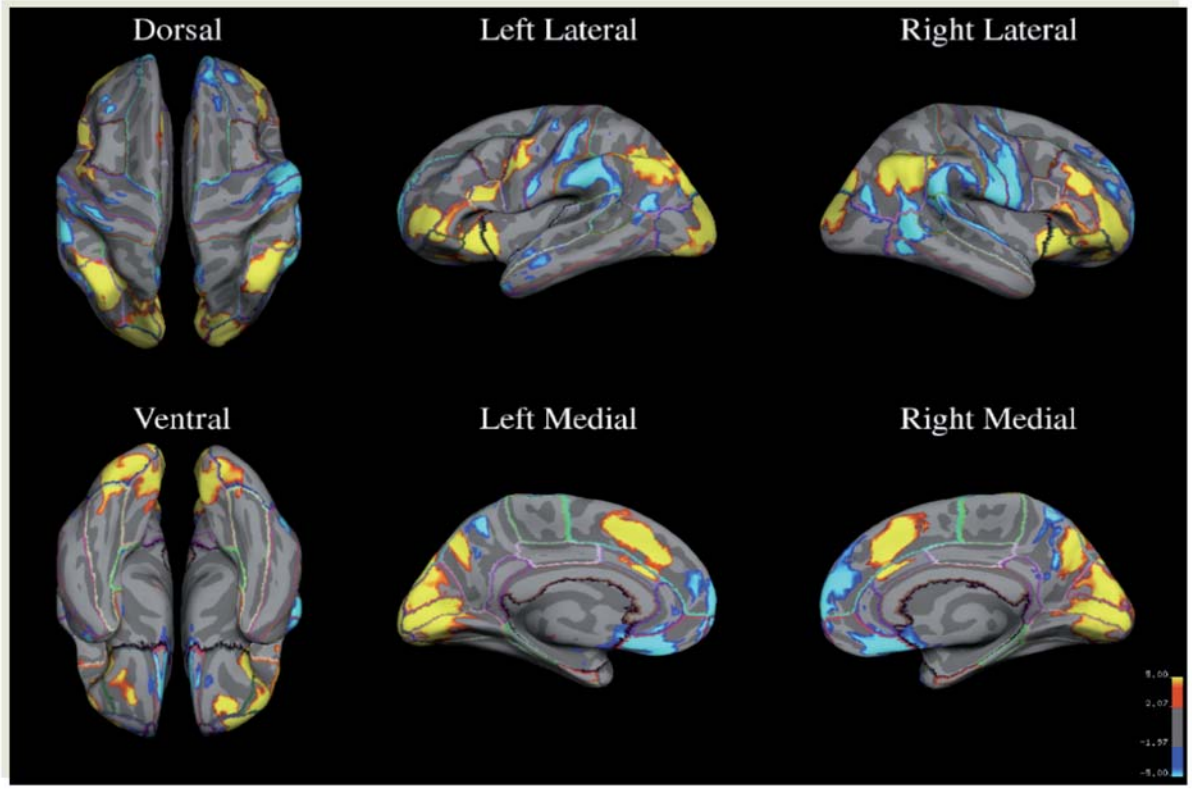

Figure 2. In yellow, task-related activations and, in blue, task-related deactivations. Maps were thresholded at $z > 4$; $p < 0.05$. The statistical results were projected onto the cortical surface of the average.

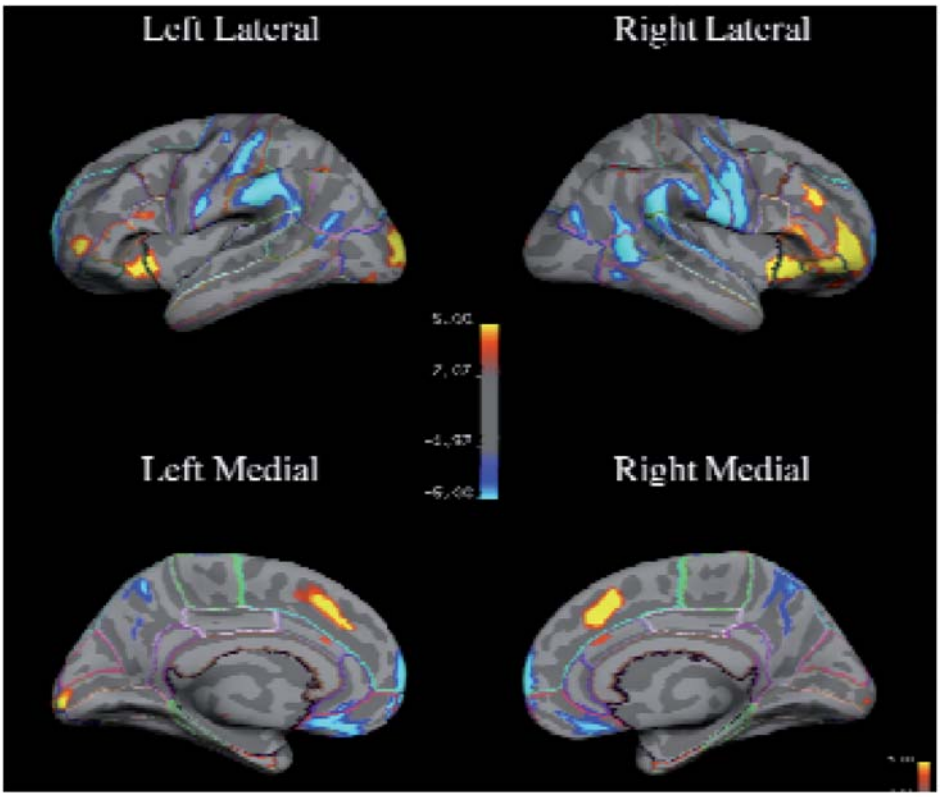

Figure 3. In yellow, areas with increased activation in control group in comparison with PD patients. In blue, areas were PD patients showed increased activation in comparison with healthy controls. Maps were thresholded at $z > 4$; $p < 0.05$. The statistical results were projected onto the cortical surface of the average.

*We would like to thank to Dr.Zarei for the design of figure 1 and 2.

5. DISCUSSION

5. Discussion

The present thesis aimed to investigate the neuroanatomical and neurofunctional correlates of declarative memory, decision-making, recognition of emotions and olfactory dysfunctions in PD. We hypothesized that all these dysfunctions are due to the limbic degenerative changes associated with PD.

The results from the cross-sectional VBM study (paper I) showed that hippocampal atrophy is present in PD. PD patients with dementia have gray matter loss involving all the hippocampus while patients who have visual hallucinations (VH) but are not demented have hippocampal gray matter loss only in the anterior regions. Focusing on the regional distribution of the atrophy in the hippocampus, we observed that changes in demented patients involved the entire hippocampal axis, but with prominent involvement of both anterior and posterior regions and relative sparing of the central region. The highest statistical significance of gray matter loss between PD patients with dementia and controls was seen in the anterior regions, mainly corresponding to the CA1 sector of the hippocampus. This pattern of hippocampal atrophy is similar to that described in the hippocampus of patients with Alzheimer's disease (Frisoni, et al. 2002, Laakso, et al. 2000). In the non-demented group of PD patients with VH, we observed gray matter loss only in the anterior part of the hippocampus. Since some studies have pointed out that PD patients with dementia have higher densities of LBs and LNs in middle parts of the hippocampus (CA2-3), and that patients with VH are at risk of developing dementia, we expected to find hippocampal atrophy in these regions in the VH group. However, in PD patients with VH we found atrophy of the head of the hippocampus, which mainly corresponds to the CA1 field. This finding agrees with a study that was published a few months before ours which showed head atrophy predominance in demented and aged PD patients (Bouchard, et al. 2008). In addition, the hippocampal head has been shown to be especially vulnerable to AD-type degenerative changes which are observed even at the early stages of the disease (Frisoni, et al. 2002, Wang, et al. 2003). Furthermore, it has been reported that smaller hippocampal volumes and specifically CA1 and subicular involvement are associated with an increased risk for converting from MCI to AD

(Apostolova, et al. 2006). In addition, individual analyses of cases showed the highest number of patients with hippocampal atrophy in the demented group (78%), followed by the PD with VH group (31%) and finally the non-VH group (26%). The regional pattern of atrophy was of anterior predominance, suggesting that the atrophy starts in the anterior region and later extends to the posterior part.

As previously reported (Grossi, et al. 2005, Ramirez-Ruiz, et al. 2006) the declarative verbal memory impairment was more pronounced in PD patients with VH than in patients without VH and this memory impairment correlated with gray matter loss in the anterior part of the hippocampus. In non-demented elderly a correlation between verbal memory and the head of the hippocampus was also found but not with the body or tail (Hackert, et al. 2002), and in PD patients a correlation of the head of hippocampus with recognition has been reported, but not with learning scores (Bouchard, et al. 2008). On the other hand, the fact that hippocampal atrophy did not correlate with motor section UPDRS scores indicated that hippocampal head atrophy is related only to verbal memory and not to the overall disease severity. We can therefore state that memory dysfunction in PD is hippocampus-dependent and that it is a storage problem, rather than a problem in retrieving the stored information as a consequence of dopamine depletion provoking a fronto-striatal dysfunction, which was the classical explanation of memory dysfunctions in PD (Cooper, et al. 1991).

After a follow-up period of 30 months (paper II), 75% of patients with VH developed dementia, but none of the patients without visual hallucinations. In addition to the decline in verbal memory, a significant cognitive decline was also observed in semantic fluency, language comprehension and visuoperceptive functions. PD patients with VH at follow-up showed the same pattern of atrophy previously reported in demented patients in the cross-sectional study, with a diffuse atrophy involving mainly the head and the tail of the hippocampus. In addition to the hippocampal atrophy, PD patients with VH showed widespread limbic, paralimbic and neocortical gray matter loss when comparing MRI images at base-line versus images at follow-up using VBM. Therefore, the significant cognitive decline and evolution to dementia of patients with VH is not only the consequence of a progressive limbic atrophy; other neocortical areas, including associative areas, are involved in the degenerative

process. In contrast, patients without VH showed progressive atrophy only in motor related areas including the primary motor cortex and the cerebellum. In addition, we found that progressive atrophy in specific brain areas was associated with a decline in several cognitive domains in patients with VH: specifically, decline in verbal learning was related to hippocampal head atrophy, and worsening in free delayed recall performance was related to progressive prefrontal cortex atrophy. The decline in other cognitive functions apart from memory was also related to progressive atrophy in other areas of the brain. Changes in semantic fluency showed a correlation with progressive thalamic atrophy, and language comprehension, which also showed a great decline over time, was related to the progressive atrophy of medial temporal structures, specifically, the amygdala. These results support the evidence that not only the dopamine deficiency affecting cortico-striatal-cortico information exchange in PD has an impact on cognitive dysfunction, but that structural gray matter changes may also play a role.

The findings in the two previous studies were related to limbic dysfunction in advanced stages of the disease and in patients who already presented a risk factor such as visual hallucinations. However, limbic dysfunctions are known to appear early in the disease course, and, in addition to verbal memory deficits, other neuropsychological dysfunctions related to the integrity of the limbic system, such as the recognition of emotions and decision-making, occur in early PD patients. Therefore, we aimed to explore deficits in these functions and looked for their neuroanatomical correlates in a sample of early PD patients with no more than 5 years of disease evolution, Hoehn and Yahr stage II or lower, and absence of other confounding factors such as dementia, VH or depression. PD patients in this study were assumed to be in Braak stage III or IV, characterized by the involvement of the mesencephalon and limbic areas, but in which neocortical regions are relatively spared (Braak, et al. 2003). However, whole brain VBM analysis showed that early PD patients presented gray matter loss not only in limbic regions (amygdala and OFC) but also in neocortical associative areas such as parieto-temporo-occipital areas. In addition, not all the limbic areas showed atrophy: for example, volumetric analysis showed that volume of entorhinal and pirifrom cortex did not show atrophy in comparison with healthy controls, which are believed to be affected earlier than prefrontal orbital areas. Reduced volume of the entorhinal cortex has been reported in PD patients with dementia but

not in PD patients without dementia and shorter disease duration (Kenny, et al. 2008). In addition, no atrophy in nucleus accumbens and paracingulate gyrus were found. It must be taken into account that the neuropathological staging proposed by Braak and Braak has recently been questioned (Jeelinger. 2008) and has not been tested by neuroimaging techniques. Braak's LB classification correlates with neurological deficits in the majority of patients with early onset and long duration of the disease (Braak, et al. 2005) but retrospective clinico-pathologic studies have shown that there is no relationship between the staging and the clinical severity of PD, or, more specifically, cognitive impairment (Jellinger. 2008).

In our study, the GM reduction in left OFC was ventrally located, but in the lateral part, and the changes in the right OFC were located in the dorsomedial region. Atrophy affecting the amygdala involved mainly the anterior nucleus and the right hemisphere only. Recent neuroimaging data point to impairments in the limbic circuitry (prefrontal orbital and amygdala) in PD. Our previous structural volumetric studies reported atrophy of amygdala in demented PD patients (Junque, et al. 2005, Ramirez-Ruiz, et al. 2005, Summerfield, et al. 2005). Nagano-Saito et al. (2005) found that, compared with advanced PD without dementia, PD patients with dementia have reductions in the medial prefrontal region (BA 10, 24 and 32). The same authors also found that BA 10 correlated with visuospatial reasoning. Reductions in the right middle frontal gyrus (BA 10) were found by Burton et al. (2004) and by Feldmann et al. (2007) when comparing depressed and non-depressed PD subgroups. PET studies have shown a significant decrease in the metabolism of the medial frontal lobe (BA 9/10) in a 2-year follow-up study of 15 patients with early PD (Huang, et al. 2007). All these studies report changes in the ventromedial OFC.

Early PD patients were impaired in decision-making and recognition of emotions, but not in other functions such as sustained attention; and we found that the gray matter loss in the OFC (but not in other areas which showed reduced gray matter loss such as the amygdala and the temporo-parieto-occipital areas) was related to these neuropsychological deficits in early PD patients. The OFC has been identified as a crucial structure in decision-making (Denburg, et al. 2007, Fellows and Farah. 2005; Wallis. 2007). Lesion studies in the OFC have reported impaired performance on the Iowa

Gambling task (Bechara. 2004). The OFC is also a crucial structure in the recognition of facial expressions of emotions (Adolphs, 2002a, Adolphs, 2002b). PET studies have reported activation in orbital regions when recognizing facial expressions of emotions (Blair, et al. 1999, Dolan, et al. 1996, Nakamura, et al. 1999), and bilateral or unilateral lesions in the OFC may impair emotional face expression identification (Heberlein, et al. 2008, Hornak, et al. 2003); however, lesions elsewhere in the frontal cortex (dorsal or lateral) do not impair this function (Heberlein, et al. 2008).

In addition to the relationship between the reduction in the OFC and the dysfunction in decision-making and recognition of emotions, we expected to find a relationship between gray matter loss in the amygdala and these functions because patients with amygdala lesions have also been reported to be impaired in IGT (Bechara, et al. 1999) and because the role of the amygdala in the recognition of fear is well supported by lesion studies (Adolphs. 2002a, Adolphs. 2002b, Calder, et al. 1996, Sato, et al, 2002) and functional studies (Morris, et al. 1996, Whalen, et al. 1998). However, we did not find a correlation between amygdalar volume and the impairment in these neuropsychological functions. The lack of correlation between the amygdala and the performance in the gambling task may corroborate recent work in non-human primates, which suggests that OFC and amygdala make distinct contributions to the decision-making process based on the findings that monkeys with lesions in the amygdala are able to flexibly change stimulus-reward associations (Rudebeck and Murray. 2008).

In addition to cognitive deficits, early PD patients are known to present olfactory dysfunction early in the disease course. Odor identification deficits appear even earlier than motor symptoms and represent a characteristic dysfunction which is known as the pre-motor phase (Tolosa, et al. 2009). Olfactory information is transmitted from peripheral olfactory structures (the olfactory epithelium) to more central structures including the olfactory bulb which, through the olfactory tracts and without thalamic relay, connects with the primary olfactory cortex which involves the piriform cortex, the anterior cortical nucleus of the amygdala, and the rostral entorhinal cortex. The OFC, the main target of the

primary olfactory cortex, is also involved in odor identification (Price, 2004). It has been proposed that PD and the associated olfactory dysfunction may be caused by the entry of environmental agents or toxins from the nasal cavity into the brain. Indeed, pesticide exposure was reported to be a risk factor for PD, increasing the risk of parkinsonism by 80% (Tanner, et al. 2009). In addition, the association of impaired olfaction with incidental LBs suggests that the cause of the deficits may be linked to the process leading to LB formation (Ponsen, et al. 2006). Olfaction-related structures (e.g., the olfactory bulb) are known to be the first sites to be affected in the course of the disease by Lewy pathology (Braak, et al. 2003) and neuropathological studies have found synuclein pathology across the central olfactory system (Silveira-Moriyama, et al. 2009). Taking into account this background, in paper IV we aimed to investigate the olfactory dysfunction in a sample of early PD patients and its association with WM microstructural changes in central olfactory areas by means of DTI. PD patients presented reduced anisotropy assessed by a voxel-wise analysis of FA maps in several brain regions including cortical and subcortical areas. However, only the reduction of the mean FA value of the WM in the central olfactory areas, specifically the WM adjacent to the gyrus rectus, was associated with olfactory dysfunction in PD patients. Voxel-based analysis of the whole brain showed that PD patients presented reduced FA in the caudal and middle corpus callosum, WM adjacent to the right posterior cingulate and parieto-occipital cortex, left WM adjacent to the fusiform gyrus, bilateral superior longitudinal fasciculus (frontal and temporal part), left WM adjacent to the temporal pole, right WM adjacent to the gyrus rectus, left thalamus and fornix, and brainstem. Previous studies using DTI have reported reductions of FA values in the substantia nigra and its projections in PD (Yoshikawa, et al. 2008, Chan, et al. 2009) even in de novo PD patients (Vaillancourt, et al. 2009). Other areas that have shown decreased FA values in early PD compared to controls are motor areas and the cingulum (Karagulle Kendi, et al. 2008). Recent studies also reported decreased FA or increased MD, localized bilaterally in the cerebellar and orbitofrontal cortex of PD patients (Zhang, et al. 2009), and in the genu of the corpus callosum and superior longitudinal fasciculus (Gattellaro, et al. 2009). PD patients with cognitive deficits, specifically executive dysfunctions, showed reduced FA in the right frontal and left parietal white matter (Matsui, et al. 2007b); in PD patients with dementia,

reductions have been reported in bilateral posterior cingulate bundles compared to PD without dementia (Matsui, et al. 2007a). From all the areas which showed reduced FA in PD patients in comparison with healthy controls, the WM adjacent to the gyrus rectus was the only area that showed a significant correlation with UPSIT scores in PD patients. When analyzing the differences in FA, focusing only on the ROIs from central olfactory areas (the primary olfactory cortex and orbitofrontal cortex) and dividing the PD group into those with hyposmia and those with anosmia, we demonstrated that both hyposmic and anosmic groups differed from controls in the white matter adjacent to gyrus rectus (BA 14) bilaterally and, in addition, anosmic PD patients differed from controls in the POC bilaterally, specifically in WM adjacent to entorhinal cortex.

A decrease in FA value of WM areas may be a sensitive indicator of histological abnormality, even if the values are derived from normal-appearing tissue by conventional MRI (Assaf and Paternak. 2008). At present, the pathological interpretation of FA reduction in the brain with neurodegenerative disease is not clear and may be due to a number of physiological factors. However, murine models of PD have demonstrated that cell loss caused by neurodegenerative disorders is likely to be accompanied by FA decreases in the deteriorated brain regions (Boska, et al. 2007). Therefore, the reduced FA values found in early PD anosmic PD patients may reflect a degeneration of the olfactory bulb and in consequence a loss of axonal connections with olfactory-related primary central cerebral structures, and also a loss of connections with the OFC, which is known to be involved in olfactory identification and therefore may contribute to the odor identification problem reported in PD. Another possible explanation for FA reduction in our early PD patients may be a demyelinating process in central olfactory areas as a consequence of the overexpression of synuclein proteins which has been previously associated with demyelinating changes in PD (Galvin, et al. 1999).

