Imaging Studies of Olfaction in Health and Parkinsonism

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TABLE OF CONTENTS

ABSTRACT	1
SVENSK SAMMANFATTNING	3
LIST OF PUBLICATIONS.....	5
AUTHOR CONTRIBUTIONS	7
ABBREVIATIONS	9
INTRODUCTION	11
OLFACTION	11
PARKINSONISM	13
IMAGING TECHNIQUES.....	15
<i>DaTSCAN® SPECT</i>	15
<i>Diffusion Tensor Imaging</i>	17
<i>Magnetization Transfer</i>	20
<i>Functional MRI</i>	21
AIMS.....	25
MATERIALS AND METHODS	27
PARTICIPANTS.....	27
<i>Study I</i>	27
<i>Study II</i>	27
<i>Study III</i>	28
<i>Study IV</i>	28
OLFACTORY EVALUATION.....	28
<i>Study I</i>	28
<i>Study II</i>	28
<i>Studies III and IV</i>	29
IMAGE ACQUISITION	29
<i>DaTSCAN SPECT (Studies I and IV)</i>	29
<i>Diffusion Tensor Imaging and Magnetization Transfer (Study II)</i>	29
<i>fMRI acquisition (Studies III and IV)</i>	30
<i>Olfactory fMRI design (Studies III and IV)</i>	30
IMAGE ANALYSIS	31
<i>DaTSCAN SPECT</i>	31

<i>Diffusion Tensor Imaging</i>	32
<i>Magnetization Transfer</i>	32
<i>Olfactory fMRI (Study III)</i>	33
<i>Olfactory fMRI (Study IV)</i>	33
<i>Resting-state fMRI (Study IV)</i>	34
STATISTICS	35
<i>Study I</i>	35
<i>Study II</i>	35
<i>Study III</i>	35
<i>Study IV</i>	36
RESULTS	37
STUDY I.....	37
STUDY II.....	39
STUDY III.....	40
STUDY IV	43
DISCUSSION	47
STUDY I.....	47
<i>Olfactory test</i>	47
<i>DaTSCAN SPECT</i>	47
<i>Discordant results</i>	49
STUDY II.....	49
<i>Diffusion Transfer Imaging</i>	49
<i>Magnetization transfer</i>	50
STUDY III.....	51
STUDY IV	52
<i>Olfactory fMRI</i>	52
<i>Resting-state fMRI</i>	54
LIMITATIONS	54
CONCLUSIONS	57
FUTURE PERSPECTIVES	59
ACKNOWLEDGMENTS	61
REFERENCES	65
APPENDIX	73

ABSTRACT

Olfactory loss is a common non-motor symptom of Parkinson's disease (PD), often preceding the cardinal motor symptoms of the disease. The aim of this thesis was to: (a) evaluate whether olfactory examination can increase diagnostic accuracy, and (b) study the structural and functional neural basis of olfactory dysfunction in PD with different applications of Magnetic Resonance Imaging (MRI).

Paper I was a comparison of the diagnostic accuracy between a simple smell identification test and DaTSCAN Single Photon Emission Computerized Tomography (SPECT), a nuclear medicine tomographic imaging technique that is commonly used in patients with suspected parkinsonism. The results indicate that smell test is inferior to DaTSCAN SPECT, but the combination of these two methods can lead to improved diagnostic accuracy.

Paper II showed that diffusion MRI could detect discrete microstructural changes in the white matter of brain areas that participate in higher order olfactory neurotransmission, whereas MRI with Magnetization Transfer contrast could not.

Paper III was a methodological study on how two different acquisition parameters can affect the activation pattern of olfactory brain areas, as observed with functional MRI (fMRI). The results indicate that brief olfactory stimulation and fast sampling rate should be preferred on olfactory fMRI studies.

Paper IV used olfactory fMRI and resting-state fMRI in order to elucidate potentially altered activation patterns and functional connectivity within olfactory brain areas, between PD patients and healthy controls. Olfactory fMRI showed that olfactory impairment in PD is associated with significantly lower recruitment of the olfactory network. Resting-state fMRI did not detect any significant changes in the functional connectivity within the olfactory network of PD patients.