Finally, in the last study we used a typical recognition fMRI paradigm, in which studied and nonstudied items are presented in a mixed sequence and the subject's task is to decide which items are old (studied) and which are new (nonstudied) (Cabeza and Nyberg. 2000), to characterise dynamic

brain activity changes in early PD using a model free-technique. Specifically, we used tensorial probabilistic ICA (T-PICA) that is a model free technique that can be used for analyzing multiple subjects and groups, extracting signals of interest (components) in the spatial, temporal, and also subject domain of fMRI data (Beckmann and Smith. 2005). The model-free approach has been proved more sensitive to detect group differences than the commonly used model-based analysis (Rombouts, et al. 2009).

There were not significant differences between PD patients and healthy controls in the performance of the recognition memory task, but PD patients showed a decreased activation in brain regions related to the performance of the task and an increased activation in areas of the default network.

There are discrepancies in the literature concerning the existence of deficits in recognition memory among PD patients. A recent study suggests that non-demented PD patients suffer from deficits in recognition memory but found no recognition deficits in early-stage PD participants at an easier level of the task suggesting that recognition memory deficits depend on task difficulty and disease severity (Whittington, et al. 2006). Our PD group is at the early stage of the disease (Hoehn and Yahr: I, II) and the task was not difficult what could explain the lack of deficits in this sample.

The episodic memory retrieval task was associated with an activation of the bilateral paracingulate cortex, bilateral dorsolateral OFC (BA 47/12), bilateral frontal pole (lateral part of BA10), bilateral angular gyrus, bilateral visual cortex (V1 and V2) and precentral gyrus (BA6) in comparison with the control task in the whole sample. Functional studies have shown that the most strongly associated regions to retrieval memory are anterior prefrontal cortex (BA10), that has been attributed to the production and maintenance of the mental set of episodic retrieval (Lepage, et al. 2000), and lateral OFC (BA47) (Cabeza and Nyberg. 2000). Other areas that have been consistently related to retrieval memory involve medial temporal areas and parietooccipital areas, anterior cingulate, occipital and cerebelar regions (Cabeza and Nyberg. 2000). In our study occipital and cerebelar areas showed task-related activations. Anterior cingulate did not show activation but the anterior paracingulate did. There are several individual morphological differences in this region that may preclude and exact localization in MRI group studies (Yucel, et al. 2001).

The areas that showed predominantly deactivating task-related signal change for both groups were bilateral ventromedial prefrontal cortex, precuneus, temporo-occipital cortex, left hippocampus and left parahippocampal gyrus, left temporal pole. This set of regions has been described previously as being involved in the default-network (Greicius, et al. 2003, Raichle, et al. 2001). Default mode network activity has been shown to be increased during resting fMRI but it is decreased during task requiring attention (Fox, et al. 2005, Raichle, et al. 2001). An intriguing functional property of the default network is that operates in opposition to other brain systems that are used for focused external attention and sensory processing. When the default network is most active, the external attention system is attenuated and vice versa (Buckner, et al. 2008, Greicius, et al. 2003, Raichle, et al. 2001).

In most of the areas that showed task-related activations PD patients showed hypoactivation in comparison with healthy controls. PD patients showed hypoactivation in bilateral paracingulate gyrus, dorsolateral OFC, lateral frontal pole, occipital pole, angular gyrus and inferior frontal gyrus. In addition, they showed a hyperactivation of areas that were supposed to be deactivated during the fMRI task. Those areas that showed more activation in the PD group involve the ventromedial prefrontal cortex and temporal areas. PD patients showed less deactivation of these areas in comparison with healthy controls. Recent findings suggest that for the successful performance of a memory or executive task the integrity of a network showing task-related deactivations might be just as relevant as a network of other structures showing activations (Buckner, et al. 2008). A recent study in PD reported that patients with PD showed less deactivation of the posterior areas involved in the default mode network but the comparable deactivation of the medial prefrontal cortex (van Eimeren, et al. 2009). In our work PD patients not only showed less deactivation in posterior temporooccipital areas and medial temporal areas but also in the ventromedial prefrontal cortex. In other neurodegenratives diseases such as AD disruption of the default network has been also reported (Celone, et al. 2006, Rombouts, et al. 2009).

However, even if PD patients did not show an efficient activation of areas related to episodic memory retrieval and did not deactivate the default network areas properly, they were able to perform the task as well as control group and showed hiperactivation of the supramarginal gyrus when performing the

task. We think that the activation of these areas is a compensating mechanism for frontal dysfunction early in the course of the disease. Indeed, though episodic memory has long been known to depend on the medial temporal lobe memory system and on prefrontal contributions to retrieval, an emerging body of functional imaging evidence suggests that parietal activations might also contribute to episodic memory retrieval (Wagner, et al. 2005). It could be that PD patients are using this network for compensating for the disruption of the OFC network (Alexander, et al. 1986). Those patients whit unaffected compensatory systems may prove more resilient to functional decline. Even with degraded metabolic pathways the cerebral cortex still works before cerebral functions eventually collapse in patients with dementia (Ferrer. 2009)

Lateral motor cortex (BA4 y BA6) also showed a hyperactivation in patients compared to controls. fMRI studies in PD assessing learning of sequential movements or automatic movements (Sabatini, et al. 2000, Wu and Hallett. 2005) have pointed that PD patients have a cortical reorganization to do the task at the same level than healthy controls. Indeed PET and fMRI studies reported that PD patients recruited parallel motor circuits including lateral and primary motor cortex in order to overcome the functional deficit of the striatocortical motor loops (Sabatini, et al. 2000). In addition, it is also of great interest the hypoactivation in PD of the left substantia nigra. With PET it was found that early parkinsonian patients showed significantly reduced F-dopa uptake or reduction in dopaminergic metabolism in the substantia nigra (Ito, et al. 1999), but with fMRI there are not studies to date showing a reduction of BOLD signal in the substantia nigra.

6. CONCLUSIONS

6. Conclusions

The main conclusions of this thesis, derived from the five studies, can be summarized as follows:

I. The pattern of atrophy in non-demented PD patients affects the anterior region of the hippocampus and progresses to the posterior part in demented patients but preserves the middle part of this structure. This pattern and evolution is similar to that seen in Mild Cognitive Impairment of amnestic type and Alzheimer's disease.

II. Declarative memory dysfunctions in PD depend on the atrophy of the head of the hippocampus.

III. Patients with visual hallucinations present progressive hippocampal atrophy and also show widespread atrophy involving the limbic, paralimbic and neocortical areas in agreement with the evolution towards dementia. In contrast, patients without VH only show gray matter loss in the motor regions and their cognitive functions remain spared.

IV. Early PD patients present gray matter loss in some limbic regions. We observed gray matter loss in the amygdala and orbitofrontal cortex but not in the anterior cingulate, enthorrinal cortex and accumbes nuclei. These results only partially support the stages proposed by Braak.

V. Impairment in decision-making and recognition of facial expressions of emotions occurs at early stages of PD. These neuropsychological deficits are accompanied by degeneration of orbitofrontal cortex (OFC) and amygdala. Bilateral OFC reductions are associated with impaired recognition of emotions, and gray matter volume loss in left lateral OFC is related to decision-making impairment in PD.

VI. PD patients presented abnormal white matter microstructural changes in several brain regions including cortical and subcortical areas early in the disease course. However, only reduction of fractional anisotropy in the white matter of the central olfactory areas, specifically the white matter adjacent to the gyrus rectus, is associated with olfactory dysfunction in PD patients.

VII. There is a disruption of functional networks involved in memory and default mode in early PD.

7. SUMMARY OF THE THESIS

RESUMEN DE LA TESIS

7. Summary of the Thesis **Resumen de la Tesis**

Alteraciones límbicas detectadas por resonancia magnética implicadas en las disfunciones de memoria, emoción y olfato en la enfermedad de Parkinson.

1. Introducción

La enfermedad de Parkinson (EP) ha sido tradicionalmente considerada como un trastorno exclusivamente motor caracterizado por temblor, rigidez, bradicinesia e inestabilidad postural. Sin embargo, estudios recientes concluyen que la EP afecta a múltiples sistemas y diferentes áreas del cerebro. Ello explica las diversas manifestaciones no motoras presentes en la enfermedad incluso en fases previas al diagnóstico. La disfunción cognitiva y evolución a demencia, la presencia de alucinaciones visuales, la reducción de la capacidad olfativa, y las alteraciones del sueño forman parte de las denominadas alteraciones no motoras en la EP (Tolosa, et al. 2009). Los síntomas que preceden a la manifestación de los síntomas motores conforman lo que se conoce como la fase pre-motora del Parkinson. El creciente interés de la EP como una enfermedad neurodegenerativa que afecta a múltiples sistemas nace de la investigación que demuestra la existencia de patología extensa además de la afectación de la sustancia negra (Lang y Obeso. 2004, Ferrer. 2009).

1.1. Neuropatología de la EP

A nivel neuropatológico, la EP se caracteriza por una pérdida masiva de neuronas dopaminérgicas y aparición supuestamente secuenciada de cuerpos y neuritas de Lewy en las células que sobreviven. Braak y colaboradores propusieron un estadiaje de progreso de la enfermedad por el cual se supone que la patología, en concreto la aparición de cuerpos de Lewy, comenzaría en la medulla oblongata y bulbo olfatorio, de ahí ascendería a áreas del cerebro medio y límbicas y finalmente en los últimos estadíos se afectaría el neocortex (Braak et al., 2003). Sin embargo, si bien los cuerpos de Lewy han sido

tradicionalmente asociados a la EP todavía no queda claro su papel en el proceso neuropatológico, siendo hoy día una de las teorías más extensas el hecho de que la patología de cuerpos de Lewy que ocurre en la EP es un evento relativamente tardío y que constituye una agregación de proteínas dañadas con poca repercusión en las manifestaciones corticales y que los múltiples déficit metabólicos ya presentes en la corteza cerebral en las fases iniciales de la enfermedad pueden tener mayor repercusión en el proceso neurodegenerativo (Ferrer. 2009). De hecho, la clasificación propuesta por Braak et al. correlaciona con los déficit neurológicos en la mayoría de pacientes con un comienzo temprano y larga duración de la enfermedad pero estudios retrospectivos clinico-patológicos demuestran que no hay relación entre el estadiaje propuesto y la gravedad de la enfermedad, o más específicamente con el deterioro cognitivo asociado a ella (Jellinger, 2008). De hecho, se han descrito casos con una considerable carga de alpha-synucleina en pacientes manifiestamente no dementes (Parkinnen, et al. 2005). Es importante tener en cuenta que la demencia en la EP también es dependiente de cambios patológicos tipo Alzheimer habiéndose descrito la presencia de ovillos neurofibrilares y places de beta-mailoide en pacientes con EP y demencia (Halliday, et al. 2008).

1.2. Disfunción cognitva y demencia en la EP

Si bien el estudio de las funciones cognitivas en la EP ha sido de alguna manera ignorado durante tiempo en favor del estudio de los síntomas motores, hoy día sabemos que el deterioro cognitivo en la EP comienza desde los estados iniciales y que es uno de los factores que más alteran la calidad de vida de los pacientes y sus familiares. En concreto se han descrito porcentajes de deterioro cognitivo que van desde el 19% hasta el 36 % de pacientes recién diagnosticados, siendo las funciones cognitivas más alteradas la memoria, funciones ejecutivas o de tipo frontal y funciones visuoperceptivas (Foltynie, et al. 2004, Muslimovic, et al. 2005, Aarsland, et al. 2009; Elgh, et al. 2009).

A medida que avanza la enfermedad un gran número de pacientes desarrollará demencia siendo incluso el 83% de los supervivientes a 20 años los que cumplan criterios para el diagnóstico de demencia (Hely, et al. 2008). Entre los factores de riesgo o predictores de demencia se encuentran la edad (Mahieux, et al.

1998, Hughes, et al. 2000, Aarsland, et al. 2001), la gravedad de los síntomas motores (Levy, et al. 2000, Aarsland, et al. 2001), el trastorno de sueño REM (Sinforiani, et al. 2008, Gagnon, et al. 2009), las alucinaciones visuales (Aarsland, et al. 2003, Galvin, et al. 2006) y las disfunciones cognitivas iniciales (Aarsland, et al. 2001, Janvin, et al. 2005) incluyendo la alteración de la fluencia verbal (Jacobs, et al. 1995, Mahieux, et al. 1998), de la memoria (Levy, et al. 2002) y de la función ejecutiva (Mahieux, et al. 1998, Levy, et al. 2002, Janvin, et al. 2005). En concreto, la presencia de alucinaciones visuales es un fenómeno frecuente en la enfermedad de Parkinson. Un estudio reciente sitúa la prevalencia alrededor del 50% (Williams and Lees. 2005). Generalmente aparecen en la segunda mitad del curso de la enfermedad y son de naturaleza persistente y progresiva. Tradicionalmente, las alucinaciones visuales habían sido descritas como una complicación secundaria a la medicación antiparkinsoniana dopaminérgica. No obstante, cada vez son más los datos que señalan que otros factores, como la extensión del proceso neuropatológico a zonas supramesencefálicas con afectación de áreas temporales mediales del cerebro, podrían ser las desencadenantes (Williams and Lees. 2005). Los pacientes con alucinaciones visuales, en comparación con los pacientes sin ellas, manifiestan mayor déficit neuropsicológico en determinadas funciones temporo-parietales tales como, como memoria verbal y visual, lenguaje, fluencia semántica y visuopercepción (Ramirez-Ruiz, et al. 2006) y estudios de neuroimagen estructural muestran reducción de sustancia gris las zonas visuales asociativas temporales y parietales en estos pacientes (Ramirez-Ruiz, et al. 2007).

1.3. Disfunción olfatoria

Los pacientes con EP presentan déficits en la identificación de olores (Doty, et al. 1988, Doty, et al. 1992, Mesholam, et al. 1998, Tissingh, et al. 2001) y estos déficits son patentes desde fases iniciales de la enfermedad (Doty, et al. 1992, Tissingh, et al. 2001). La disfunción olfatoria es independiente de la magnitud de los síntomas motores de la enfermedad, estatus cognitivo, medicación, duración y gravedad de la enfermedad (Doty, et al. 1988, Doty. 2007). La disfunción olfatoria está asociada a un mayor riesgo

de desarrollo de EP (Ponsen, et al. 2004) y se ha descrito que dicha disfunción aparece 4 años antes del comienzo de los síntomas motores (Ross, et al. 2008). Estos hallazgos indican que la disfunción olfatoria es un marcador preclínico y predictivo de la EP (Tolosa, et al. 2009, Berendse, et al. 2001, Ross, et al. 2008). Sin embargo, no todos los síndromes parkinsonianos muestran disfunción olfatoria. Por ejemplo, los pacientes con parkinsonismo vascular (Katsenschlager, et al. 2006), parálisis supranuclear progresiva (Doty, et al. 1993) o parkinsonismo inducido por intoxicación de MPTP (Doty, et al. 1992) presentan olfacción normal.

La información olfatoria se transmite de las estructuras periféricas (el epitelio olfativo) a estructuras cerebrales centrales incluyendo el bulbo que a través del tracto olfatorio conecta directamente con la corteza olfatoria primaria la cual conforman la corteza piriforme y entorrinal y la amígdala. La corteza olfatoria primaria conecta con la secundaria, la corteza orbitofrontal, la cual también está implicada en la identificación de olores (Price, 2004). Diversas teorías proponen que la EP y la consecuente disfunción olfatoria pueden estar causadas por la entrada de toxinas ambientales a través de la cavidad nasal (Del tredici, et al. 2002, Hawkes, et al. 2007). De hecho, la exposición a pesticidas incrementa hasta en un 80% el riesgo de padecer EP (Tanner, et al. 2009). Por otro lado, existe una asociación del deterioro de la capacidad olfativa con la presencia de cuerpos de Lewy (Ponsen, et al. 2006); y las estructuras cerebrales relacionadas con la olfacción, en concreto el bulbo olfatorio, son unas de las primeras áreas en presentar cambios patológicos (Braak, et al. 2003). Los estudios neuropatológicos también muestran mayor carga de alpha-sinucleína en la corteza olfatoria primaria y secundaria (Silveira-Moriyama, et al. 2009).

Existen varios estudios de neuroimagen funcional que han identificado anormalidades en el sistema nervioso central asociadas al déficits de identificación de olores en la EP. Se ha descrito una correlación significativa entre la hipocaptación del transportador de dopamina en el estriado y la puntuación en el test de olfacción UPSIT (University of Pennsylvania Smell Identification Test) en pacientes en fases inciales de la EP (Sideworf, et al. 2005). Un estudio de RM funcional con estimulación olfatoria dentro del

escáner demostró que la actividad neuronal del hipocampo y la amígdala estaban reducidas en los pacientes con EP respecto a los sujetos control (Westermann, et al. 2008).

Por otro lado, los estudios de RM estructural dan soporte a la presencia de anormalidades en estructuras cerebrales centrales. Utilizando *diffusion weighted imaging* (DWI) se encontró un decremento de la difusión en el tracto olfatorio de EP iniciales; y un estudio reciente con *diffusion tensor imaging* (DTI) ha descrito la correlación entre índices de anisotropía fraccional en el cerebelo y los umbrales de identificación de olores (Zhang, et al. 2009).

1.4. Estudios mediante resonancia magnética en la EP

Los estudios de volumetría de región de interés delimitada manualmente en la EP se han centrado principalmente en estructuras temporales mediales porque estas estructuras presentan atrofia en otras enfermedades neurodegenerativas tales como el Alzheimer y la demencia por cuerpos de Lewy. En los pacientes con EP y demencia ha sido descrita atrofia en el hipocampo (Laakso, et al. 1996, Camicioli, et al. 2003, Junque, et al. 2005, Bouchard, et al. 2008), la amígdala (Junque, et al. 2005, Bouchard, et al. 2008) y la corteza entorrinal (Kenny, et al. 2008). Estos hallazgos sugieren que aunque la atrofia de estructuras temporales mediales sea característica principal de la enfermedad de Alzheimer (Scheltens, et al. 2002), puede que también subyazca a la presencia de demencia en la EP. Los estudios de *voxel-based morphometry* (VBM) también han descrito atrofia de regiones temporales mediales en la demencia en la EP; pero también en regiones subcorticales, paralímbicas y neocorticales (Burton, et al. 2004, Summerfield, et al. 2005, Beyer, et al. 2007). El grado de atrofia en los pacientes dementes varía en función de un comienzo precoz o tardío del desarrollo de demencia. El comienzo precoz está asociado a una mayor degeneración de estructuras subcorticales y corticales (Beyer, et al. 2008).

En los enfermos de Parkinson sin demencia también se ha descrito una atrofia del hipocampo (Laakso, et al. 1995, Camicioli, et al. 2003, Junque, et al. 2005, Bouchard, et al. 2008) y de la amígdala (Junque, et al.

2005, Bouchard, et al. 2008) que se puede objetivar mediante volumetría manual y mediante escalas del grado de atrofia a nivel visual (Bruck, et al. 2004). Los estudios de neuroimagen estructural en los que se usado la técnica de la *voxel-based morphometry* en pacientes no dementes han demostrado reducciones en múltiples estructuras cerebrales. En estos pacientes se ha descrito atrofia del núcleo caudado (Brenneis, et al. 2003), regiones frontales e ínsula (Burton, et al. 2004), corteza orbitofrontal y parahipocampo (Nagano-Saito, et al. 2005), hipocampo y cingulado anterior (Summerfield, et al. 2005), surco intraparietal (Cordato, et al. 2005), giro superior temporal y frontal (Beyer, et al. 2006, Ramirez-Ruiz, et al. 2007) y cerebelo (Camicioli, et al. 2009). Una posible explicación para la gran variabilidad entre resultados radica en la heterogeneidad de las muestras de EP examinadas. En general, los enfermos de Parkinson en estos estudios se consideran como un grupo uniforme pero sin diferenciar aquellos que presentan algún déficit cognitivo o factores de riesgo de desarrollo de demencia. De hecho, un estudio encontró que los pacientes con EP y diagnóstico de deterioro cognitivo leve presentaban reducciones de sustancia gris en regiones frontales y temporales en comparación con pacientes cognitivamente intactos (Beyer, et al. 2006).