In conclusion, the included studies provide evidence of: (a) disease-related structural and functional changes in olfactory brain areas, and (b) beneficial addition of olfactory tests in the clinical work-up of patients with parkinsonism.

Keywords: Smell, Parkinsonism, Parkinson disease, Neuroimaging

SVENSK SAMMANFATTNING

Parkinsons sjukdom drabbar 1% av befolkningen över 60 årsålder och kännetecknas av typiska motoriska rubbningar. Dessutom ger Parkinson upphov till icke-motoriska symtom som kraftigt påverkar patienternas livskvalitet. Nedsatt luktsinne är ett mycket vanligt icke-motoriskt symtom, som oftast uppträder flera år innan de första motoriska symtomen. Därutöver finns en rad olika sjukdomar (som tillsammans kallas atypisk parkinsonism) med tämligen liknande klinisk bild, framför allt tidigt i sjukdomsförloppet. Syftet med studierna som presenteras här, har varit att använda olika utbildningsmetoder för att närmare kartlägga hur luktspecifika hjärnområden drabbas vid Parkinson och hur undersökning av luktsinnet kan vara behjälplig vid diagnostiken av parkinsonism.

Denna avhandling fokuserar på SPECT-undersökning (fotonemissionstomografi, en metod som ofta används vid klinisk utredning av parkinsonism) och olika metoder av magnetresonanstomografi (MR). Resultaten från den första studien visar att SPECT är effektiv för att skilja parkinsonism från icke parkinsonism. En kombination av SPECT med vanligt dofttest kan öka den diagnostiska säkerheten och skilja Parkinson från atypisk parkinsonism. Den andra studien har använt en speciell MR-metod som kvantifierar vattnets slumpmässiga termiska rörelser; denna metod har identifierat mycket diskreta strukturella förändringar i vissa luktspecifika hjärnområden.

Vi har dessutom etablerat en pålitlig MR-metod som kan användas för att titta på hjärnans funktion när man luktar på olika dofter. Med hjälp av denna metod har vi kunnat demonstrera två nätverk med luktspecifika hjärnområden i såväl stor- som lillhjärnan. Dessa nätverk framträder mindre involverade efter doftstimulering hos patienter med Parkinson än hos friska personer.

Sammanfattningsvis visar denna avhandling att nedsatt luktsinne vid Parkinsons sjukdom är associerat med såväl strukturella som funktionella förändringar i hjärnan. Dessutom kan klinisk utredning av parkinsonism gynnas av ett enkelt dofttest.

LIST OF PUBLICATIONS

Papers included in the thesis, referred to as their Roman numerals:

I. The diagnostic value of dopamine transporter imaging and olfactory testing in patients with parkinsonian syndromes.

Georgiopoulos, C., Davidsson, A., Engstrom, M., Larsson, E. M., Zachrisson, H., Dizdar, N. (2015).

Journal of Neurology, 262 (9), p. 2154-63, doi: 10.1007/s00415-015-7830-4

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II. Olfactory Impairment in Parkinson's Disease Studied with Diffusion Tensor and Magnetization Transfer Imaging.

Georgiopoulos, C., Warntjes, M., Dizdar, N., Zachrisson, H., Engstrom, M., Haller, S., Larsson, E. M. (2017).

Journal of Parkinson's Disease, 7 (2), p. 301-311, doi: 10.3233/JPD-161060

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III. Olfactory fMRI: Implications of Stimulation Length and Repetition Time.

Georgiopoulos, C., Witt, S. T., Haller, S., Dizdar, N., Zachrisson, H., Engstrom, M., Larsson, E. M. (2018).

Chemical Senses, 43 (6), p. 389-398, doi: 10.1093/chemse/bjy025

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IV. A study of neural activity and functional connectivity within the olfactory brain network in Parkinson's disease.

Georgiopoulos, C., Witt, S. T., Haller, S., Dizdar, N., Zachrisson, H., Engstrom, M., Larsson, E. M. (2019). *Submitted*

Paper not included in the thesis:

- Comparison between visual assessment of dopaminergic degeneration pattern and semi-quantitative ratio calculations in patients with Parkinson's disease and Atypical Parkinsonian syndromes using DaTSCAN(R) SPECT.

Davidsson, A., **Georgiopoulos, C.**, Dizdar, N., Granerus, G., Zachrisson, H. (2014).

Annals of Nuclear Medicine, 28 (9), p. 851-9, doi: 10.1007/s12149-014-0878-x

© The Japanese Society of Nuclear Medicine (2014).

Conference papers published as part of the project:

- DaTSCAN SPECT evaluation of patients with movement disorders

Georgiopoulos, C., Davidsson, A., Granerus, G., Dizdar, N., Zachrisson, H. (2011).

15th Congress of the European-Federation-of-Neurological-Societies (EFNS), September 10-13, 2011, Budapest, Hungary, doi: 10.1111/j.1468-1331.2011.03552.x

- Methodological considerations in designing olfactory fMRI studies

Georgiopoulos C., Witt T. S., Haller S., Dizdar N., Zachrisson H., Engström M, Larsson E.M. (2017)

European Society for Magnetic Resonance in Medicine and Biology (ESMRMB) Annual Scientific Meeting, October 19 - 21, 2017, Barcelona, Spain

AUTHOR CONTRIBUTIONS

Paper I:

	CG	AD	ME	EML	HZ	ND
Study conception and design	x	x	x	x	x	x
Data acquisition	x	x	-	-	-	-
Data analysis	x	x	-	-	x	-
Data interpretation	x	x	-	-	x	x

Manuscript

First draft	x	-	-	-	-	-
Revision	x	x	x	x	x	x
Writing of final version	x	-	-	-	-	-
Correspondence with journal	x	-	-	-	-	-

Paper II:

	CG	MW	ND	HZ	ME	SH	EML
Study conception and design	x	x	x	x	x	x	x
Data acquisition	x	-	-	-	-	-	-
Data analysis	x	x	-	-	-	x	-
Data interpretation	x	x	-	-	-	x	x

Manuscript

First draft	x	-	-	-	-	-	-
Revision	x	x	x	x	x	x	x
Writing of final version	x	-	-	-	-	-	-
Correspondence with journal	x	-	-	-	-	-	-

Paper III:

	CG	STW	SH	ND	HZ	ME	EML
Study conception and design	x	x	x	x	x	x	x
Data acquisition	x	-	-	-	-	-	-
Data analysis	x	x	-	-	-	-	-
Data interpretation	x	x	x	-	-	x	x

Manuscript

First draft	x	-	-	-	-	-	-
Revision	x	x	x	x	x	x	x
Writing of final version	x	-	-	-	-	-	-
Correspondence with journal	x	-	-	-	-	-	-

Paper IV:

	CG	STW	SH	ND	HZ	ME	EML
Study conception and design	x	x	x	x	x	x	x
Data acquisition	x	-	-	-	-	-	-
Data analysis	x	x	x	-	-	-	-
Data interpretation	x	x	x	-	-	x	x

Manuscript

First draft	x	-	-	-	-	-	-
Revision	x	x	x	x	x	x	x
Writing of final version	x	-	-	-	-	-	-
Correspondence with journal	x	-	-	-	-	-	-

ABBREVIATIONS

3D: Three-dimensional
AD: Axial Diffusivity
APS: Atypical Parkinsonian Syndromes
BET: Brain Extraction Toolbox
BOLD: Blood Oxygen Level Dependent
B-SIT: Brief 12-item Smell Identification Test
CBD: Corticobasal Degeneration
CI: Confidence Intervals
CMIV: Center for Medical Imaging and Visualization
DTI: Diffusion Tensor Imaging
DaTSCAN: SPECT with the presynaptic ligand ioflupane
DLB: Dementia with Lewy bodies
FA: Fractional Anisotropy
FIR: Finite Impulse Response
FLAIR: Fluid Attenuated Inversion Recovery
fMRI: functional Magnetic Resonance Imaging
FSL: FMRIB Software Library, University of Oxford, United Kingdom
GLM: General Linear Model
HRF: Hemodynamic Response Function
H&Y: Hoehn and Yahr Staging
ICA: Independent Component Analysis
MD: Mean Diffusivity
MMSE: Mini-Mental State Examination
MNI: Montreal Neurological Institute
MRI: Magnetic Resonance Imaging
ms: milliseconds
MSA: Multiple System Atrophy
MT: Magnetization Transfer
PD: Parkinson's disease
PSP: Progressive Supranuclear Palsy
RD: Radial Diffusivity
ROI: Region of Interest
S&E: Schwab and England activities of daily living scale