Tanto la técnica de volumetría manual como la VBM permiten la investigación de los correlatos neuroanatómicos de las disfunciones neuropsicológicas. El volumen del hipocampo ha sido previamente relacionado con medidas de memoria verbal en la EP (Camicioli, et al. 2003, Junque, et al. 2005, Bouchard, et al. 2008). Recientemente, también se ha descrito una correlación significativa entre la fluencia semántica y la reducción de sustancia gris en regiones temporales, frontales y cerebelo (Pereira, et al. 2009a) y la correlación entre déficits visuo-espaciales y reducción de sustancia gris en el lóbulo parietal superior y giro occipital (Pereira, et al. 2009b).

Los estudios de *diffusion tensor imaging* (DTI) han descrito reducción de la anisotropía fraccional (FA) en la sustancia negra y en las proyecciones nigroestriatales (Yoshikawa, et al. 2008, Chan, et al. 2009) incluso en pacientes *de novo* (Vaillancourt, et al. 2009). Otras áreas que han mostrado decrementos de FA en la EP son regiones motoras y el cingulado (Karagulle Kendi, et al. 2008); cerebelo y corteza

orbitofrontal (Zhang, et al. 2009); cuerpo calloso y fascículo longitudinal superior (Gattellaro, et al. 2009). Pacientes con disfunciones ejecutivas muestran FA reducida en sustancia blanca de la corteza prefrontal y parietal (Matsui, et al. 2007a); y en pacientes con demencia la reducción de FA afecta al cingulado posterior bilateralmente en comparación con los pacientes no dementes (Matsui, et al. 2007b).

Finalmente, estudios de resonancia magnética funcional, si bien aún muy escasos en la EP se han utilizados para evaluar la actividad neuronal durante la realización de tareas motoras (Sabatini, et al. 2000), memoria (Mattay, et al. 2002, Lewis, et al. 2003), procesamiento de recompensa (Schott, et al. 2007), percepción de expresiones de miedo (Tessitore, et al. 2002), memoria implícita (Moody, et al. 2004) y funciones ejecutivas (Van Eimeren, et al. 2009).

2. Objetivos e hipótesis de la tesis

El interés general de esta tesis radica en estudiar las bases cerebrales subyacentes algunas de las manifestaciones no-motoras de la enfermedad de Parkinson. En concreto, el estudio de las disfunciones límbicas y sus correlatos neuroanatómicos y funcionales incluyendo los déficits de memoria declarativa, reconocimiento de emociones y toma de decisiones, y las disfunciones olfatorias.

Objetivos específicos

Estudios I y III

Antecedentes

La memoria declarativa se ve afectada en la EP, pero la base de los déficits de memoria en la EP es controvertida. Los estudios iniciales clásicos proponían que la causa del déficit de memoria en la EP era el mal funcionamiento de la circuitería fronto-estriatal a causa de la depleción dopaminérgica (Cooper, et al., 1991). Sin embargo, son cada vez más los trabajos que apuntan hacia una disfunción y atrofia de estructuras temporales mediales que podrían justificar las alteraciones de memoria observadas. El estudio de los déficits de memoria en la EP es fundamental porque se aprecia desde los comienzos de la enfermedad (Muslimovic, et al. 2005), progresan con el tiempo (Muslimovic, et al. 2007), es más acentuado en los pacientes con alucinaciones visuales (Grossi, et al. 2005, Ramirez-Ruiz, et al. 2006), y predice evolución a demencia (Lewy, et al. 2002). Los pacientes con EP y demencia presentan mayores densidades de cuerpos y neuritas de Lewy en el sector CA 2-3 del hipocampo. Por otro lado, la presencia de alucinaciones visuales también predice evolución a demencia y está asociada con cambios en estructuras temporales mediales (Williams and Lees. 2005).

Objetivos

El estudio I es transversal y fue diseñado con los siguientes objetivos:

1. Examinar los diferentes grados de atrofia del hipocampo por medio de la técnica *voxel-based morphometry* en pacientes con EP y demencia y en pacientes no dementes con y sin alucinaciones visuales en comparación con una muestra de controles sanos.
2. Determinar el patrón de atrofia del hipocampo en cada uno de los casos individualmente.
3. Investigar los correlatos neuroanatómicos de la disfunción de la memoria verbal en la EP.

El estudio II es un estudio longitudinal de la muestra anterior con los siguientes objetivos:

4. Estudiar la evolución de los déficits de memoria y la progresión a demencia
5. Establecer la progresiva atrofia del hipocampo como un marcador de evolución a demencia, además de investigar si está progresión se extiende a otras áreas cerebrales.

Hipótesis

En el primer estudio hipotetizamos que:

1. Dado que los pacientes con alucinaciones visuales está en mayor riesgo de desarrollar demencia su parón de atrofia será similar al presentado por los pacientes con demencia.

2. La frecuencia de atrofia hippocampal será mayor en los pacientes con alucinaciones en comparación con aquellos que no presentan alucinaciones, y dicha atrofia debería afectar a la sección CA2 del hipocampo y en consecuencia observarse más en regiones hipocampales medias en las IRM.

3. La disfunción de memoria en la EP es hipocampo-dependiente. La atrofia del hipocampo estará relacionada con una peor ejecución de pruebas de memoria verbal declarativa.

En el estudio longitudinal (estudio II) hipotetizamos lo siguiente:

4. Los pacientes con EP y alucinaciones visuales presentarán desarrollo de demencia y un marcado deterioro en las funciones de memoria verbal.

5. La atrofia cerebral en los pacientes con VH que han evolucionado a demencia abarcará el hipocampo de una manera y con un patrón similar a lo encontrado en los pacientes con demencia en el estudio anterior.

6. Además del hipocampo, otras áreas cerebrales incluyendo regiones límbicas, paralímbicas y neocorticales se verán progresivamente afectadas en estos pacientes.

Estudios III y IV

Antecedentes

Las disfunciones límbicas ocurren en fases iniciales de la EP. Estudios previos han hallado deterioro de la toma de decisiones, el reconocimiento de expresiones faciales y disfunciones olfatorias en pacientes con EP en estado inicial. Estudios funcionales y de lesión señalan la corteza orbitofrontal y la amígdala como estructuras clave para la toma de decisiones y el reconocimiento de emociones. Por otro lado, la información olfatoria se transmite de estructuras periféricas a estructura cerebrales centrales incluyendo el bulbo olfatorio que a través del tracto olfatorio conecta con la corteza olfatoria primaria la cual incluye la corteza piriforme y entorrinal y la amígdala. La corteza orbitofrontal también está implicada en la identificación de olores ya que es la principal estructura diana de la corteza olfatoria primaria.

Objetivos

1. El objetivo del estudio III radica en investigar los correlatos neuroanatómicos de las disfunciones en reconocimiento de expresiones emocionales y toma de decisiones de los pacientes en estado inicial de la EP mediante *voxel-based morphometry*.
2. En el estudio IV investigamos los correlatos neuroanatómicos de la disfunción olfatoria en enfermos iniciales de EP pero con *diffusion tensor imaging* para observar cambios microestructurales en las conexiones entre estructuras relacionadas con la identificación de olores.

Hipótesis

En el estudio III las hipótesis eran las siguientes:

5. Los pacientes en fases iniciales de la EP mostrarán atrofia en áreas límbicas y paralímbicas y en consiguiente déficits asociados en la toma de decisiones y reconocimiento de emociones.

6. Habrá una correlación entre el volumen de la corteza orbitofrontal y la amígdala y la disfunción en la toma de decisiones y reconocimiento de expresiones emocionales.

En el estudio IV hipotetizamos que:

7. Los pacientes en estado inicial de la EP mostrarán déficits en la identificación de olores y además mostrarán una conectividad estructural anormal entre áreas límbicas y neocorticales.

8. La disfunción olfatoria se verá asociada a una reducción de la anisotropía fraccional de las conexiones entre la corteza olfatoria primaria y la corteza orbitofrontal.

Estudio V

Antecedentes

Las funciones cognitivas se porcesan por medio de redes funcionales cerebrales distribuidas que interactuan entre sí. La conectividad functional está determinada por la activación coordinada de dichas areas. Desde las fases iniciales de la enfermedad de Parkinson ocurren cambios metabólicos que podrían afectar a estas conexiones funcionales.

Objetivos

1. Investigar los patrones diferenciales de conectividad functional mediante RMf durante una tarea de memoria de reconocimiento en pacientes en fases inciales de EP y controles sanos.

Hipótesis

1. Habrá una activación y deactivación sincrónica de regions cerebrales involucradas en la relaización de la tarea de memoria .

2. Los pacientes con EP mostrarán conectividad functional diferente a los controles. Hipotetizamos que mostrarán una hiperactivación de regiones preservadas para poder desempeñar la tarea al mismo nivel que los controles.

3. Metodología

La presente tesis consiste en cinco estudios que examinan las bases neuroanatómicas y neurofuncionales de las disfunciones de memoria, emoción y olfato en la enfermedad de Parkinson. Para la realización de los estudios se han utilizado diversas muestras y diversas aproximaciones neuropsicológicas y de resonancia magnética. Todos los estudios fueron aprobados por el Comité Ético de la Universidad de Barcelona (UB) y todos los participantes firmaron el consentimiento informado previamente.

Cada uno de los estudios cuenta con una descripción detallada de las características de las muestras, la metodología de análisis de las imágenes obtenidas por RM y las evaluaciones cognitivas y conductuales llevadas a cabo.

Las **muestras** del estudio I y II forman parte de un proyecto dedicado al estudio de pacientes de Parkinson con factores de riesgo de demencia, en concreto pacientes con alucinaciones visuales, llevado a cabo por el grupo de Neuropsicología del Departamento de Psiquiatría y Psicobiología Clínica de la Universidad de Barcelona, en colaboración con la Unidad de Parkinson y Trastornos del Movimiento del Hospital Clínic de Barcelona. La muestra el primer estudio está compuesta por un total de cien participantes divididos en subgrupos de la siguiente manera: 9 pacientes con EP y demencia, 16 pacientes con EP y alucinaciones visuales, 19 pacientes con EP sin alucinaciones visuales y 56 controles. El segundo estudio consiste en un seguimiento de esta muestra inicial y está compuesto por 12 pacientes con EP y alucinaciones visuales, 14 pacientes con EP y sin alucinaciones visuales y 12 controles. El tercer y cuarto estudio se centran en una muestra de 24 enfermos de Parkinson en fases iniciales de la enfermedad. Las características de estos pacientes considerados iniciales son las siguientes: i) EP menor a 5 años de evolución; ii) estadío de Hoehn y Yahr menor a II; iii) ausencia de fluctuaciones motoras. El grupo control de esta muestra comprendía 24 sujetos sanos apareados a la muestra de enfermos por diferentes variables demográficas tales como la edad, el género y los años de escolaridad.

La **evaluación neuropsicólogica** consistía en las siguientes pruebas: i) subtests de Vocabulario, Información y Semejanzas de la escala Wechsler de Inteligencia para adultos (WAIS-III) para estimar el cociente intelectual premórbido; ii) Test Auditivo Verbal de Rey para evaluar la memoria verbal (aprendizaje, recuerdo demorado y reconocimiento); iii) test de memoria de caras de Warrington para la memoria visual; iv) test de discriminación de formas de Benton para examinar las funciones visuoperceptivas; v) evaluación de las fluencias verbales (semántica y fonética); vi) el test de Token para la comprensión del lenguaje; vii) el test de Boston para la denominación; viii) el *Iowa Gambling Task* para la toma de decisiones; ix) el test de reconocimiento de expresiones emocionales de Ekman; x) el test de la Universidad de Pennsylvania para la identificación de olores.

Las **imágenes de RM** de los cinco estudios se adquirieron en el Centre de Diagnòstic per la Imatge (CDIC), del Hospital Clínic de Barcelona. Para el primer y segundo estudio se adquirieron imágenes de resonancia magnética estructural potenciadas en t1 en una RM de 1.5 tesla. Para los estudios II, IV y V las imágenes fueron obtenidas en una resonancia de 3 tesla y no sólo imágenes potenciadas en t1 sino que también imágenes de DTI y de resonancia magnética funcional aplicando un paradigma de memoria verbal.

Las **técnicas de neuroimagen** empleadas en cada uno de los estudios fueron las siguientes:
i) técnica de "voxel-based morphometry" (VBM) para evaluar las diferencias grupales volumétricas y/o de densidad de la sustancia gris. El procesamiento de las imágenes se realizó mediante los software SPM2 y SPM5 (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, University College London, UK, http://www.fil.ion.ucl.ac.uk/spm) con Matlab 6.5 y Matlab 7.0 (MathWorks, Natick, MA), respectivamente; ii) técnica de "Tract-Based Spatial Statistics" (TBSS) para analizar las imágenes de DTI y evaluar las diferencias de anisotropía de la sustancia blanca mediante el software FSL (FMRIB's Software Library, http://www.fmrib.ox.ac.uk/fsl/); iii) para el estudio de las imágenes obtenidas por resonancia magnética funcional utilizamos una herramienta basada en el análisis de

componentes independientes parate del FSL que se conoce como "*Multivariate Exploratory Linear Optimized Decomposition into Independent Components" (*MELODIC) con la cual se consigue una descomposición de los datos en variaciones dependientes del tiempo, del espacio, y de los sujetos; proporcionando más datos que aquellos que se puedan extraer mediante el análisis clásico de RM funcional mediante análisis de modelo lineal general.

4. Resultados

Estudio I: *Predominio de atrofia de la cabeza del hipocampo en enfermos de Parkinson con alucinaciones visuales y con demencia*

El primer estudio es un estudio de voxel-based morphometry pero delimitando una región de interés concreta, el hipocampo.

Los análisis grupales mostraron que la atrofia del hipocampo en los pacientes con EP y demencia es generalizada, afectando sobre todo a regiones anteriores y posteriores de dicha estructura y quedando las áreas centrales relativamente preservadas. Los pacientes no dementes pero con alucinaciones visuales también presentan atrofia del hipocampo pero limitada a la cabeza del hipocampo; mientras que los pacientes no dementes sin alucinaciones visuales no presentaban atrofia del hipocampo en el análisis grupal.

El análisis individual de cada uno de los sujetos demostró que el 78% de los pacientes con demencia, 31% de los pacientes no dementes con alucinaciones visuales y el 26% de los pacientes sin demencia y sin alucinaciones presentaban atrofia del hipocampo.

La pérdida de sustancia gris en la cabeza del hipocampo correlacionó con los déficits de memoria ($r= 0.54$ en el hipocampo izquierdo; $r= 0.65$ en el hipocampo derecho) pero no con otras variables clínicas en los pacientes con EP, lo que sugiere que estos déficits están relacionados con la atrofia de esta estructura y no son secundarios a alteraciones en los circuitos fronto-estriatales.

Estudio II: ***Progresión diferencial de la atrofia cerebral en enfermos de Parkinson con y sin alucinaciones visuales***

El segundo trabajo es un estudio longitudinal de la muestra anterior. Tras un periodo de 2 años y medio de seguimiento el porcentaje de conversión a demencia en los pacientes con EP y alucinaciones visuales ascendía a un 75%, y el hipocampo de estos pacientes mostraba el mismo patrón de atrofia descrito en los pacientes con demencia en el estudio previo. El estudio comparativo de las imágenes de resonancia magnética adquiridas en el inicio con las imágenes obtenidas en el seguimiento reveló que los pacientes con alucinaciones visuales presentaban una no sólo una atrofia progresiva de las áreas límbicas, sino también paralímbicas y neocorticales, mientras que los enfermos sin alucinaciones visuales, de los cuales ninguno había desarrollado demencia, sólo mostraron pequeños decrementos de sustancia gris en regiones motoras.

La progresiva pérdida de sustancia gris en determinadas áreas cerebrales correlacionó con el progresivo deterioro de funciones cognitivas concretas. El declive en aprendizaje verbal estaba asociado con una mayor atrofia de la cabeza del hipocampo (r= 0.88), y la memoria verbal demorada con una mayor atrofia de áreas frontales mediales (r= 0.95). El declive en fluencia semántica estaba asociado a atrofia progresiva del tálamo (r= 0.95) y por último el progresivo deterioro en la comprensión del lenguaje correlacionó con la pérdida progresiva de sustancia gris en la amígdala (r= 0.89).

Estudio III: ***Correlatos neuroanatómicos de las disfunciones en el reconocimiento de expresiones faciales emocionales y toma de decisiones en las fases iniciales de la EP***

Los pacientes en fases iniciales de la EP obtuvieron perores puntuaciones en el test de Ekman de reconocimiento de expresiones faciales emocionales (F=,2.48 p= 0.042) y en el *Iowa Gambling Test* (F= 7.55, p= 0.001) en comparación con los controles sanos apareados por género, edad y años de educación. Sin embargo, otras medidas tales como el desempeño en una prueba de atención sostenida no mostraron diferencias entre grupos.

Analizamos las diferencias cerebrales entre grupos utilizando regiones de interés definidas *a priori* de la corteza orbitofrontal y amígdala. Los pacientes con EP mostraron pérdida de volumen de sustancia gris en la amígdala derecha y en la corteza orbitofrontal bilateralmente en comparación con los controles ($p < 0.05$ corregido por múltiples comparaciones).

El análisis exploratorio de diferencias cerebrales en todo el cerebro mostró que además de la amígdala y la corteza orbitofrontal los pacientes con EP mostraban áreas de reducción de sustancia gris en regiones temporo-parieto-occipitales, parahipocampo y cerebelo ($p < 0.001$ sin corregir).

La puntuación en el reconocimiento de emociones faciales de Ekman en los pacientes con EP correlacionó con la corteza orbitofrontal bilatealmente (r=0.58). Las puntuaciones en el *Iowa Gambling test* también mostraron una correlación significativa con la corteza orbitofrontal, pero sólo en el hemisferio izquierdo y un cluster reducido a la parte lateral (r= 0.49). Ninguna de estas pruebas neuropsicológicas mostró correlación con la amígdala o con otras estructuras que mostraron diferencias entre pacientes y controles en la comparación grupal.

Estudio IV: ***Relación entre la disfunción olfatoria y reducción de la anisotropía fraccional de regiones centrales olfatorias en fases iniciales de la enfermedad de Parkinson.***

Los pacientes con EP mostraron puntuaciones menores a los controles en el test de identificación de olores UPSIT ($t= 6.22$; $p < 0.001$). Las puntuaciones del UPSIT no correlacionaron con escalas clínicas tales como el UPDRS-III ($r= 0.21$; $p= 0.39$), los años de evolución de la enfermedad ($r= -0.21$; $p= 0.41$) o el estadío de Hoehn y Yahr ($r= -0.31$; $p= 0.22$) en el grupo de pacientes.

La comparación grupal de las imágenes de DTI mostró que los pacientes presentaban una anisotropía fraccional (FA) reducida en el cuerpo calloso, la sustancia blanca adyacente al cingulado posterior y regiones parieto-occcipitales, el fascículo longitudinal superior, parte anterior del lóbulo temporal, sustancia blanca adyacente al gyrus rectus, tálamo, fornix y tronco cerebral ($t > 3$; 0.001 sin corregir). Introdujimos la media de FA de todas estas áreas en un análisis de regresión lineal únicamente la FA media de la sustancia blanca adyacente al gyrus rectus explicaba las disfunciones olfatorias ($F = 5.762$, $p < 0.029$).

Al analizar las diferencias de FA centrándonos solamente en regiones de interés relacionadas con la capacidad olfativa tales como, la corteza olfatoria primaria y orbitofrontal y el fascículo uncinado y dividiendo el grupo de pacientes de acuerdo al grado de deterioro de la capacidad olfativa, demostramos que tanto los pacientes con anosmia (puntuaciones menores a 18 en el UPSIT) como hiposmia (puntuaciones entre 18 y 25) presentaban reducción de FA en la sustancia blanca subyacente al gyrus rectus y que además, los pacientes con anosmia diferían de los controles en la FA de la corteza olfatoria primaria, en concreto de la sustancia blanca adyacente a la corteza entorrinal ($p < 0.05$ corregido por múltiples comparaciones).