SP: Secondary Parkinsonism

SPECT: Single Photon Emission Computed Tomography

SWEDD: Scans Without Evidence of Dopaminergic Deficit

TBSS: Tract-Based Spatial Statistics

TE: Echo Time

TR: Repetition Time

UPDRS: Unified Parkinson's Disease Rating Scale

UPSIT: University of Pennsylvania Smell Identification Test

INTRODUCTION

OLFACTION

The sense of smell has a significant impact on quality of life; odors play a major role in the enjoyment of eating, they impact people's cognition, emotions and social behavior, they play a role in memory formation and they may even contribute to the choice of one's sexual partner [1]. However, olfaction has been understudied compared to the other four senses of perception. A recent (January 2019) database query in the medical search engine PubMed yielded 24,175 results for the term "olfaction" and 19,724 results for the term "smell". In contrast, "vision" yielded 180,978 results, "hearing" 129,295 results, "touch" 34,225 results and "taste" 38,225 results.

The initial olfactory processing between the olfactory receptor neurons and the olfactory bulb has for years been the main focus of basic research. Odor perception starts in the olfactory epithelium of the nose, where odorants bind to the sensory endings of the olfactory sensory neurons, which then terminate in the olfactory bulb glomeruli, where they synapse with the dendritic terminals of the mitral cells [2]. Recent advances in neuroimaging and increasing interest on the central olfactory system has shed light on how odor perception is encoded beyond the olfactory bulb. Output from the mitral cells is transferred through the lateral olfactory tract and projects monosynaptically to the olfactory cortex of the brain [3]. The olfactory cortex comprises areas of the basal frontal and medial temporal lobes, such as the anterior olfactory

Figure 1: Olfactory regions of interest studied in this thesis

nucleus, olfactory tubercle, piriform cortex, amygdala and rostral entorhinal cortex, all of which are reciprocally interconnected. Moreover, the olfactory cortex is bidirectionally connected with the olfactory bulb, allowing modulation of the bulbar output. Main projections of the olfactory cortex include the orbitofrontal cortex, anterior insula, hypothalamus, hippocampus and striatum. Figure 1 illustrates the anatomical location of the olfactory brain regions that are studied in this thesis.

The piriform cortex is located at the medial junction of the frontal and temporal lobes and is the largest structure within the olfactory cortex. It can be divided anatomically and functionally into the anterior piriform cortex and the posterior piriform cortex. Previous research in both rats and humans indicates that odor identity is encoded in the anterior piriform cortex, whereas odor quality is encoded in the posterior piriform cortex [4-6]. Moreover, the anterior piriform cortex has been found to respond preferentially to attended sniffs, indicating that it is involved in the attentional modulation of odor perception [7]. There is, also, evidence that the anterior piriform cortex is responsible for discriminating an odor against its background (odor-background segmentation) [8].

The orbitofrontal cortex is one of the main projections beyond the olfactory cortex, with a direct monosynaptic pathway to and from the piriform cortex. Interestingly, there is evidence that only the right orbitofrontal cortex is involved in conscious olfaction; the right orbitofrontal cortex is also predominant in odor memory and discrimination [9, 10]. The orbitofrontal cortex is, additionally, part of a transthalamic route that connects the piriform to the orbitofrontal cortex through the mediodorsal thalamus. This transthalamic route is considered to be involved in olfactory attentional modulation [11]. It should, however, be highlighted that olfaction is the only sense where thalamus is not the primary relay station in the brain. Apart from olfaction, the orbitofrontal cortex responds to gustatory, visual and somatosensory stimuli, and it is involved in a variety of higher-order functions, which are beyond the scope of this study.

The role of the entorhinal cortex and anterior insula in olfaction is not yet well-defined. The entorhinal cortex serves as the gateway of the hippocampus, with a suggested role in odor discrimination memory and a robust modulatory effect on the activity of the piriform cortex [12]. Recent evidence supports that successful odor identification depends on the activation of the entorhinal cortex, whereas impaired odor identification is associated with medial temporal lobe degeneration [13, 14]. Anterior insula is considered as a cognitive-evaluative area, which is often highly activated in studies requiring a task to be performed while smelling the odorous stimulus [15].