Estudio V: Alteaciones de redes funcionales cerebrales durante la realización de una tarea de memoria episódica en la enfermedad de Parkinson

Los pacientes no mostraron diferencias con los controles en la ejecución de la tarea. El número de palabras correctamente reconocidas, de falsos reconocimientos, omisiones y el tiempo medio de respuesta no mostraron diferencias significativas entre grupos; si bien las puntuaciones en el grupo de pacientes eran algo menores.

El análisis de componentes de las imágenes de RMf mostró que la tarea de memoria de reconocimiento estaba asociada con una serie de activaciones y deactivaciones de regiones cerebrales. La activación correspondía al paracingulado, la corteza orbiofrontal lateral (BA 47/12), la corteza prefrontal anterior, el giro angular, la corteza visual y el cerebelo.

La tarea también estaba relacionada con la deactivación de ciertas regiones cerebrales, específicamente, la corteza prefrontal ventromedial, precuneus, la encrucijada temporo-occipital y áreas temporales mediales. Estas regiones son las implicadas en la red conocida como *default network*

Los pacientes con EP mostraron hipoactivación de todas las áreas implicadas en la tarea de memoria en comparación con los controles, además de una hipoactivación en la sustancia negra, y mostraron una deactivación reducida de las áreas implicadas en la *default network.*

Sin embargo, aunque los pacientes presentaban hipoactivación de redes funcionales implicadas en la tarea y reducida deactivación de la red que se supone debería permanecer suspendida durante la ejecución de la tarea, mostraron un desempeño parecido al de los controles; y una hiperactivación en áreas supramarginales.

5. Discusión

La presente tesis está dirigida a investigar los correlatos neuroanatómicos y neurofuncionales de las disfunciones de memoria declarativa, reconocimiento de emociones y toma de decisiones y los déficits de identificación de olores en la enfermedad de Parkinson. Hipotetizamos que estas disfunciones se verán asociadas a la degeneración del sistema límbico desde las fases iniciales de la enfermedad.

Los resultados de las comparaciones grupales del primer estudio mostraron que la atrofia del hipocampo detectada por análisis de *voxel-based morphometry* está presente en la EP. Los pacientes con demencia tienen pérdida de sustancia gris que implica la parte anterior y posterior de dicha estructura quedando la parte media relativamente preservada, mientras que los pacientes sin demencia pero con alucinaciones visuales muestran atrofia solo en la cabeza del hipocampo. Por otro lado, los análisis individuales de cada uno de los pacientes pusieron en evidencia que un 78% de los pacientes con EP y demencia y un 31% y 26% de los pacientes no dementes con y sin alucinaciones visuales respectivamente mostraban atrofia predominante en la cabeza del hipocampo. Estos hallazgos coincidían con los llevados a cabo por otro grupo independiente en el mismo periodo en el que hallaron los pacientes no dementes de avanzada edad con EP mostraron atrofia predominante también en la cabeza del hipocampo (Bouchard, et al. 2008). La cabeza del hipocampo se corresponde básicamente con la región CA1 y esta región es especialmente vulnerable a cambios de tipo Alzheimer incluso en las fases iniciales de la enfermedad (Frisoni, et al. 2002, Wang, et al. 2003). Además, se ha descrito que la reducción del volumen del hipocampo y especialmente la implicación del sector CA1 están asociados a un mayor riesgo de conversión de deterioro cognitivo leve a demencia de tipo Alzheimer (Apostolova, et al. 2006b).

Los déficits de memoria, en concreto en la medida de aprendizaje verbal, correlacionaron con la pérdida de sustancia gris en la parte anterior del hipocampo. En ancianos no dementes también se ha descrito una correlación de la atrofia del hipocampo con medidas de memoria verbal (Hackert, et al. 2002). En la EP, se han hallado correlaciones con medidas de memoria de reconocimiento pero no con medidas de aprendizaje (Bouchard, et al. 2008). Por otro lado, el hecho de que la atrofia el hipocampo correlacionase

con la ejecución en pruebas de memoria verbal, pero no con escalas de tipo clínico sugiere que esta atrofia está específicamente relacionada con el deterioro en memoria, pero no con la evolución general de la enfermedad. Los hallazgos de este estudio nos llevan a cuestionar la asunción clásica de los déficits de memoria en la EP como consecuencia de la depleción dopaminérgica afectando a los circuitos fronto-estriatales (Cooper, et al. 1991); y sugerimos que la causa de la disfunción radica en la integridad de estructuras temporales mediales, en concreto en una estructura clave dentro del sistema límbico, el hipocampo.

En el estudio II, después de un seguimiento de 30 meses, el 75% de los pacientes con alucinaciones visuales inicialmente no dementes mostraban criterios de demencia en la exploración final. Además de un declive exacerbado en memoria verbal, los pacientes mostraron un deterioro cognitivo significativo en fluencia semántica, comprensión del lenguaje y funciones visuoperceptivas. Los pacientes con EP y alucinaciones visuales mostraban exactamente el mismo patrón de atrofia descrito en los pacientes con demencia en el estudio transversal inicial con una mayor afectación de la cola y la cabeza del hipocampo. Además de la atrofia del hipocampo los pacientes mostraron un patrón de atrofia progresiva en otras áreas límbicas y regiones paralímbicas y neocorticales. Por lo tanto, la evolución a demencia en la EP no es solo consecuencia de la degeneración de áreas límbicas pero también de la progresiva afectación de áreas neocorticales, sobre todo áreas terciarias o de asociación. En contraste con la evolución de los pacientes con alucinaciones, los pacientes sin alucinaciones visuales no mostraron evolución a demencia y la progresiva pérdida de sustancia gris sólo afecto a áreas motoras. La progresiva pérdida de sustancia gris en determinadas áreas cerebrales de los pacientes que evolucionaron a demencia correlacionó con el progresivo deterioro de funciones cognitivas concretas. El declive en aprendizaje verbal mostró correlación con la pérdida de sustancia gris en del hipocampo, y la memoria verbal demorada con una mayor atrofia de áreas frontales mediales. El declive en fluencia semántica estaba asociado a atrofia progresiva del tálamo, y por último el progresivo deterioro en la comprensión del lenguaje correlacionó con la pérdida progresiva de sustancia gris en la amígdala. Los dos primeros patrones de correlación son

esperables por los correlatos neurofuncionales conocidos. La relación entre lenguaje y amígdala es de difícil explicación y es probablemente casual.

Los hallazgos de los dos estudios previos abordan las disfunciones límbicas en etapas avanzadas de la enfermedad y en pacientes con factores de riesgo de evolución a demencia tales como la presencia de alucinaciones visuales. Sin embargo, las disfunciones límbicas están presentes en las fases iniciales de la enfermedad, y otros funciones cognitivas dependientes de la integridad del sistema límbico, al margen de la memoria, tales como el reconocimiento de expresiones emocionales y la toma de decisiones se ven afectadas. Por lo tanto, los siguientes estudios se centraron en una muestra de pacientes en fases iniciales de la enfermedad con no más de 5 años de evolución, Hoehn y Yahr igual o menor a II, y ningún factor de riesgo conocido. Estos pacientes, se corresponderían con un estadios III o IV de la clasificación propuesta por Braak (Braak, et al. 2003) por la cual se supone que habría una afectación de áreas límbicas y del mesencéfalo pero relativa preservación de áreas neocorticales. Sin embargo, el análisis de *voxel-based morphometry* mostró que los pacientes en fases iniciales no sólo mostraban atrofia de áreas límbicas, incluyendo la amígdala y la corteza orbitofrontal, sino que también había reducción de sustancia gris en áreas de la encrucijada parieto-temporo-occipital. Además, no todas las áreas límbicas mostraron atrofia, por ejemplo, no había diferencias en el volumen de la corteza entorrinal entre pacientes y controles; y este área se supone que tendría que mostrar una afectación previa a la corteza orbitofrontal. El estadiaje propuesto por Braak ha sido recientemente cuestionado y se ha demostrado que el estadío no se corresponde generalmente con el grado de deterioro cognitivo en la EP (Jellinger. 2008).

En nuestro estudio la reducción de sustancia gris afectaba a la parte ventrolateral y dorsomedial de la corteza orbitofrontal y la atrofia de la amígdala implicaba la amígdala derecha y mayormente el núcleo anterior. Los estudios previos de neuroimagen estructural (Junque, et al. 2005, Ramirez-Ruiz, et al. 2005, Summerfield, et al. 2005, Nagano-Saito, et al. 2005) y funcional (Huang, et al. 2007) también apuntan a una degeneración de la circuitería límbica incluyendo la amígdala y la corteza orbitofrontal en la EP.

Los pacientes con EP mostraron peor ejecución que lo controles en la pruebas de reconocimiento de emociones y toma de decisiones, pero no en otras pruebas neuropsicológicas tales como medidas de atención sostenida, y los déficits neuropsicológicos correlacionaron con la perdida de sustancia gris en la corteza orbitofrontal, pero no con la atrofia de la amígdala y de otras estructuras que mostraron atrofia en la comparación grupal. La corteza orbitofrontal, considerada corteza de asociación límbica, ha demostrado tanto en estudios funcionales como en estudios de lesión ser una estructura crítica para el desempeño adecuado del reconocimiento de emociones (Adolphs. 2002a, Adolphs. 2002b, Blair, et al. 1999, Dolan, et al. 1996, Heberlein, et al. 2008, Hornak, et al. 2003, Nakamura, et al. 1999) y la toma de decisiones (Bechara. 2004, Denburg, et al. 2007, Fellows and Farah. 2005, Wallis. 2007) y nuestro estudio sugiere que la pérdida de sustancia gris en esta región es la responsable de los déficits emocionales en la EP.

Además de déficits cognitivos es sabido que los pacientes con EP presentan disfunciones de la capacidad de identificar olores en las fases iniciales de la enfermedad. Los déficits en la capacidad de olfacción aparecen incluso antes que los síntomas motores y son característica esencial de lo que se conoce como la fase pre-motora del Parkinson (Tolosa, et al. 2009). La información olfatoria se transmite de las estructuras periféricas (el epitelio olfativo) a estructuras cerebrales centrales incluyendo el bulbo que a través del tracto olfatorio conecta directamente con la corteza olfatoria primaria la cual conforman la corteza piriforme y entorrinal y la amígdala. La corteza olfatoria primaria conecta con la secundaria, la corteza orbitofrontal, la cual también está implicada en la identificación de olores (Price. 2004). Diversas teorías proponen que la EP y la consecuente disfunción olfatoria pueden estar causadas por la entrada de toxinas ambientales a través de la cavidad nasal (Del tredici, et al. 2002, Hawkes, et al. 2007). De hecho, la exposición a pesticidas incrementa el riesgo de padecer EP (Tanner, et al. 2009). Por otro lado, existe una asociación del deterioro de la capacidad olfativa con la presencia de cuerpos de Lewy (Ponsen, et al. 2006); y las estructuras cerebrales relacionadas con la olfacción, en concreto el bulbo olfatorio, son unas de las primeras áreas en presentar cambios patológicos (Braak, et al. 2003). Otros estudios

neuropatológicos también muestran mayor carga de alpha-sinucleína en la corteza olfatoria primaria y secundaria (Silveira-Moriyama, et al. 2009). En el estudio IV investigamos la relación entre los déficits en la identificación de olores en una muestra de pacientes con EP en fases iniciales con cambios microestructurales de la sustancia blanca en regiones centrales olfatorias mediante el análisis de imágenes por tensor de difusión (DTI). Los pacientes con EP mostraron reducción de la anisotropía fraccional (FA) en diversas regiones corticales y subcorticales. Sin embargo, únicamente la reducción de la FA de la sustancia blanca adyacente a áreas olfatorias primarias mostró correlación con la puntuación en el test de identificación de olores UPSIT. Al analizar las diferencias de FA centrándonos solamente en regiones de interés relacionadas con la capacidad olfativa tales como, la corteza olfatoria primaria y orbitofrontal y el fascículo uncinado y dividiendo el grupo de pacientes de acuerdo al grado de deterioro de la capacidad olfativa, demostramos que tanto los pacientes con anosmia (puntuaciones menores a 18 en el UPSIT) como hiposmia (puntuaciones entre 18 y 25) presentaban reducción de FA en la sustancia blanca subyacente al gyrus rectus y que además, los pacientes con anosmia diferían de los controles en la FA de la corteza olfatoria primaria, en concreto de la sustancia blanca adyacente a la corteza entorrinal. Un decremento en FA es un indicador sensible de anormalidad histológica, si bien a día de hoy la interpretación de dicha reducción en las enfermedades neurodegenerativas puede ser atribuida a diferentes factores fisiológicos (Assaf et al., 2008). Interesantemente, el modelo animal de la EP ha demostrado que la pérdida neuronal se corresponde con un decremento de FA de las regiones afectadas (Boska, et al. 2007). Por lo tanto, la reducción de FA encontrada en nuestra muestra de pacientes con EP y capacidad olfativa disminuida podría estar reflejando una degeneración de las regiones olfatorias cerebrales centrales inicialmente en el bulbo olfatorio y a partir de ahí una pérdida de conexiones axonales con la corteza olfatoria primaria y secundaria. Otra posible explicación para la reducción de FA en nuestros pacientes implicaría un proceso de desmielinización de regiones olfatorias a raíz de una sobreexpresión de proteínas de la familia de las sinucleinas lo cual ya ha sido relacionado con cambios en la mielinización previamente en la EP (Galvin, et al. 1999).

Por último, en el estudio V evaluamos las redes funcionales implicadas en una tarea de memoria de reconocimiento mediante resonancia magnética funcional (RMf) en pacientes con EP en fase inicial. Si bien tanto los pacientes como los controles mostraron una ejecución similar en la tarea, el análisis de componentes de las imágenes de RMf reveló una activación cerebral reducida de los pacientes en comparación con los controles en redes funcionales implicadas en la memoria, incluyendo la corteza orbitofrontal lateral y el paracingulado, y en la sustancia negra. Sin embargo, los pacientes con EP mostraron una mayor activación en el área supramarginal bilateralmente lo que podría ser considerado como un mecanismo de compensación para poder ejecutar la tarea adecuadamente. Por otro lado, los pacientes mostraron una deactivación menor a los controles en áreas implicadas en la *default network*.

Los pacientes no mostraron diferencias con los controles en la ejecución de la tarea. El número de palabras correctamente reconocidas, de falsos reconocimientos, omisiones y el tiempo medio de respuesta no mostraron diferencias significativas entre grupos; si bien las puntuaciones en el grupo de pacientes eran algo menores. Hay discrepancias en la literatura respecto al hecho de si los pacientes no dementes presentan déficits en la memoria de reconocimiento. Un estudio reciente sugiere que los pacientes con EP aunque no presenten demencia sí que muestran una alteración de la memoria de reconocimiento pero no encontró diferencias entre controles y pacientes en fases iniciales al hacer una versión sencilla de la tarea deduciendo por tanto que la memoria de reconocimiento depende de la dificultad de la tarea y la severidad de la enfermedad (Whittington, et al. 2006). Nuestro grupo de pacientes se encuentra en fases iniciales de la enfermedad y el diseño de la tarea era sencillo lo que puede explicar la adecuada ejecución de la tarea en nuestra muestra.

El análisis de componentes de las imágenes de RMf mostró que la tarea de memoria de reconocimiento estaba asociada con una serie de activaciones y deactivaciones de regiones cerebrales. La activación correspondía al paracingulado, la corteza orbiofrontal lateral (BA 47/12), la corteza prefrontal anterior, el giro angular, la corteza visual y el cerebelo. Estas activaciones se corresponden con lo encontrado en sujetos sanos en la literatura (Cabeza and Nyberg. 2000, Lepage, et al. 2000). La tarea también estaba

relacionada con la deactivación de ciertas regiones cerebrales, específicamente, la corteza prefrontal ventromedial, precuneus, la encrucijada temporo-occipital y áreas temporales mediales. Estas regiones son las implicadas en la red conocida como *default network* (Buckner, et al. 2008). La actividad de la *default network* se ve incrementada en experimentos que utilizan RMf de reposo, pero su actividad se ve decrementada en tareas que requieren atención (Greicius, et al. 2003, Raichle, et al. 2001).

Los pacientes con EP mostraron hipoactivación de todas las áreas implicadas en la tarea de memoria en comparación con los controles, además de una hipoactivación en la sustancia negra, y mostraron una deactivación reducida de las áreas implicadas en la default. Estudios recientes sugieren que una adecuada deactivación de la default network durante la ejecución de una tarea de memoria es tan importante como una buena activación de las áreas implicadas en la tarea (D'Esposito, et al. 2003). Un estudio reciente en la EP mostró que los pacientes mostraban menor deactivación en áreas posteriores implicadas en la default (van Eimeren, et al. 2009). En nuestro trabajo los pacientes no sólo mostraban una menor deactivación de areas posteriores pero también de la corteza prefrontal ventromedial. Otras enfermedades neurodegenerativas tales como el Alzheimer también muestran disrupción de esta red neuronal (Celone, et al. 2006, Rombouts, et al. 2009).

Sin embargo, aunque los pacientes presentaban hipoactivación de redes funcionales implicadas en la tarea y reducida deactivación de la red que se supone debería permanecer suspendida durante la ejecución de la tarea, mostraron un desempeño parecido al de los controles; y una hiperactivación en áreas supramarginales. Creemos que el reclutamiento de estas áreas podría suponer un mecanismo de compensación para suplir la disfunción en el circuito límbico (Alexander, et al. 1986). De hecho, hay cada vez más evidencia del rol del lóbulo parietal en la memoria episódica (Wagner, et al. 2005).

Por último, es de gran interés la hipoactivación mostrada en la sustancia negra en los pacientes con EP; ya que si bien está hipoactivación ha sido previamente detectada en estudios de PET (Ito, et al. 1999), hasta la fecha ningún estudio con fMRI la había descrito.

6. Conclusiones

Las principales conclusiones de esta tesis derivadas de los 5 estudios se pueden resumir de la siguiente manera:

I. La atrofia del hipocampo en la enfermedad de Parkinson afecta a la parte anterior de dicha estructura en los enfermos sin demencia y en los pacientes con demencia progresa a la parte posterior quedando la parte media relativamente preservada. Este patrón de atrofia es similar al encontrado en la enfermedad de Alzheimer.

II. Las disfunciones de memoria correlacionan con la pérdida de sustancia gris en la cabeza del hipocampo y sugieren un déficit de codificación de información más que de recuperación.

III. Los pacientes con alucinaciones visuales presentan evolución a demencia y atrofia progresiva del hipocampo y de otras áreas cerebrales incluyendo regiones límbicas, paralímbicas y neocorticales; mientras que los pacientes sin alucinaciones solo muestran atrofia progresiva de áreas motoras.

IV. Los pacientes en fases iniciales de la enfermedad muestran pérdida de sustancia gris afectando a la corteza orbitofrontal y amígdala pero quedando preservadas areas paralímbicas. Estos patrones no se ajustan al estadiaje propuesto por Braak y Braak.

V. El deterioro del reconocimiento de expresiones emocionales y toma de decisiones ocurre en los estados iniciales de la enfermedad. La reducción de sustancia gris en la corteza orbitofrontal bilateralmente está asociada al déficit en reconocimiento de emociones y la pérdida de sustancia gris en la corteza orbitofrontal lateral izquierda está asociada a la disfunción en la toma de decisiones.

VI. Los pacientes con EP presentan cambios microestructurales de la sustancia blanca en regiones corticales y subcorticales. Sin embargo, solo la anisotropía fraccional reducida de las áreas olfativas centrales, específicamente de la sustancia blanca adyacente al gyrus rectus, muestran relación con la disfunción olfatoria.

VII. Los pacientes con EP muestran una menor activación de redes funcioanales implicadas en la memoria de reconociemiento y una reducción de la deactivación de areas de la *default network.*

Los estudios descritos en esta tesis aporten evidencia de que las disfunciones de memoria, reconocimiento de expresiones emocionales y toma de decisiones y déficits olfativos están relacionados con cambios estructurales y funcionales de la sustancia gris y blanca de áreas relacionadas con el sistema límbico.