Olfactory dysfunction is associated with depression, neurodegenerative and neuroimmunological disorders [16-18]. In particular, olfactory impairment in Parkinson's disease (PD) is implicated with faster disease progression and cognitive decline [19, 20]. Despite the recent advances in understanding how olfaction is encoded beyond the olfactory bulb, there is still a lot of ground to cover in order to fully elucidate higher-order olfactory perception in both health and disease.

PARKINSONISM

PD is the second most common neurodegenerative disorder after Alzheimer's disease, with approximately 1% prevalence in people over 60 years old of age [21]. PD is characterized by classical motor symptoms, such as rigidity, bradykinesia, resting tremor and postural imbalance, which have monopolized clinical research for years. However, already in 1817, James Parkinson described several non-motor symptoms, such as sleep disturbance, constipation, sialorrhea and dysarthria [22]. Even though non-motor symptoms are often overshadowed by the dominance of motor symptoms, they have significant impact on quality of life and life expectancy [23]. The presence of olfactory dysfunction in PD was first described in 1975 by Ansari et al. who studied this phenomenon in 22 patients [24]. It has since been well-established that olfactory dysfunction in PD affects more than 90% of patients, often precedes motor symptoms and is independent of age, gender, treatment, duration and state of the disease [25-27]. PD patients demonstrate decreased performance on odor detection, identification and discrimination [28].

Progressive dopaminergic neuronal depletion and presence of Lewy bodies in substantia nigra pars compacta is considered to be the hallmark of PD, resulting in the motor deficits of the disease [29]. Post-mortem studies have additionally revealed the presence of Lewy bodies in the olfactory bulb, the entorhinal cortex and the piriform cortex [30, 31]. Interestingly, according to a study by Huisman et al., the total number of dopaminergic neurons in the olfactory bulb is twice as high in PD patients compared to healthy controls [32]. Because dopamine inhibits olfactory transmission between the olfactory receptor neurons and the mitral cells of the olfactory bulb, the authors of that study suggested that the increase of dopaminergic neurons in the olfactory bulb could explain olfactory dysfunction in PD.

There are two forms of PD: familial and sporadic. Familial PD is caused by genetic mutations, whereas the cause of sporadic PD remains largely unknown, despite the fact that both

Figure 2: Spreading of Lewy pathology in Parkinson's disease according to Braak hypothesis. At stages 1 and 2 Lewy pathology is located in the medulla, pontine tegmentum (via the vagus nerve) and the anterior olfactory nucleus (via the olfactory bulb). At stages 3 and 4, there is progression of Lewy pathology in the midbrain, basal prosencephalon and mesocortex. Finally, at stages 5 and 6 Lewy pathology reaches the neocortex, affecting gradually high order sensory association areas and, occasionally, primary motor areas.

genetic and environmental factors have been implicated. In 2003, Braak et al. suggested that sporadic PD could be initiated by an unknown pathogen in the gut, and they proposed a staging system for PD, based on the spreading of Lewy pathology in the brain (Figure 2) [33, 34]. These publications were followed by the so-called “dual-hit” hypothesis, suggesting that the above-mentioned pathogen enters the brain via two routes: (a) anterogradely via olfactory pathways, and (b) retrogradely via the enteric plexus and the vagus nerve [35, 36]. This is now known as Braak hypothesis, and despite criticism that it may not be applied to all patients, there is both clinical and preclinical evidence supporting it [37]. For instance, there has been evidence that vagotomy is associated with a reduced risk of developing PD [38]. Moreover, appendix is found to be rich in PD pathology-associated α -synuclein aggregates, that are known to accumulate in Lewy bodies; interestingly, early removal of appendix is suggested to have protective effect against the development of PD [39]. A recent study by Sampson et al. demonstrated that colonization of α -synuclein-overexpressing mice with human gut microbiota from PD patients induce enhanced motor dysfunction [40].