7. Conclusions

Les principals conclusions d'aquesta tesi derivades dels cinc estudis es poden resumir de la següent manera:

I. L'atròfia de l'hipocamp en la malaltia de Parkinson (MP) afecta a la part anterior de aquesta estructura en els malats sense demència i, en els pacients amb demència, progressa a la part posterior, mentre la part mitja es manté relativament preservada. Aquest patró d'atròfia és similar al descrit en el deteriorament cognitiu lleu amnèsic i la malaltia d'Alzheimer.

II. Les disfuncions de memòria declarativa en la MP correlacionen amb la pèrdua de substància gris en el cap de l'hipocamp.

III. Els pacients amb al·lucinacions visuals presenten una evolució cap a la demència i atròfia progressiva de l'hipocamp i d'altres estructures cerebrals que inclouen regions límbiques i neocorticals; mentre que els pacients sense al·lucinacions sols postren atròfia progressiva de les àrees motores.

IV. Els pacients en les fases inicials de la malaltia presenten pèrdua de substància gris en algunes regions límbiques tals com el l'escorça orbitofrontal i amígdala, però no en d'altres com l'escorça entorrinal, nucli accumbens i el paracingulat. Aquests patrons tan sols s'ajusten en part al estadiatje proposat per Braak.

V. El deteriorament del reconeixement d'expressions emocionals i presa de decisions apareix en els estadis inicials de la malaltia. La reducció de la substància gris en l'escorça orbitofrontal de forma bilateral està associada al dèficit en el reconeixement d'emocions. Mentre la pèrdua de substància gris en l'escorça orbitofrontal lateral esquerra està associada a la disfunció en la presa de decisions.

VI. Els pacients amb MP presenten canvis microestructurals de la substància blanca en regions corticals i subcorticals. Tanmateix, sols l'anisotropia fraccional reduïda de les àres olfactives, especialmente de la substància blanca adjacent al gyrus rectus, mostren relació amb la disfunción olfactiva.

VII. En fases inicials de la MP existeix una disrupció de les reds funcionals cerebrals implicades en la memòria de reconeixement i la *default network*.

Els estudis descrits en aquesta tesi aporten evidència de que les disfuncions de memòria, reconeixement d'expressions emocionals, presa de decisions i dèficits olfactius estan relacionats amb canvis estructurals i funcionals de la substància gris i blanca d'àrees relacionades amb el sistema límbic.

8. Ondorioak

Tesi honetatik eta bere bost ikerketetatik ateratako ondoriak horrela laburtu daitezke:

I. Dementzia gabeko Parkinson gaixoetan atrofia hipokampoaren aurreko zatian ikusten da. Dementzia daukaten Parkinson gaixoetan, hipokampozko atrofia hedatua ikusten da, aurreko eta atzeko zatian baina erdiko tartea preserbatuta dago. Atrofia patroi hau Alzheimer gaixotasunean gertatzen den bezalakoa da.

II. Hitzezko oroimenaren galerak Parkinsonean hipokampoaren aurreko zatiaren osotasunean dependitzen dira.

III. Ikusmen haluzinazioak dituzten Parkinson gaixoetan, hipokampozko atrofia progresiboa ta dementziarako bilakaeraz gain, atrofia hedatua ere ematen da, eremu limbikora, paralimbikora eta gune neokortikaletara zabaltzen dena.

IV. Parkinson goiztiarreko gaixoek gai griseko galera dute, eremu limbiko batzuetan (amigdala eta orbitofrontalean) baina ez beste batzuetan (entorrinalean, nucleus accumebens eta paracinguate). Hau ez dator bat Brakek proposatutako eritasun aroekin.

V. Erabakiak hartzeko eta aurpegi espresioak bereizteko ezintasuna gertatzen da PD goiztiarreko faseetan. Gabezia neuropsikologiko hauek amigdalaren eta OFC-ren endekapenarekin batera datoz. OFC aldebiko gutxitzea emozioak bereizteko ezintasunarekin asoziatuta dago, eta gai griseko bolumen galera ezker aldeko OFC-an erabakiak hartzeko ezintasunarekin asoziatuta dago.

VI. Parkinson gaixoek ohiz-kanpoko gai txuriko aldaketa mikroestrukturalak ageri zituzten gaixotasunaren aro goiztiarretatik hainbat garun eremutan, gune kortikalak eta subkortikalak barne. Alabaina, bakarrik anisotropia frakzionalezko gutxipena dago usaimen area zentraletako gai txurian, bereziki gyrus rectus aldamenean dagoen gai txurian, asoziatuta PD gaixoetako usaimen disfuntzioarekin.

VI. Parkinson gaixotasuenan garun sare funtzionalen jarduera akatzduna dago oroimenari eta default modeari eraginez.

8. Appendix

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MRI and Cognitive Impairment in Parkinson's Disease

Naroa Ibarretxe-Bilbao, PhD,[1,2,3] Eduardo Tolosa, MD,[1,2,4]* Carme Junque, PhD,[1,2,3] and Maria-Jose Marti, MD[1,2,4]

[1]*Centro de Investigación en Red de Enfermedades Neurodegenerativas (CIBERNED), Hospital Clínic de Barcelona, Barcelona, Spain*
[2]*Institute of Biomedical Research August Pi i Sunyer, Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain*
[3]*Department of Psychiatry and Clinical Psychobiology, University of Barcelona, Barcelona, Spain*
[4]*Movement Disorders Unit, Neurology Service, Institut Clínic de Neurociències (ICN), Hospital Clínic de Barcelona, Barcelona, Spain*

Abstract: Patients with Parkinson's disease (PD) may present impairment in cognitive functions even at early stages of the disease. When compared with the general population, their risk of dementia is five to six times higher. Recent investigations using structural MRI have shown that dementia in PD is related to cortical structural changes and that specific cognitive dysfunctions can be attributed to atrophy in specific structures. We review the structural MRI studies carried out in PD using either a manual region of interest (ROI) approach or voxel-based morphometry (VBM). ROI studies have shown that hippocampal volume is decreased in patients with PD with and without dementia; in addition, hippocampal atrophy correlated with deficits in verbal memory. VBM studies have demonstrated that dementia in PD involves structural changes in limbic areas and widespread cortical atrophy. Findings in nondemented patients with PD are less conclusive, possibly because cognitively heterogeneous groups of patients have been studied. Patients with PD with cognitive impairment and/or visual hallucinations present greater brain atrophy than patients without these characteristics. These findings suggest that cortical atrophy is related to cognitive dysfunction in PD and precedes the development of dementia. Structural MRI might therefore provide an early marker for dementia in PD. © 2009 Movement Disorder Society

Key words: Parkinson's disease; dementia; hallucinations; MRI; cognitive functions

Parkinson's disease (PD) has traditionally been considered as a motor disorder. However, cognitive dysfunctions are known to occur even at early stages[1] and most patients develop dementia over the course of the disease.[2] Dementia in PD was initially described as subcortical, and cognitive dysfunctions in nondemented patients have been attributed to dopaminergic depletion affecting the fronto-striatal circuit[3] or the dopamine-acetylcholine synaptic imbalance.[4] Nevertheless, recent investigations using MRI suggest that specific cognitive deficits, such as memory deficits, and dementia in PD may also be explained by cortical structural changes. The methods most widely used to assess structural MRI changes in PD have been region of interest (ROI) and voxel-based morphometry (VBM). The ROI method consists in measuring manually delineated and anatomically defined regions within the brain based on an a priori hypothesis. ROI approach takes into consideration the variability across subjects, but as it also depends on the subjective criteria of the investigator, time-consuming inter and intrarater validations are mandatory. VBM is a fully automated whole brain measurement technique that maps the statistical probability of differences in regional tissue volume or density between groups.[5] It provides a nonbiased measure of regions that may be neglected in hypothesis-based studies using ROI. However, the normalization stage within the VBM analysis, which is required to ensure that the same brain regions can be

*Correspondence to: Eduardo Tolosa, Movement Disorders Unit, Neurology Service, Hospital Clínic de Barcelona, Villarroel 173, Barcelona, 08036, Spain. E-mail: etolosa@clinic.ub.es

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in the bilateral prefrontal cortex and hippocampus. The right hippocampus atrophy score was 1.15 in PD versus 0.45 in controls, and the left hippocampus score was 1.05 in PD versus 0.64 in controls.[28] Another study from the same group[29] in a sample of nondemented patients with PD but at a more advanced stage of the disease and with impairment in several cognitive domains also reported atrophy in the hippocampus and the prefrontal cortex when compared with controls. Rated visually, more severe medial temporal atrophy has also been reported in patients with PD versus controls, but less than in subjects with DLB and AD.[30]

Results of VBM studies in patients with PD without dementia are heterogeneous, but most findings suggest the involvement of neocortical areas. In these patients, atrophy has been reported in caudate nucleus,[31] frontal areas and insula,[22] prefrontal cortex and parahippocampus,[32] hippocampus and anterior cingulate,[17] left intraparietal sulcus,[33] superior temporal and frontal gyrus,[34,35] and cerebellum.[36] A possible explanation for this high variability might be the heterogeneous characteristics of patients with PD who are often considered as one uniform group, without differentiating between cognitively intact patients and those with neuropsychological deficits or with hallucinations.

MRI IN PATIENTS WITH PD WITH COGNITIVE IMPAIRMENT AND HALLUCINATIONS

Cognitive impairment is common even in newly diagnosed patients with PD, occurring in 25 to 30% of cases.[1] Patients with cognitive deficits have an increased risk of developing dementia.[37,38] One study found that patients with PD with MCI diagnosis according to the criteria proposed by Petersen et al.[39] had gray matter reductions in temporal and frontal areas when compared with patients without MCI.[35] Another imaging study that identified MCI subtypes before conversion to various kinds of dementia found that the subtype most closely associated with conversion to dementia in PD was characterized by third ventricular enlargement and similar, though less severe, atrophy of the medial temporal lobe when compared with patients with MCI who converted to AD. Corrected hippocampal volumes in patients with MCI converting to AD were 0.084 mm^3 (left) and 0.078 mm^3 (right) when compared with values of 0.109 mm^3 (left) and 0.099 mm^3 (right) in patients with MCI converting to PD.[40] Unfortunately, MCI criteria for PD are not well defined and modified criteria used for AD[39] have mainly been used for the diagnosis. New criteria have recently been proposed for the diagnosis of dementia in PD,[41] and similar efforts should be made to create standardized criteria for MCI diagnosis in this condition.

Patients with visual hallucinations (VHs) present greater neuropsychological impairment in domains such as verbal memory, language, semantic fluency, and visuoperceptive functions than those without.[42–44] Longitudinal studies have pointed out the presence of VH as a predictor of dementia in PD.[45–47] We found that nondemented patients with PD with VH had gray matter loss in occipito-parietal regions when compared with patients without VH[34] and also presented hippocampal atrophy when compared with healthy controls.[48] These results suggest that pathological changes occurring in PD with VH are more marked and severe than those occurring in nonhallucinating patients with PD. As in AD,[7] hippocampal atrophy mainly affected the head of the structure and correlated with verbal memory.[48] Smaller hippocampal volumes and specifically the involvement of the hippocampal head have been identified as predictors of conversion to dementia in AD.[8] The same may apply to PD; in this initially nondemented sample of patients with PD with VH, nearly half met the criteria for dementia after 1 year follow-up.[49]

Manual ROI studies also provide evidence that specific cognitive deficits are related to specific structural changes in PD. Hippocampus volumes have been reported to correlate with memory scores[12–14,28,29] and overall cognitive performance scores,[12,13] but not with frontal functions.[50] In addition, amygdalar volumes have been reported to correlate with scores on these cognitive tests.[13,14] The atrophy of medial temporal structures probably runs in parallel and may underlie the memory dysfunctions associated with PD.

LONGITUDINAL MRI STUDIES IN PATIENTS WITH PD

The progression of regional brain atrophy with VBM in PD has only been investigated in two studies.[51,52] The first study showed limbic and temporo-occipital areas of gray matter reduction after a 25-month follow-up,[51] but the other study found no areas of gray matter loss in patients with PD after a follow-up period of 1.4 years.[52] The differences in these results may be explained by longer disease duration, increased age of patients, more prolonged follow-up, and use of uncorrected *P* values in the study in which atrophy was documented. In agreement with the first study, an earlier report by Hu et al.[53] found that annual brain volume loss was greater in patients with PD than in controls and these changes correlated with cognitive decline. However, other serial MRI studies using meas-

ures of global atrophy for monitoring disease progression reported no differences in atrophy rates between controls and nondemented patients with PD,[54,55] but found significantly increased atrophy in patients with PD with dementia when compared with nondemented patients with PD and controls.[54]

Longitudinal MRI studies should focus on tracking regional cortical changes in PD. Neuropathological studies in PD have proposed a six-stage system[56] of brain pathology to indicate a predictable sequence of ascending lesions that correlates with neurological deficits in the majority of patients with early onset and long duration of the disease, but this classification often fails to correlate with clinical severity and dementia in PD.[57] MRI studies permit assessment of morphological changes in vivo, allowing us to establish where morphological changes begin, and for which kind of patients these changes become more marked and which ones remain stable over time. Furthermore, if the areas that suffer the most atrophy over time are related to cognitive dysfunctions that lead to dementia, we would be able to determine objective markers for the development of dementia and provide evidence of therapeutic effect when modifying treatments related to the onset of dementia are available.

CONCLUSIONS

MRI studies have reported cortical atrophy in PD. ROI imaging studies have shown reduced hippocampal and amygdala volumes even in nondemented patients, and atrophy in these structures has been related to overall cognitive performance and memory deficits in PD. VBM studies have demonstrated that patients with PD with dementia present limbic and widespread neocortical gray matter loss, whereas patients with PD without dementia mainly present atrophy in frontal and temporal areas. Nondemented patients with PD with a higher risk of developing dementia, for example those with cognitive impairment and/or VHs, show greater atrophy than patients who do not present these risk factors. The involvement of the hippocampus in patients with PD with cognitive deficits has been identified in both ROI and VBM studies. Evidence of neocortical involvement is currently available only from whole brain VBM studies because to date no ROI studies have focused on the relationship between specific neocortical regions and cognitive domains. Overall, these findings suggest that cortical atrophy is related to cognitive dysfunction in PD and precedes the development of dementia. Cortical atrophy assessed by MRI may therefore be a useful early marker for the development of dementia in PD. The imaging findings reviewed here suggest that the term "subcortical dementia" is not adequate to describe the dementia occurring in PD. However, it should be borne in mind that the results from MRI structural studies in PD vary widely. The main problems are as follows: (1) the heterogeneity of samples studied; it is not always well defined whether the patients with PD included present cognitive deficits and/or psychiatric symptoms, and in fact the criteria used to diagnose dementia vary from study to study; (2) the inconsistency in applying correction for global cerebral volume in whole brain VBM and ROI studies; and (3) the inconsistency in the protocol used in VBM: optimized versus classical protocols, use of modulated versus unmodulated images, and the size of the Gaussian kernel in the smoothing.

To overcome these difficulties, we make the following recommendations: (1) cognitive and psychiatric symptoms in the sample should be defined using the criteria proposed by the Movement Disorder Task Force for the diagnosis of dementia in PD; (2) both raw and corrected volumes of the selected structures should be reported in ROI studies; (3) the standard guidelines[58] for reporting VBM studies should be followed, and a modulation of gray matter volume should be used based on the warping applied during normalization so as to minimize the danger of distortion. We also favor the use of the recently developed Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) algorithm, a nonlinear warping technique to minimize structural variation between subjects.[59]

Major challenges that remain in the field of MRI structural studies in PD are the need to establish patterns of atrophy that can predict different disease outcomes and to combine their use with other approaches, such as PET studies, using different tracers (FDG, MPPF, and FDDNP) to determine the etiopathogenesis of PD.

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REFERENCES

1. Muslimovic D, Post B, Speelman JD, Schmand B. Cognitive profile of patients with newly diagnosed Parkinson disease. Neurology 2005;65:1239–1245.
2. Hely MA, Reid WGJ, Adena MA, Halliday GM, Morris JGL. The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years. Mov Disord 2008;6:837–844.
3. Owen AM. Cognitive dysfunction in Parkinson's disease: the role of frontostriatal circuitry. Neuroscientist 2004;10:525–537.
4. Calabresi C, Picconi B, Parnetti L, Di Filippo M. A convergent model for cognitive dysfunctions in Parkinson's disease: the critical dopamine-acetylcholine synaptic balance. Lancet Neurol 2006;5:974–983.
5. Ashburner J, Friston KJ. Voxel-based morphometry: the methods. Neuroimage 2000;11:805–821.
6. Mechelli A, Price CJ, Friston KJ, Ashburner J. Voxel-based morphometry of the human brain: methods and applications. Curr Med Imaging Rev 2005;1:105–113.
7. Scher AI, Xu Y, Korf ESC, et al. Hippocampal shape analysis in Alzheimer's disease: a population-based study. Neuroimage 2007;36:8–18.
8. Apostolova L, Dutton RA, Dinos ID, et al. Conversion of mild cognitive impairment to Alzheimer's disease predicted by hippocampal atrophy maps. Arch Neurol 2006;63:693–699.
9. Hashimoto M, Kitagaki H, Imamura T, et al. Medial temporal and whole-brain atrophy in dementia with Lewy bodies: a volumetric MRI study. Neurology 1998;51:357–362.
10. Barber R, Ballard C, McKeith IG, Gholkar A, O'Brien JT. MRI volumetric study of dementia with Lewy bodies: a comparison with AD and vascular dementia. Neurology 2000;54:1304–1309.
11. Laakso MP, Partanen K, Riekkinen P. Hippocampal volumes in Alzheimer's disease, Parkinson's disease with and without dementia, and in vascular dementia: an MRI study. Neurology 1995;46:678–681.
12. Camicioli R, Moore MM, Kinney A, Corbridge E, Glassberg K, Kaye JA. Parkinson's disease is associated with hippocampal atrophy. Mov Disord 2003;18:784–790.
13. Junque C, Ramirez-Ruiz B, Tolosa E, et al. Amygdalar and hippocampal MRI volumetric reductions in Parkinson's disease with dementia. Mov Disord 2005;20:540–544.
14. Bouchard TP, Malykhin N, Martin WR, et al. Age and dementia-associated atrophy predominates in the hippocampal head and amygdala in Parkinson's disease. Neurobiol Aging 2008;29:1027–1039.
15. Kenny ER, Burton EJ, O'Brien JT. A volumetric magnetic resonance imaging study of entorhinal cortex volume in dementia with Lewy bodies. A comparison with Alzheimer's disease and Parkinson's disease with and without dementia. Dement Geriatr Cogn Disord 2008;26:218–225.
16. Scheltens P, Fox N, Barkhof F, De Carli C. Structural magnetic resonance imaging in the practical assessment of dementia: beyond exclusion. Lancet Neurol 2002;1:13–21.
17. Summerfield C, Junque C, Tolosa E, et al. Structural brain changes in Parkinson disease with dementia: a voxel-based morphometry study. Arch Neurol 2005;62:281–285.
18. Churchyard A, Lees AJ. The relationship between dementia and direct involvement of the hippocampus and amygdala in Parkinson's disease. Neurology 1997;49:1570–1576.
19. Apaydin H, Ahlskog E, Parisi JE, Boeve BF, Dickson DW. Parkinson disease neuropathology. Later-developing dementia and loss of the levodopa response. Arch Neurol 2002;59:102–112.
20. Mattila PM, Rinne JO, Helenius H, Dickson DW, Röyttä M. Alpha-synuclein-immureactive cortical Lewy bodies are associated with cognitive impairment in Parkinson's disease. Acta Neuropathol 2000;100:285–290.
21. Kövari E, Gold G, Herrmann FR. Lewy body densities in the entorhinal and anterior cingulated cortex predict cognitive deficits in Parkinson's disease. Acta Neuropathol 2003;106:83–88.
22. Burton EJ, McKeith IG, Burn DJ, Williams ED, O'Brien JT. Cerebral atrophy in Parkinson's disease with and without dementia: a comparison with Alzheimer's disease, dementia with Lewy bodies and controls. Brain 2004;127:791–800.
23. Beyer MK, Larsen JP, Aarsland D. Gray matter atrophy in Parkinson disease with dementia and dementia with Lewy bodies. Neurology 2007;69:747–754.
24. Beyer MK, Aarsland D. Grey matter atrophy in early versus late dementia in Parkinson's disease. Parkinsonism Relat Disord 2008;14:620–625.
25. Ballard C, Ziabreva I, Perry R, et al. Differences in neuropathologic characteristics across the Lewy body dementia spectrum. Neurology 2006;67:1931–1934.
26. Jellinger KA, Seppi K, Wenning GK, Poewe W. Impact of coexistent Alzheimer pathology on the natural history of Parkinson's disease. J Neural Transm 2002;109:329–339.
27. Scheltens P, Leys D, Barkhof F, et al. Atrophy of medial temporal lobes on MRI in probable Alzheimer's disease and normal aging: diagnostic value and neuropsychological correlates. J Neurol Neurosurg Psychiatry 1992;55:967–972.
28. Brück A, Kurki T, Kaasinen V, Vahlberg T, Rinne JO. Hippocampal and prefrontal atrophy in patients with early non-demented Parkinson's disease is related to cognitive impairment. J Neurol Neurosurg Psychiatry 2004;75:1467–1469.
29. Jokinen P, Brück A, Aalto S, Forsback S, Parkkola R, Rinne JO. Impaired cognitive performance in Parkinson's disease is related to caudate dopaminergic hypofunction and hippocampal atrophy. Parkinsonism Relat Disord 2009;15:88–93.
30. Tam CWC, Burton EJ, McKeith IG, Burn DJ, O'Brien JT. Temporal lobe atrophy on MRI in Parkinson disease with dementia. A comparison with Alzheimer disease and dementia with Lewy bodies. Neurology 2005;64:861–865.
31. Brenneis C, Seppi K, Schocke MF, et al. Voxel-based morphometry detects cortical atrophy in the Parkinson variant of multiple system atrophy. Mov Disord 2003;18:1132–1138.
32. Nagano-Saito A, Washimi Y, Arahata Y, Kachi T, Lerch JP, Evans AC. Cerebral atrophy and its relation to cognitive impairment in Parkinson disease. Neurology 2005;64:224–229.
33. Cordato NJ, Duggins AJ, Halliday GM, Morris JGL, Pantelis C. Clinical deficits correlate with regional cerebral atrophy in progressive supranuclear palsy. Brain 2005;128:1259–1266.
34. Ramirez-Ruiz B, Marti MJ, Tolosa E, et al. Cerebral atrophy in Parkinson's disease patients with visual hallucinations. Eur J Neurol 2007;14:750–756.
35. Beyer MK, Janvin CC, Larsen JP, Aarsland D. A magnetic resonance imaging study of patients with Parkinson's disease with mild cognitive impairment and dementia using voxel-based morphometry. J Neurol Neurosurg Psychiatry 2007;78:254–259.
36. Camicioli R, Gee M, Fisher NJ, et al. Voxel-based morphometry reveals extra-nigral atrophy patterns associated with dopamine refractory cognitive and motor impairment in parkinsonism. Parkinsonism Relat Disord 2009;15:187–195.
37. Janvin C, Aarsland D, Larsen JP, Hugdahl K. Neuropsychological profile of patients with Parkinson's disease without dementia. Dement Geriatr Cogn Disord 2003;15:126–131.
38. Williams-Gray CH, Foltynie T, Brayne CEG, Robbins TW, Barker RA. Evolution of cognitive dysfunction in an incident Parkinson's disease cohort. Brain 2007;130:1787–1798.
39. Petersen RC, Doody R, Kurz A, et al. Current concepts in mild cognitive impairment. Arch Neurol 2001;58:1985–1992.
40. Meyer JS, Huang J, Chowdhury MH. MRI confirms mild cognitive impairments prodromal for Alzheimer's, vascular and Parkinson-Lewy bodies dementias. J Neurol Sci 2007;257:97–104.
41. Dubois B, Burn D, Goetz C, et al. Diagnostic procedures for Parkinson's disease dementia: recommendations from the movement disorder society task force. Mov Disord 2007;22:2314–2324.
42. Grossi D, Trojano L, Pellecchia MT, Amboni M, Fragassi NA, Barone P. Frontal dysfunction contributes to the genesis of hallu-

cinations in non-demented Parkinsonian patients. Int J Geriatr Psychiatry 2005;20:668–673.
43. Ramirez-Ruiz B, Junque C, Marti MJ, Vallderiola F, Tolosa E. Neuropsychological deficits in Parkinson's disease patients with visual hallucinations. Mov Disord 2006;21:1483–1487.
44. Sinforiani E, Zangaglia R, Manni R, et al. REM sleep behavior disorder, hallucinations, and cognitive impairment in Parkinson's disease. Mov Disord 2006;21:462–466.
45. Aarsland D, Andersen K, Larsen JP, Lolk A, Kragh-Sørensen P. Prevalence and characteristics of dementia in Parkinson disease: an 8-year prospective study. Arch Neurol 2003;60:387–392.
46. Galvin JE, Pollack J, Morris JC. Clinical phenotype of Parkinson disease dementia. Neurology 2006;67:1605–1611.
47. Santangelo G, Trojano L, Vitale C, et al. A neuropsychological longitudinal study in Parkinson's patients with and without visual hallucinations. Mov Disord 2007;16:2418–2425.
48. Ibarretxe-Bilbao N, Ramirez-Ruiz B, Tolosa E, et al. Hippocampal head atrophy predominance in Parkinson's disease with hallucinations and with dementia. J Neurol 2008;255:1324–1331.
49. Ramirez-Ruiz B, Junque C, Marti MJ, Valldeoriola F, Tolosa E. Cognitive changes in Parkinson's disease patients with visual hallucinations. Dement Geriatr Cogn Disord 2007;23:281–288.
50. Riekkinen P, Kejonen K, Laakso MP, Soininen H, Partanen K, Riekkinen M. Hippocampal atrophy is related to impaired memory, but not frontal functions in non-demented Parkinson's disease patients. Neuroreport 1998;9:1507–1511.
51. Ramirez-Ruiz B, Marti MJ, Tolosa E, et al. Longitudinal evaluation of cerebral morphological changes in Parkinson's disease with and without dementia. J Neurol 2005;252:1345–1352.
52. Brenneis C, Egger K, Scherfler C, et al. Progression of brain atrophy in multiple system atrophy: a longitudinal VBM study. J Neurol 2007;254:191–196.
53. Hu MTM, White SJ, Chaudhuri KR, Morris RG, Bydder GM, Brooks DJ. Correlating rates of cerebral atrophy in Parkinson's disease with measures of cognitive decline. J Neural Transm 2001;108:571–580.
54. Burton EJ, McKeith IG, Burn DJ, O'Brien JT. Brain atrophy rates in Parkinson's disease with and without dementia using serial magnetic resonance imaging. Mov Disord 2005;20:1571–1576.
55. Paviour DC, Price SL, Jahanshashi M, Lees AJ, Fox NC. Longitudinal MRI in progressive supranuclear palsy and multiple system atrophy: rates and regions of atrophy. Brain 2006;129:1040–1049.
56. Braak H, Del tredici K, Rüb U, de Vos RAI, Jansen Steur EHN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003;24:197–211.
57. Jellinger KA. A critical reappraisal of current staging of Lewy-related pathology in human brain. Acta Neuropathol 2008;116:1–16.
58. Ridgway GR, Henley SMD, Rohrer JD, Scahill RI, Warren JD, Fox NC. Ten simple rules for reporting voxel-based morphometry studies. Neuroimage 2008;40:1429–1435.
59. Ashburner J. A fast diffeomorphic image registration algorithm. Neuroimage 2007;38:95–113.

9. References

Aarsland D, Kurz MW. The epidemiology of dementia associated with parkinson disease. J Neurol Sci 2009.

Aarsland D, Zaccai J, Brayne C. A systematic review of prevalence studies of dementia in parkinson's disease. Mov Disord 2005a; 20: 1255-63.

Aarsland D, Perry R, Brown A, Larsen JP, Ballard C. Neuropathology of dementia in parkinson's disease: A prospective, community-based study. Ann Neurol 2005b; 58: 773-6.

Aarsland D, Andersen K, Larsen JP, Lolk A, Kragh-Sorensen P. Prevalence and characteristics of dementia in parkinson disease: An 8-year prospective study. Arch Neurol 2003; 60: 387-92.

Aarsland D, Larsen JP, Karlsen K, Lim NG, Tandberg E. Mental symptoms in parkinson's disease are important contributors to caregiver distress. Int J Geriatr Psychiatry 1999; 14: 866-74.

Aarsland D, Bronnick K, Larsen JP, Tysnes OB, Alves G, Norwegian ParkWest Study Group. Cognitive impairment in incident, untreated parkinson disease: The norwegian ParkWest study. Neurology 2009; 72: 1121-6.

Aarsland D, Andersen K, Larsen JP, Lolk A, Nielsen H, Kragh-Sorensen P. Risk of dementia in parkinson's disease: A community-based, prospective study. Neurology 2001; 56: 730-6.

Aarsland D, Andersen K, Larsen JP, Perry R, Wentzel-Larsen T, Lolk A, et al. The rate of cognitive decline in parkinson disease. Arch Neurol 2004; 61: 1906-11.

Adolphs R. Recognizing emotion from facial expressions: Psychological and neurological mechanisms. Behav Cogn Neurosci Rev 2002a; 1: 21-62.

Adolphs R. Neural systems for recognizing emotion. Curr Opin Neurobiol 2002b; 12: 169-77.

Adolphs R, Schul R, Tranel D. Intact recognition of facial emotion in parkinson's disease. Neuropsychology 1998; 12: 253-8.

Alexander GE, DeLong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 1986; 9: 357-81.

Alves G, Larsen JP, Emre M, Wentzel-Larsen T, Aarsland D. Changes in motor subtype and risk for incident dementia in parkinson's disease. Mov Disord 2006; 21: 1123-30.

Apaydin H, Ahlskog JE, Parisi JE, Boeve BF, Dickson DW. Parkinson disease neuropathology: Later-developing dementia and loss of the levodopa response. Arch Neurol 2002; 59: 102-12.

Apostolova LG, Dinov ID, Dutton RA, Hayashi KM, Toga AW, Cummings JL, et al. 3D comparison of hippocampal atrophy in amnestic mild cognitive impairment and alzheimer's disease. Brain 2006a; 129: 2867-73.

Apostolova LG, Dutton RA, Dinov ID, Hayashi KM, Toga AW, Cummings JL, et al. Conversion of mild cognitive impairment to alzheimer disease predicted by hippocampal atrophy maps. Arch Neurol 2006b; 63: 693-9.

Ariatti A, Benuzzi F, Nichelli P. Recognition of emotions from visual and prosodic cues in parkinson's disease. Neurol Sci 2008; 29: 219-27.

Ashburner J, Friston KJ. Voxel-based morphometry--the methods. Neuroimage 2000; 11: 805-21.

Assaf Y, Pasternak O. Diffusion tensor imaging (DTI)-based white matter mapping in brain research: A review. J Mol Neurosci 2008; 34: 51-61.

Assogna F, Pontieri FE, Caltagirone C, Spalletta G. The recognition of facial emotion expressions in parkinson's disease. Eur Neuropsychopharmacol 2008; 18: 835-48.

Athey RJ, Porter RW, Walker RW. Cognitive assessment of a representative community population with parkinson's disease (PD) using the cambridge cognitive assessment-revised (CAMCOG-R). Age Ageing 2005; 34: 268-73.

Ballard C, Ziabreva I, Perry R, Larsen JP, O'Brien J, McKeith I, et al. Differences in neuropathologic characteristics across the lewy body dementia spectrum. Neurology 2006; 67: 1931-4.

Barber R, Ballard C, McKeith IG, Gholkar A, O'Brien JT. MRI volumetric study of dementia with lewy bodies: A comparison with AD and vascular dementia. Neurology 2000; 54: 1304-9.

Barnes J, Boubert L. Executive functions are impaired in patients with parkinson's disease with visual hallucinations. J Neurol Neurosurg Psychiatry 2008; 79: 190-2.

Barnes J, David AS. Visual hallucinations in parkinson's disease: A review and phenomenological survey. J Neurol Neurosurg Psychiatry 2001; 70: 727-33.

Barnes J, Boubert L, Harris J, Lee A, David AS. Reality monitoring and visual hallucinations in parkinson's disease. Neuropsychologia 2003; 41: 565-74.

Bechara A. The role of emotion in decision-making: Evidence from neurological patients with orbitofrontal damage. Brain Cogn 2004; 55: 30-40.

Bechara A, Damasio H, Damasio AR, Lee GP. Different contributions of the human amygdala and ventromedial prefrontal cortex to decision-making. J Neurosci 1999; 19: 5473-81.

Bechara A, Damasio H, Tranel D, Damasio AR. Deciding advantageously before knowing the advantageous strategy. Science 1997; 275: 1293-5.

Beckmann CF, Smith SM. Tensorial extensions of independent component analysis for multisubject FMRI analysis. Neuroimage 2005; 25: 294-311.

Berendse HW, Booij J, Francot CM, Bergmans PL, Hijman R, Stoof JC, et al. Subclinical dopaminergic dysfunction in asymptomatic parkinson's disease patients' relatives with a decreased sense of smell. Ann Neurol 2001; 50: 34-41.

Beyer MK, Aarsland D. Grey matter atrophy in early versus late dementia in parkinson's disease. Parkinsonism Relat Disord 2008; 14: 620-5.

Beyer MK, Larsen JP, Aarsland D. Gray matter atrophy in parkinson disease with dementia and dementia with lewy bodies. Neurology 2007a; 69: 747-54.

Beyer MK, Janvin CC, Larsen JP, Aarsland D. A magnetic resonance imaging study of patients with parkinson's disease with mild cognitive impairment and dementia using voxel-based morphometry. J Neurol Neurosurg Psychiatry 2007b; 78: 254-9.

Beyer MK, Aarsland D, Greve OJ, Larsen JP. Visual rating of white matter hyperintensities in parkinson's disease. Mov Disord 2006; 21: 223-9.

Blair RJ, Morris JS, Frith CD, Perrett DI, Dolan RJ. Dissociable neural responses to facial expressions of sadness and anger. Brain 1999; 122 (Pt 5): 883-93.

Boecker H, Ceballos-Baumann AO, Volk D, Conrad B, Forstl H, Haussermann P. Metabolic alterations in patients with parkinson disease and visual hallucinations. Arch Neurol 2007; 64: 984-8.

Boska MD, Hasan KM, Kibuule D, Banerjee R, McIntyre E, Nelson JA, et al. Quantitative diffusion tensor imaging detects dopaminergic neuronal degeneration in a murine model of parkinson's disease. Neurobiol Dis 2007; 26: 590-6.

Bouchard TP, Malykhin N, Martin WR, Hanstock CC, Emery DJ, Fisher NJ, et al. Age and dementia-associated atrophy predominates in the hippocampal head and amygdala in parkinson's disease. Neurobiol Aging 2008; 29: 1027-39.

Braak H, Rub U, Jansen Steur EN, Del Tredici K, de Vos RA. Cognitive status correlates with neuropathologic stage in parkinson disease. Neurology 2005; 64: 1404-10.

Braak H, Ghebremedhin E, Rub U, Bratzke H, Del Tredici K. Stages in the development of parkinson's disease-related pathology. Cell Tissue Res 2004; 318: 121-34.

Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic parkinson's disease. Neurobiol Aging 2003; 24: 197-211.

Brenneis C, Egger K, Scherfler C, Seppi K, Schocke M, Poewe W, et al. Progression of brain atrophy in multiple system atrophy. A longitudinal VBM study. J Neurol 2007; 254: 191-6.

Brenneis C, Seppi K, Schocke MF, Muller J, Luginger E, Bosch S, et al. Voxel-based morphometry detects cortical atrophy in the parkinson variant of multiple system atrophy. Mov Disord 2003; 18: 1132-8.

Bruck A, Kurki T, Kaasinen V, Vahlberg T, Rinne JO. Hippocampal and prefrontal atrophy in patients with early non-demented parkinson's disease is related to cognitive impairment. J Neurol Neurosurg Psychiatry 2004; 75: 1467-9.

Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: Anatomy, function, and relevance to disease. Ann N Y Acad Sci 2008; 1124: 1-38.

Burton EJ, McKeith IG, Burn DJ, O'Brien JT. Brain atrophy rates in parkinson's disease with and without dementia using serial magnetic resonance imaging. Mov Disord 2005; 20: 1571-6.

Burton EJ, McKeith IG, Burn DJ, Williams ED, O'Brien JT. Cerebral atrophy in parkinson's disease with and without dementia: A comparison with alzheimer's disease, dementia with lewy bodies and controls. Brain 2004; 127: 791-800.

Buter TC, van den Hout A, Matthews FE, Larsen JP, Brayne C, Aarsland D. Dementia and survival in parkinson disease: A 12-year population study. Neurology 2008; 70: 1017-22.

Cabeza R, Nyberg L. Imaging cognition II: An empirical review of 275 PET and fMRI studies. J Cogn Neurosci 2000; 12: 1-47.

Camicioli R, Moore MM, Kinney A, Corbridge E, Glassberg K, Kaye JA. Parkinson's disease is associated with hippocampal atrophy. Mov Disord 2003; 18: 784-90.

Camicioli R, Gee M, Bouchard TP, Fisher NJ, Hanstock CC, Emery DJ, et al. Voxel-based morphometry reveals extra-nigral atrophy patterns associated with dopamine refractory cognitive and motor impairment in parkinsonism. Parkinsonism Relat Disord 2009; 15: 187-95.

Caviness JN, Driver-Dunckley E, Connor DJ, Sabbagh MN, Hentz JG, Noble B, et al. Defining mild cognitive impairment in parkinson's disease. Mov Disord 2007; 22: 1272-7.

Celone KA, Calhoun VD, Dickerson BC, Atri A, Chua EF, Miller SL, et al. Alterations in memory networks in mild cognitive impairment and alzheimer's disease: An independent component analysis. J Neurosci 2006; 26: 10222-31.

Chan LL, Rumpel H, Yap K, Lee E, Loo HV, Ho GL, et al. Case control study of diffusion tensor imaging in parkinson's disease. J Neurol Neurosurg Psychiatry 2007; 78: 1383-6.

Churchyard A, Lees AJ. The relationship between dementia and direct involvement of the hippocampus and amygdala in parkinson's disease. Neurology 1997; 49: 1570-6.

Clark US, Neargarder S, Cronin-Golomb A. Specific impairments in the recognition of emotional facial expressions in parkinson's disease. Neuropsychologia 2008; 46: 2300-9.

Cooper JA, Sagar HJ, Jordan N, Harvey NS, Sullivan EV. Cognitive impairment in early, untreated parkinson's disease and its relationship to motor disability. Brain 1991; 114 (Pt 5): 2095-122.

Cordato NJ, Halliday GM, Caine D, Morris JG. Comparison of motor, cognitive, and behavioral features in progressive supranuclear palsy and parkinson's disease. Mov Disord 2006; 21: 632-8.

Cummings JL. Behavioral complications of drug treatment of parkinson's disease. J Am Geriatr Soc 1991; 39: 708-16.

Dalaker TO, Larsen JP, Dwyer MG, Aarsland D, Beyer MK, Alves G, et al. White matter hyperintensities do not impact cognitive function in patients with newly diagnosed parkinson's disease. Neuroimage 2009; 47: 2083-9.

de Lau LM, Schipper CM, Hofman A, Koudstaal PJ, Breteler MM. Prognosis of parkinson disease: Risk of dementia and mortality: The rotterdam study. Arch Neurol 2005; 62: 1265-9.

Del Tredici K, Rub U, De Vos RA, Bohl JR, Braak H. Where does parkinson disease pathology begin in the brain? J Neuropathol Exp Neurol 2002; 61: 413-26.

Denburg NL, Cole CA, Hernandez M, Yamada TH, Tranel D, Bechara A, et al. The orbitofrontal cortex, real-world decision making, and normal aging. Ann N Y Acad Sci 2007; 1121: 480-98.

D'Esposito M, Deouell LY, Gazzaley A. Alterations in the BOLD fMRI signal with ageing and disease: A challenge for neuroimaging. Nat Rev Neurosci 2003; 4: 863-72.

Diederich NJ, Goetz CG, Stebbins GT. Repeated visual hallucinations in parkinson's disease as disturbed external/internal perceptions: Focused review and a new integrative model. Mov Disord 2005; 20: 130-40.

Diederich NJ, Fenelon G, Stebbins G, Goetz CG. Hallucinations in parkinson disease. Nat Rev Neurol 2009; 5: 331-42.

Dolan RJ, Fletcher P, Morris J, Kapur N, Deakin JF, Frith CD. Neural activation during covert processing of positive emotional facial expressions. Neuroimage 1996; 4: 194-200.

Doty RL. The olfactory system and its disorders. Semin Neurol 2009; 29: 74-81.

Doty RL. The olfactory vector hypothesis of neurodegenerative disease: Is it viable? Ann Neurol 2008; 63: 7-15.

Doty RL, Deems DA, Stellar S. Olfactory dysfunction in parkinsonism: A general deficit unrelated to neurologic signs, disease stage, or disease duration. Neurology 1988; 38: 1237-44.

Doty RL, Stern MB, Pfeiffer C, Gollomp SM, Hurtig HI. Bilateral olfactory dysfunction in early stage treated and untreated idiopathic parkinson's disease. J Neurol Neurosurg Psychiatry 1992; 55: 138-42.

Dubois B, Burn D, Goetz C, Aarsland D, Brown RG, Broe GA, et al. Diagnostic procedures for parkinson's disease dementia: Recommendations from the movement disorder society task force. Mov Disord 2007; 22: 2314-24.

Elgh E, Domellof M, Linder J, Edstrom M, Stenlund H, Forsgren L. Cognitive function in early parkinson's disease: A population-based study. Eur J Neurol 2009.

Emre M, Aarsland D, Brown R, Burn DJ, Duyckaerts C, Mizuno Y, et al. Clinical diagnostic criteria for dementia associated with parkinson's disease. Mov Disord 2007; 22: 1689,707; quiz 1837.

Factor SA, Feustel PJ, Friedman JH, Comella CL, Goetz CG, Kurlan R, et al. Longitudinal outcome of parkinson's disease patients with psychosis. Neurology 2003; 60: 1756-61.

Feldmann A, Illes Z, Kosztolanyi P, Illes E, Mike A, Kover F, et al. Morphometric changes of gray matter in parkinson's disease with depression: A voxel-based morphometry study. Mov Disord 2008; 23: 42-6.

Fellows LK, Farah MJ. Different underlying impairments in decision-making following ventromedial and dorsolateral frontal lobe damage in humans. Cereb Cortex 2005; 15: 58-63.

Fenelon G, Mahieux F, Huon R, Ziegler M. Hallucinations in parkinson's disease: Prevalence, phenomenology and risk factors. Brain 2000; 123 (Pt 4): 733-45.

Ferrer I. Early involvement of the cerebral cortex in parkinson's disease: Convergence of multiple metabolic defects. Prog Neurobiol 2009; 88: 89-103.

Foltynie T, Brayne CE, Robbins TW, Barker RA. The cognitive ability of an incident cohort of parkinson's patients in the UK. the CamPaIGN study. Brain 2004; 127: 550-60.

Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc Natl Acad Sci U S A 2005; 102: 9673-8.

Frisoni GB, Testa C, Zorzan A, Sabattoli F, Beltramello A, Soininen H, et al. Detection of grey matter loss in mild alzheimer's disease with voxel based morphometry. J Neurol Neurosurg Psychiatry 2002; 73: 657-64.

Gagnon JF, Vendette M, Postuma RB, Desjardins C, Massicotte-Marquez J, Panisset M, et al. Mild cognitive impairment in rapid eye movement sleep behavior disorder and parkinson's disease. Ann Neurol 2009; 66: 39-47.

Galvin JE, Pollack J, Morris JC. Clinical phenotype of parkinson disease dementia. Neurology 2006; 67: 1605-11.

Galvin JE, Uryu K, Lee VM, Trojanowski JQ. Axon pathology in parkinson's disease and lewy body dementia hippocampus contains alpha-, beta-, and gamma-synuclein. Proc Natl Acad Sci U S A 1999; 96: 13450-5.

Gattellaro G, Minati L, Grisoli M, Mariani C, Carella F, Osio M, et al. White matter involvement in idiopathic parkinson disease: A diffusion tensor imaging study. AJNR Am J Neuroradiol 2009; 30: 1222-6.

Goetz CG, Leurgans S, Pappert EJ, Raman R, Stemer AB. Prospective longitudinal assessment of hallucinations in parkinson's disease. Neurology 2001; 57: 2078-82.

Greicius MD, Supekar K, Menon V, Dougherty RF. Resting-state functional connectivity reflects structural connectivity in the default mode network. Cereb Cortex 2009; 19: 72-8.

Greicius MD, Krasnow B, Reiss AL, Menon V. Functional connectivity in the resting brain: A network analysis of the default mode hypothesis. Proc Natl Acad Sci U S A 2003; 100: 253-8.

Grossi D, Trojano L, Pellecchia MT, Amboni M, Fragassi NA, Barone P. Frontal dysfunction contributes to the genesis of hallucinations in non-demented parkinsonian patients. Int J Geriatr Psychiatry 2005; 20: 668-73.

Hackert VH, den Heijer T, Oudkerk M, Koudstaal PJ, Hofman A, Breteler MM. Hippocampal head size associated with verbal memory performance in nondemented elderly. Neuroimage 2002; 17: 1365-72.

Halliday G, Hely M, Reid W, Morris J. The progression of pathology in longitudinally followed patients with parkinson's disease. Acta Neuropathol 2008; 115: 409-15.

Harding AJ, Broe GA, Halliday GM. Visual hallucinations in lewy body disease relate to lewy bodies in the temporal lobe. Brain 2002; 125: 391-403.

Hashimoto M, Kitagaki H, Imamura T, Hirono N, Shimomura T, Kazui H, et al. Medial temporal and whole-brain atrophy in dementia with lewy bodies: A volumetric MRI study. Neurology 1998; 51: 357-62.

Haslinger B, Erhard P, Kampfe N, Boecker H, Rummeny E, Schwaiger M, et al. Event-related functional magnetic resonance imaging in parkinson's disease before and after levodopa. Brain 2001; 124: 558-70.

Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: A dual-hit hypothesis. Neuropathol Appl Neurobiol 2007; 33: 599-614.

Hawkes CH, Shephard BC, Daniel SE. Olfactory dysfunction in parkinson's disease. J Neurol Neurosurg Psychiatry 1997; 62: 436-46.

Heberlein AS, Padon AA, Gillihan SJ, Farah MJ, Fellows LK. Ventromedial frontal lobe plays a critical role in facial emotion recognition. J Cogn Neurosci 2008; 20: 721-33.

Hely MA, Morris JG, Reid WG, Trafficante R. Sydney multicenter study of parkinson's disease: Non-L-dopa-responsive problems dominate at 15 years. Mov Disord 2005; 20: 190-9.

Hely MA, Reid WG, Adena MA, Halliday GM, Morris JG. The sydney multicenter study of parkinson's disease: The inevitability of dementia at 20 years. Mov Disord 2008; 23: 837-44.

Hilker R, Schweitzer K, Coburger S, Ghaemi M, Weisenbach S, Jacobs AH, et al. Nonlinear progression of parkinson disease as determined by serial positron emission tomographic imaging of striatal fluorodopa F 18 activity. Arch Neurol 2005a; 62: 378-82.

Hilker R, Thomas AV, Klein JC, Weisenbach S, Kalbe E, Burghaus L, et al. Dementia in parkinson disease: Functional imaging of cholinergic and dopaminergic pathways. Neurology 2005b; 65: 1716-22.

Hobson P, Meara J. Risk and incidence of dementia in a cohort of older subjects with parkinson's disease in the united kingdom. Mov Disord 2004; 19: 1043-9.

Holroyd S, Wooten GF. Preliminary FMRI evidence of visual system dysfunction in parkinson's disease patients with visual hallucinations. J Neuropsychiatry Clin Neurosci 2006; 18: 402-4.

Holroyd S, Currie L, Wooten GF. Prospective study of hallucinations and delusions in parkinson's disease. J Neurol Neurosurg Psychiatry 2001; 70: 734-8.

Hornak J, Bramham J, Rolls ET, Morris RG, O'Doherty J, Bullock PR, et al. Changes in emotion after circumscribed surgical lesions of the orbitofrontal and cingulate cortices. Brain 2003; 126: 1691-712.

Hosokai Y, Nishio Y, Hirayama K, Takeda A, Ishioka T, Sawada Y, et al. Distinct patterns of regional cerebral glucose metabolism in parkinson's disease with and without mild cognitive impairment. Mov Disord 2009; 24: 854-62.

Hu MT, White SJ, Chaudhuri KR, Morris RG, Bydder GM, Brooks DJ. Correlating rates of cerebral atrophy in parkinson's disease with measures of cognitive decline. J Neural Transm 2001; 108: 571-80.

Huang C, Mattis P, Perrine K, Brown N, Dhawan V, Eidelberg D. Metabolic abnormalities associated with mild cognitive impairment in parkinson disease. Neurology 2008; 70: 1470-7.

Huang C, Tang C, Feigin A, Lesser M, Ma Y, Pourfar M, et al. Changes in network activity with the progression of parkinson's disease. Brain 2007; 130: 1834-46.

Hughes TA, Ross HF, Musa S, Bhattacherjee S, Nathan RN, Mindham RH, et al. A 10-year study of the incidence of and factors predicting dementia in parkinson's disease. Neurology 2000; 54: 1596-602.

Hummel T, Damm M, Vent J, Schmidt M, Theissen P, Larsson M, et al. Depth of olfactory sulcus and olfactory function. Brain Res 2003; 975: 85-9.

Imamura K, Wada-Isoe K, Kitayama M, Nakashima K. Executive dysfunction in non-demented parkinson's disease patients with hallucinations. Acta Neurol Scand 2008; 117: 255-9.

Ito K, Morrish PK, Rakshi JS, Uema T, Ashburner J, Bailey DL, et al. Statistical parametric mapping with 18F-dopa PET shows bilaterally reduced striatal and nigral dopaminergic function in early parkinson's disease. J Neurol Neurosurg Psychiatry 1999; 66: 754-8.

Jacobs DM, Marder K, Cote LJ, Sano M, Stern Y, Mayeux R. Neuropsychological characteristics of preclinical dementia in parkinson's disease. Neurology 1995; 45: 1691-6.

Janvin CC, Aarsland D, Larsen JP. Cognitive predictors of dementia in parkinson's disease: A community-based, 4-year longitudinal study. J Geriatr Psychiatry Neurol 2005; 18: 149-54.

Janvin CC, Larsen JP, Aarsland D, Hugdahl K. Subtypes of mild cognitive impairment in parkinson's disease: Progression to dementia. Mov Disord 2006; 21: 1343-9.

Jellinger KA. A critical reappraisal of current staging of lewy-related pathology in human brain. Acta Neuropathol 2008; 116: 1-16.

Jellinger KA. Neuropathology of dementia in parkinson's disease. Ann Neurol 2006; 59: 727.

Jellinger KA. Lewy body-related alpha-synucleinopathy in the aged human brain. J Neural Transm 2004; 111: 1219-35.

Jellinger KA, Seppi K, Wenning GK, Poewe W. Impact of coexistent alzheimer pathology on the natural history of parkinson's disease. J Neural Transm 2002; 109: 329-39.

Jokinen P, Bruck A, Aalto S, Forsback S, Parkkola R, Rinne JO. Impaired cognitive performance in parkinson's disease is related to caudate dopaminergic hypofunction and hippocampal atrophy. Parkinsonism Relat Disord 2009; 15: 88-93.

Junque C, Ramirez-Ruiz B, Tolosa E, Summerfield C, Marti MJ, Pastor P, et al. Amygdalar and hippocampal MRI volumetric reductions in parkinson's disease with dementia. Mov Disord 2005; 20: 540-4.

Kan Y, Kawamura M, Hasegawa Y, Mochizuki S, Nakamura K. Recognition of emotion from facial, prosodic and written verbal stimuli in parkinson's disease. Cortex 2002; 38: 623-30.

Karagulle Kendi AT, Lehericy S, Luciana M, Ugurbil K, Tuite P. Altered diffusion in the frontal lobe in parkinson disease. AJNR Am J Neuroradiol 2008; 29: 501-5.

Kenny ER, Burton EJ, O'Brien JT. A volumetric magnetic resonance imaging study of entorhinal cortex volume in dementia with lewy bodies. A comparison with alzheimer's disease and parkinson's disease with and without dementia. Dement Geriatr Cogn Disord 2008; 26: 218-25.

Kim JY, Lee WY, Chung EJ, Dhong HJ. Analysis of olfactory function and the depth of olfactory sulcus in patients with parkinson's disease. Mov Disord 2007; 22: 1563-6.

Kobayakawa M, Koyama S, Mimura M, Kawamura M. Decision making in parkinson's disease: Analysis of behavioral and physiological patterns in the iowa gambling task. Mov Disord 2008; 23: 547-52.

Kollensperger M, Seppi K, Liener C, Boesch S, Heute D, Mair KJ, et al. Diffusion weighted imaging best discriminates PD from MSA-P: A comparison with tilt table testing and heart MIBG scintigraphy. Mov Disord 2007; 22: 1771-6.

Kovari E, Gold G, Herrmann FR, Canuto A, Hof PR, Bouras C, et al. Lewy body densities in the entorhinal and anterior cingulate cortex predict cognitive deficits in parkinson's disease. Acta Neuropathol 2003; 106: 83-8.

Laakso MP, Partanen K, Riekkinen P, Lehtovirta M, Helkala EL, Hallikainen M, et al. Hippocampal volumes in alzheimer's disease, parkinson's disease with and without dementia, and in vascular dementia: An MRI study. Neurology 1996; 46: 678-81.

Lang AE, Obeso JA. Time to move beyond nigrostriatal dopamine deficiency in parkinson's disease. Ann Neurol 2004; 55: 761-5.

Lawrence AD, Goerendt IK, Brooks DJ. Impaired recognition of facial expressions of anger in parkinson's disease patients acutely withdrawn from dopamine replacement therapy. Neuropsychologia 2007; 45: 65-74.

Le Bihan D, Mangin JF, Poupon C, Clark CA, Pappata S, Molko N, et al. Diffusion tensor imaging: Concepts and applications. J Magn Reson Imaging 2001; 13: 534-46.

Lees AJ, Hardy J, Revesz T. Parkinson's disease. Lancet 2009; 373: 2055-66.

Lehrner J, Brucke T, Kryspin-Exner I, Asenbaum S, Podreka I. Impaired olfactory function in parkinson's disease. Lancet 1995; 345: 1054-5.

Lepage M, Ghaffar O, Nyberg L, Tulving E. Prefrontal cortex and episodic memory retrieval mode. Proc Natl Acad Sci U S A 2000; 97: 506-11.

Levy G, Tang MX, Cote LJ, Louis ED, Alfaro B, Mejia H, et al. Motor impairment in PD: Relationship to incident dementia and age. Neurology 2000; 55: 539-44.

Levy G, Jacobs DM, Tang MX, Cote LJ, Louis ED, Alfaro B, et al. Memory and executive function impairment predict dementia in parkinson's disease. Mov Disord 2002; 17: 1221-6.

Lewis SJ, Dove A, Robbins TW, Barker RA, Owen AM. Cognitive impairments in early parkinson's disease are accompanied by reductions in activity in frontostriatal neural circuitry. J Neurosci 2003; 23: 6351-6.

Lewis SJ, Foltynie T, Blackwell AD, Robbins TW, Owen AM, Barker RA. Heterogeneity of parkinson's disease in the early clinical stages using a data driven approach. J Neurol Neurosurg Psychiatry 2005; 76: 343-8.

Logothetis NK. The neural basis of the blood-oxygen-level-dependent functional magnetic resonance imaging signal. Philos Trans R Soc Lond B Biol Sci 2002; 357: 1003-37.

Logothetis NK, Pfeuffer J. On the nature of the BOLD fMRI contrast mechanism. Magn Reson Imaging 2004; 22: 1517-31.

Mahieux F, Fenelon G, Flahault A, Manifacier MJ, Michelet D, Boller F. Neuropsychological prediction of dementia in parkinson's disease. J Neurol Neurosurg Psychiatry 1998; 64: 178-83.

Masliah E, Rockenstein E, Veinbergs I, Sagara Y, Mallory M, Hashimoto M, et al. Beta-amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking alzheimer's disease and parkinson's disease. Proc Natl Acad Sci U S A 2001; 98: 12245-50.

Matsui H, Nishinaka K, Oda M, Niikawa H, Kubori T, Udaka F. Dementia in parkinson's disease: Diffusion tensor imaging. Acta Neurol Scand 2007a; 116: 177-81.

Matsui H, Nishinaka K, Oda M, Niikawa H, Komatsu K, Kubori T, et al. Wisconsin card sorting test in parkinson's disease: Diffusion tensor imaging. Acta Neurol Scand 2007b; 116: 108-12.

Matsui H, Nishinaka K, Oda M, Hara N, Komatsu K, Kubori T, et al. Hypoperfusion of the visual pathway in parkinsonian patients with visual hallucinations. Mov Disord 2006; 21: 2140-4.

Mattay VS, Tessitore A, Callicott JH, Bertolino A, Goldberg TE, Chase TN, et al. Dopaminergic modulation of cortical function in patients with parkinson's disease. Ann Neurol 2002; 51: 156-64.

Mattila PM, Rinne JO, Helenius H, Dickson DW, Roytta M. Alpha-synuclein-immunoreactive cortical lewy bodies are associated with cognitive impairment in parkinson's disease. Acta Neuropathol 2000; 100: 285-90.

McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, et al. Diagnosis and management of dementia with lewy bodies: Third report of the DLB consortium. Neurology 2005; 65: 1863-72.

Mechelli A, Price CJ, Friston KJ, Ashburner J. Voxel-based morphometry of the human brain: Methods and applications. Current Medical Imaging Reviews 2005; 1: 105-113

Meppelink AM, Koerts J, Borg M, Leenders KL, van Laar T. Visual object recognition and attention in parkinson's disease patients with visual hallucinations. Mov Disord 2008; 23: 1906-12.

Mesholam RI, Moberg PJ, Mahr RN, Doty RL. Olfaction in neurodegenerative disease: A meta-analysis of olfactory functioning in alzheimer's and parkinson's diseases. Arch Neurol 1998; 55: 84-90.

Meyer JS, Huang J, Chowdhury MH. MRI confirms mild cognitive impairments prodromal for alzheimer's, vascular and parkinson-lewy body dementias. J Neurol Sci 2007; 257: 97-104.

Mimura M, Oeda R, Kawamura M. Impaired decision-making in parkinson's disease. Parkinsonism Relat Disord 2006; 12: 169-75.

Moody TD, Bookheimer SY, Vanek Z, Knowlton BJ. An implicit learning task activates medial temporal lobe in patients with parkinson's disease. Behav Neurosci 2004; 118: 438-42.

Morris JS, Frith CD, Perrett DI, Rowland D, Young AW, Calder AJ, et al. A differential neural response in the human amygdala to fearful and happy facial expressions. Nature 1996; 383: 812-5.

Muslimovic D, Post B, Speelman JD, Schmand B. Cognitive profile of patients with newly diagnosed parkinson disease. Neurology 2005; 65: 1239-45.

Muslimovic D, Schmand B, Speelman JD, de Haan RJ. Course of cognitive decline in Parkinson's disease: A meta-analysis. J Int Neuropsychol Soc 2007; 13: 920-932.

Muslimovic D, Post B, Speelman JD, De Haan RJ, Schmand B. Cognitive decline in parkinson's disease: A prospective longitudinal study. J Int Neuropsychol Soc 2009; 15: 426-37.

Nagano-Saito A, Washimi Y, Arahata Y, Kachi T, Lerch JP, Evans AC, et al. Cerebral atrophy and its relation to cognitive impairment in parkinson disease. Neurology 2005; 64: 224-9.

Nagano-Saito A, Washimi Y, Arahata Y, Iwai K, Kawatsu S, Ito K, et al. Visual hallucination in parkinson's disease with FDG PET. Mov Disord 2004; 19: 801-6.

Nakamura K, Kawashima R, Ito K, Sugiura M, Kato T, Nakamura A, et al. Activation of the right inferior frontal cortex during assessment of facial emotion. J Neurophysiol 1999; 82: 1610-4.

Nicoletti G, Lodi R, Condino F, Tonon C, Fera F, Malucelli E, et al. Apparent diffusion coefficient measurements of the middle cerebellar peduncle differentiate the parkinson variant of MSA from parkinson's disease and progressive supranuclear palsy. Brain 2006; 129: 2679-87.

Oishi N, Udaka F, Kameyama M, Sawamoto N, Hashikawa K, Fukuyama H. Regional cerebral blood flow in parkinson disease with nonpsychotic visual hallucinations. Neurology 2005; 65: 1708-15.

Okada K, Suyama N, Oguro H, Yamaguchi S, Kobayashi S. Medication-induced hallucination and cerebral blood flow in parkinson's disease. J Neurol 1999; 246: 365-8.

Olanow CW, Perl DP, DeMartino GN, McNaught KS. Lewy-body formation is an aggresome-related process: A hypothesis. Lancet Neurol 2004; 3: 496-503.

Ouchi Y, Yoshikawa E, Okada H, Futatsubashi M, Sekine Y, Iyo M, et al. Alterations in binding site density of dopamine transporter in the striatum, orbitofrontal cortex, and amygdala in early parkinson's disease: Compartment analysis for beta-CFT binding with positron emission tomography. Ann Neurol 1999; 45: 601-10.

Owen AM, James M, Leigh PN, Summers BA, Marsden CD, Quinn NP, et al. Fronto-striatal cognitive deficits at different stages of parkinson's disease. Brain 1992; 115 (Pt 6): 1727-51.

Pagonabarraga J, Kulisevsky J, Llebaria G, Garcia-Sanchez C, Pascual-Sedano B, Gironell A. Parkinson's disease-cognitive rating scale: A new cognitive scale specific for parkinson's disease. Mov Disord 2008; 23: 998-1005.

Pagonabarraga J, Garcia-Sanchez C, Llebaria G, Pascual-Sedano B, Gironell A, Kulisevsky J. Controlled study of decision-making and cognitive impairment in parkinson's disease. Mov Disord 2007; 22: 1430-5.

Papapetropoulos S, Mash DC. Psychotic symptoms in parkinson's disease. from description to etiology. J Neurol 2005; 252: 753-64.

Parashos SA, Maraganore DM, O'Brien PC, Rocca WA. Medical services utilization and prognosis in parkinson disease: A population-based study. Mayo Clin Proc 2002; 77: 918-25.

Parkkinen L, Pirttila T, Tervahauta M, Alafuzoff I. Widespread and abundant alpha-synuclein pathology in a neurologically unimpaired subject. Neuropathology 2005; 25: 304-14.

Paviour DC, Thornton JS, Lees AJ, Jager HR. Diffusion-weighted magnetic resonance imaging differentiates parkinsonian variant of multiple-system atrophy from progressive supranuclear palsy. Mov Disord 2007; 22: 68-74.

Paviour DC, Price SL, Jahanshahi M, Lees AJ, Fox NC. Longitudinal MRI in progressive supranuclear palsy and multiple system atrophy: Rates and regions of atrophy. Brain 2006; 129: 1040-9.

Pell MD, Leonard CL. Facial expression decoding in early parkinson's disease. Brain Res Cogn Brain Res 2005; 23: 327-40.

Pereira JB, Junque C, Marti MJ, Ramirez-Ruiz B, Bartres-Faz D, Tolosa E. Structural brain correlates of verbal fluency in parkinson's disease. Neuroreport 2009a.

Pereira JB, Junque C, Marti MJ, Ramirez-Ruiz B, Bargallo N, Tolosa E. Neuroanatomical substrate of visuospatial and visuoperceptual impairment in parkinson's disease. Mov Disord 2009b; 24: 1193-9.

Perretta JG, Pari G, Beninger RJ. Effects of parkinson disease on two putative nondeclarative learning tasks: Probabilistic classification and gambling. Cogn Behav Neurol 2005; 18: 185-92.

Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern Med 2004; 256: 183-94.

Petersen RC, Doody R, Kurz A, Mohs RC, Morris JC, Rabins PV, et al. Current concepts in mild cognitive impairment. Arch Neurol 2001; 58: 1985-92.

Ponsen MM, Stoffers D, Booij J, van Eck-Smit BL, Wolters EC, Berendse HW. Idiopathic hyposmia as a preclinical sign of parkinson's disease. Ann Neurol 2004; 56: 173-81.

Post B, Speelman JD, de Haan RJ, CARPA-study group. Clinical heterogeneity in newly diagnosed parkinson's disease. J Neurol 2008; 255: 716-22.

Postuma RB, Montplaisir J. Potential early markers of parkinson's disease in idiopathic rapid-eye-movement sleep behaviour disorder. Lancet Neurol 2006; 5: 552-3.

Price JL. Olfaction. In: Paxinos G, Mai JK. The human nervous system, 2nd edition. 2004. Elsevier

Qiu D, Tan LH, Zhou K, Khong PL. Diffusion tensor imaging of normal white matter maturation from late childhood to young adulthood: Voxel-wise evaluation of mean diffusivity, fractional anisotropy, radial and axial diffusivities, and correlation with reading development. Neuroimage 2008; 41: 223-32.

Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. Proc Natl Acad Sci U S A 2001; 98: 676-82.

Rajput AH, Voll A, Rajput ML, Robinson CA, Rajput A. Course in parkinson disease subtypes: A 39-year clinicopathologic study. Neurology 2009; 73: 206-12.

Ramirez-Ruiz B, Junque C, Marti MJ, Valldeoriola F, Tolosa E. Cognitive changes in parkinson's disease patients with visual hallucinations. Dement Geriatr Cogn Disord 2007; 23: 281-8.

Ramirez-Ruiz B, Junque C, Marti MJ, Valldeoriola F, Tolosa E. Neuropsychological deficits in parkinson's disease patients with visual hallucinations. Mov Disord 2006; 21: 1483-7.

Ramirez-Ruiz B, Marti MJ, Tolosa E, Falcon C, Bargallo N, Valldeoriola F, et al. Brain response to complex visual stimuli in parkinson's patients with hallucinations: A functional magnetic resonance imaging study. Mov Disord 2008; 23: 2335-43.

Ramirez-Ruiz B, Marti MJ, Tolosa E, Gimenez M, Bargallo N, Valldeoriola F, et al. Cerebral atrophy in parkinson's disease patients with visual hallucinations. Eur J Neurol 2007; 14: 750-6.

Ramirez-Ruiz B, Marti MJ, Tolosa E, Bartres-Faz D, Summerfield C, Salgado-Pineda P, et al. Longitudinal evaluation of cerebral morphological changes in parkinson's disease with and without dementia. J Neurol 2005; 252: 1345-52.

Reid WG, Hely MA, Morris JG, Broe GA, Adena M, Sullivan DJ, et al. A longitudinal of parkinson's disease: Clinical and neuropsychological correlates of dementia. J Clin Neurosci 1996; 3: 327-33.

Riekkinen P,Jr, Kejonen K, Laakso MP, Soininen H, Partanen K, Riekkinen M. Hippocampal atrophy is related to impaired memory, but not frontal functions in non-demented parkinson's disease patients. Neuroreport 1998; 9: 1507-11.

Rochet JC, Conway KA, Lansbury PT,Jr. Inhibition of fibrillization and accumulation of prefibrillar oligomers in mixtures of human and mouse alpha-synuclein. Biochemistry 2000; 39: 10619-26.

Rombouts SA, Damoiseaux JS, Goekoop R, Barkhof F, Scheltens P, Smith SM, et al. Model-free group analysis shows altered BOLD FMRI networks in dementia. Hum Brain Mapp 2009; 30: 256-66.

Roosendaal SD, Geurts JJ, Vrenken H, Hulst HE, Cover KS, Castelijns JA, et al. Regional DTI differences in multiple sclerosis patients. Neuroimage 2009; 44: 1397-403.

Ross GW, Petrovitch H, Abbott RD, Tanner CM, Popper J, Masaki K, et al. Association of olfactory dysfunction with risk for future parkinson's disease. Ann Neurol 2008; 63: 167-73.

Rudebeck PH, Murray EA. Amygdala and orbitofrontal cortex lesions differentially influence choices during object reversal learning. J Neurosci 2008; 28: 8338-43.

Sabatini U, Boulanouar K, Fabre N, Martin F, Carel C, Colonnese C, et al. Cortical motor reorganization in akinetic patients with parkinson's disease: A functional MRI study. Brain 2000; 123 (Pt 2): 394-403.

Sandmann-Keil D, Braak H. Postmortal diagnosis of parkinson's disease. Pathologe 2005; 26: 214-20.

Sandmann-Keil D, Braak H, Okochi M, Haass C, Braak E. Alpha-synuclein immunoreactive lewy bodies and lewy neurites in parkinson's disease are detectable by an advanced silver-staining technique. Acta Neuropathol 1999; 98: 461-4.

Santangelo G, Trojano L, Vitale C, Ianniciello M, Amboni M, Grossi D, et al. A neuropsychological longitudinal study in parkinson's patients with and without hallucinations. Mov Disord 2007; 22: 2418-25.

Sato W, Kubota Y, Okada T, Murai T, Yoshikawa S, Sengoku A. Seeing happy emotion in fearful and angry faces: Qualitative analysis of facial expression recognition in a bilateral amygdala-damaged patient. Cortex 2002; 38: 727-42.

Scheltens P, Leys D, Barkhof F, Huglo D, Weinstein HC, Vermersch P, et al. Atrophy of medial temporal lobes on MRI in "probable" alzheimer's disease and normal ageing: Diagnostic value and neuropsychological correlates. J Neurol Neurosurg Psychiatry 1992; 55: 967-72.

Scher AI, Xu Y, Korf ES, White LR, Scheltens P, Toga AW, et al. Hippocampal shape analysis in alzheimer's disease: A population-based study. Neuroimage 2007; 36: 8-18.

Scherfler C, Schocke MF, Seppi K, Esterhammer R, Brenneis C, Jaschke W, et al. Voxel-wise analysis of diffusion weighted imaging reveals disruption of the olfactory tract in parkinson's disease. Brain 2006; 129: 538-42.

Schocke MF, Seppi K, Esterhammer R, Kremser C, Mair KJ, Czermak BV, et al. Trace of diffusion tensor differentiates the parkinson variant of multiple system atrophy and parkinson's disease. Neuroimage 2004; 21: 1443-51.

Schott BH, Niehaus L, Wittmann BC, Schutze H, Seidenbecher CI, Heinze HJ, et al. Ageing and early-stage parkinson's disease affect separable neural mechanisms of mesolimbic reward processing. Brain 2007; 130: 2412-24.

Schrag A, Jahanshahi M, Quinn N. How does parkinson's disease affect quality of life? A comparison with quality of life in the general population. Mov Disord 2000; 15: 1112-8.

Seeley WW, Crawford RK, Zhou J, Miller BL, Greicius MD. Neurodegenerative diseases target large-scale human brain networks. Neuron 2009; 62: 42-52.

Seppi K, Schocke MF, Esterhammer R, Kremser C, Brenneis C, Mueller J, et al. Diffusion-weighted imaging discriminates progressive supranuclear palsy from PD, but not from the parkinson variant of multiple system atrophy. Neurology 2003; 60: 922-7.

Shimada H, Hirano S, Shinotoh H, Aotsuka A, Sato K, Tanaka N, et al. Mapping of brain acetylcholinesterase alterations in lewy body disease by PET. Neurology 2009; 73: 273-8.

Siderowf A, Jennings D, Connolly J, Doty RL, Marek K, Stern MB. Risk factors for parkinson's disease and impaired olfaction in relatives of patients with parkinson's disease. Mov Disord 2007; 22: 2249-55.

Siderowf A, Newberg A, Chou KL, Lloyd M, Colcher A, Hurtig HI, et al. 99mTc]TRODAT-1 SPECT imaging correlates with odor identification in early parkinson disease. Neurology 2005; 64: 1716-20.

Silveira-Moriyama L, Holton JL, Kingsbury A, Ayling H, Petrie A, Sterlacci W, et al. Regional differences in the severity of lewy body pathology across the olfactory cortex. Neurosci Lett 2009; 453: 77-80.

Sinforiani E, Pacchetti C, Zangaglia R, Pasotti C, Manni R, Nappi G. REM behavior disorder, hallucinations and cognitive impairment in parkinson's disease: A two-year follow up. Mov Disord 2008; 23: 1441-5.

Sinforiani E, Zangaglia R, Manni R, Cristina S, Marchioni E, Nappi G, et al. REM sleep behavior disorder, hallucinations, and cognitive impairment in parkinson's disease. Mov Disord 2006; 21: 462-6.

Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. Neuroimage 2002; 17: 1429-36.

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in lewy bodies. Nature 1997; 388: 839-40.

Spottke AE, Reuter M, Machat O, Bornschein B, von Campenhausen S, Berger K, et al. Cost of illness and its predictors for parkinson's disease in germany. Pharmacoeconomics 2005; 23: 817-36.

Sprengelmeyer R, Young AW, Mahn K, Schroeder U, Woitalla D, Buttner T, et al. Facial expression recognition in people with medicated and unmedicated parkinson's disease. Neuropsychologia 2003; 41: 1047-57.

Stebbins GT, Goetz CG, Carrillo MC, Bangen KJ, Turner DA, Glover GH, et al. Altered cortical visual processing in PD with hallucinations: An fMRI study. Neurology 2004; 63: 1409-16.

Stefanova E, Potrebic A, Ziropadja L, Maric J, Ribaric I, Kostic VS. Depression predicts the pattern of cognitive impairment in early parkinson's disease. J Neurol Sci 2006; 248: 131-7.

Stern MB, Doty RL, Dotti M, Corcoran P, Crawford D, McKeown DA, et al. Olfactory function in parkinson's disease subtypes. Neurology 1994; 44: 266-8.

Summerfield C, Junque C, Tolosa E, Salgado-Pineda P, Gomez-Anson B, Marti MJ, et al. Structural brain changes in parkinson disease with dementia: A voxel-based morphometry study. Arch Neurol 2005; 62: 281-5.

Suzuki A, Hoshino T, Shigemasu K, Kawamura M. Disgust-specific impairment of facial expression recognition in parkinson's disease. Brain 2006; 129: 707-17.

Takeda A, Saito N, Baba T, Kikuchi A, Sugeno N, Kobayashi M, et al. Functional imaging studies of hyposmia in parkinson's disease. J Neurol Sci 2009.

Tam CW, Burton EJ, McKeith IG, Burn DJ, O'Brien JT. Temporal lobe atrophy on MRI in parkinson disease with dementia: A comparison with alzheimer disease and dementia with lewy bodies. Neurology 2005; 64: 861-5.

Tanner CM, Ross GW, Jewell SA, Hauser RA, Jankovic J, Factor SA, et al. Occupation and risk of parkinsonism: A multicenter case-control study. Arch Neurol 2009; 66: 1106-13.

Tessitore A, Hariri AR, Fera F, Smith WG, Chase TN, Hyde TM, et al. Dopamine modulates the response of the human amygdala: A study in parkinson's disease. J Neurosci 2002; 22: 9099-103.

Tissingh G, Berendse HW, Bergmans P, DeWaard R, Drukarch B, Stoof JC, et al. Loss of olfaction in de novo and treated parkinson's disease: Possible implications for early diagnosis. Mov Disord 2001; 16: 41-6.

Tolosa E, Gaig C, Santamaria J, Compta Y. Diagnosis and the premotor phase of parkinson disease. Neurology 2009; 72: S12-20.

Toro R, Fox PT, Paus T. Functional coactivation map of the human brain. Cereb Cortex 2008; 18: 2553-9.

Troster AI. Neuropsychological characteristics of dementia with lewy bodies and parkinson's disease with dementia: Differentiation, early detection, and implications for "mild cognitive impairment" and biomarkers. Neuropsychol Rev 2008; 18: 103-19.

Vaillancourt DE, Spraker MB, Prodoehl J, Abraham I, Corcos DM, Zhou XJ, et al. High-resolution diffusion tensor imaging in the substantia nigra of de novo parkinson disease. Neurology 2009; 72: 1378-84.

van Eimeren T, Monchi O, Ballanger B, Strafella AP. Dysfunction of the default mode network in parkinson disease: A functional magnetic resonance imaging study. Arch Neurol 2009; 66: 877-83.

Wagner AD, Shannon BJ, Kahn I, Buckner RL. Parietal lobe contributions to episodic memory retrieval. Trends Cogn Sci 2005; 9: 445-53.

Wallin A, Ekberg S, Lind K, Milos V, Granerus AK, Granerus G. Posterior cortical brain dysfunction in cognitively impaired patients with parkinson's disease--a rCBF scintigraphy study. Acta Neurol Scand 2007; 116: 347-54.

Wallis JD. Orbitofrontal cortex and its contribution to decision-making. Annu Rev Neurosci 2007; 30: 31-56.

Wang L, Swank JS, Glick IE, Gado MH, Miller MI, Morris JC, et al. Changes in hippocampal volume and shape across time distinguish dementia of the alzheimer type from healthy aging. Neuroimage 2003; 20: 667-82.

Westermann B, Wattendorf E, Schwerdtfeger U, Husner A, Fuhr P, Gratzl O, et al. Functional imaging of the cerebral olfactory system in patients with parkinson's disease. J Neurol Neurosurg Psychiatry 2008; 79: 19-24.

Whalen PJ, Rauch SL, Etcoff NL, McInerney SC, Lee MB, Jenike MA. Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. J Neurosci 1998; 18: 411-8.

Whitehouse PJ, Hedreen JC, White CL,3rd, Price DL. Basal forebrain neurons in the dementia of parkinson disease. Ann Neurol 1983; 13: 243-8.

Whittington CJ, Podd J, Stewart-Williams S. Memory deficits in parkinson's disease. J Clin Exp Neuropsychol 2006; 28: 738-54.

Williams DR, Lees AJ. Visual hallucinations in the diagnosis of idiopathic parkinson's disease: A retrospective autopsy study. Lancet Neurol 2005; 4: 605-10.

Williams-Gray CH, Foltynie T, Brayne CE, Robbins TW, Barker RA. Evolution of cognitive dysfunction in an incident parkinson's disease cohort. Brain 2007; 130: 1787-98.

Winblad B, Palmer K, Kivipelto M, Jelic V, Fratiglioni L, Wahlund LO, et al. Mild cognitive impairment--beyond controversies, towards a consensus: Report of the international working group on mild cognitive impairment. J Intern Med 2004; 256: 240-6.

Woods SP, Troster AI. Prodromal frontal/executive dysfunction predicts incident dementia in parkinson's disease. J Int Neuropsychol Soc 2003; 9: 17-24.

Wu T, Hallett M. A functional MRI study of automatic movements in patients with parkinson's disease. Brain 2005; 128: 2250-9.

Yip JT, Lee TM, Ho SL, Tsang KL, Li LS. Emotion recognition in patients with idiopathic parkinson's disease. Mov Disord 2003; 18: 1115-22.

Yoshikawa K, Nakata Y, Yamada K, Nakagawa M. Early pathological changes in the parkinsonian brain demonstrated by diffusion tensor MRI. J Neurol Neurosurg Psychiatry 2004; 75: 481-4.

Yucel M, Stuart GW, Maruff P, Velakoulis D, Crowe SF, Savage G, et al. Hemispheric and gender-related differences in the gross morphology of the anterior cingulate/paracingulate cortex in normal volunteers: An MRI morphometric study. Cereb Cortex 2001; 11: 17-25.

Zhang K, Yu C, Zhang Y, Wu X, Zhu C, Chan P, et al. Voxel-based analysis of diffusion tensor indices in the brain in patients with parkinson's disease. Eur J Radiol 2009.