The terms atypical parkinsonian syndromes (APS) and secondary parkinsonism (SP) describe parkinsonian syndromes that share similar signs and symptoms with PD [41]. APS includes Multiple System Atrophy (MSA), Progressive Supranuclear Palsy (PSP), Corticobasal Degeneration (CBD) and Dementia with Lewy bodies (DLB). SP refers to patients with

vascular or drug-induced parkinsonism. These disorders are clinically heterogeneous and demonstrate significant phenotypic overlap, making their diagnosis challenging. In general, patients with MSA, PSP and CBD show a faster clinical deterioration, with a shorter survival time compared to PD patients [42-44]. As for olfaction, it is markedly impaired in PD, mildly impaired in MSA and LBD, whereas PSP and CBD are not associated with olfactory dysfunction [45, 46]. Therefore, olfactory examination is officially recommended by the European Federation of Neurological Societies and the Movement Disorder Society to differentiate PD from APS and SP [47].

IMAGING TECHNIQUES

A broad spectrum of imaging techniques can be employed for the examination of patients with parkinsonism, spanning from Single Photon Emission Computed Tomography (SPECT) to Magnetic Resonance Imaging (MRI). MRI in particular can be employed for the evaluation of olfaction.

DaTSCAN® SPECT

SPECT is a nuclear medicine tomographic imaging technique, in which an array of gamma cameras rotates around the patient, providing 3D spatial information on the distribution of an intravenously injected radionuclide. Hence, SPECT yields better detail, contrast and spatial information than planar nuclear imaging. DaTSCAN® (GE Healthcare B.V.) is the commercial name of ioflupane [iodine-123-fluoropropyl-carbomethoxy-3-β-(4-iodophenyl)tropane (FP-CIT)]. It is currently the only approved *in vivo* imaging agent, used clinically for the evaluation of adult patients suspected of having a parkinsonian syndrome. Following intravenous injection, ioflupane distributes to the striatum, where it binds with high affinity to the presynaptic dopamine transporter (DaT). The DaT is a sodium chloride-dependent protein on the presynaptic dopaminergic nerve terminal, controlling dopamine levels by active reuptake of dopamine after its interaction with the postsynaptic receptor (Figure 3) [48]. Ioflupane imaging can demonstrate striatal dopamine activity *in vivo* and is, thus, considered a biomarker for nigrostriatal dopaminergic degeneration [49].

Figure 3: Schematic representation of the dopaminergic nerve terminal. The dopamine transporter (DaT) controls dopamine levels by active reuptake of dopamine after its interaction with the postsynaptic receptor. The radioligand DaTSCAN® attaches to the dopamine transporter, allowing in vivo demonstration of DaT abnormalities.

Visual assessment of DaTSCAN® SPECT is usually sufficient for evaluating the integrity of DaT binding; additional semiquantitative analysis is recommended to objectively assess the DaT availability [50]. A normal scan will show two symmetric ‘comma’ or ‘crescent’ shaped focal areas of activity mirrored about the median plane, corresponding to the striatum. Parkinsonism is associated with decreased uptake, and ioflupane SPECT can efficiently distinguish PD patients from patients with essential tremor [51]. However, the term “scans without evidence of dopaminergic deficit” (SWEDD) has emerged because of patients with suspected parkinsonism, who have normal scans. A previous study compared the clinical and imaging characteristics between SWEDD patients and patients with abnormal DaTSCAN during a 22-month follow-up, and concluded that SWEDD patients had minimal evidence of clinical or imaging progression, suggesting that SWEDD patients are unlikely to have parkinsonism [52]. Hence, patients with normal striatal DAT availability are highly unlikely to suffer from nigrostriatal degeneration, and alternative diagnoses should be considered in those cases.

In general, DaTSCAN® SPECT is considered to be efficient in separating PD from SP, but its accuracy in distinguishing PD from APS is considered to be relatively low [53, 54]. However, a voxelwise analysis of ioflupane uptake, found reduced midbrain uptake in patients with the Parkinson variant of MSA, compared to PD patients [55]. Additionally, Kahraman et al. found significantly different dopaminergic degeneration patterns between PD and APS patients, suggesting that APS is associated with more severe uptake reduction [56]. They classified the scans of PD and APS patients in five predefined visual patterns: (a) *burst striatum*, severe bilateral

Figure 4: Classification of striatal DaTSCAN® uptake as proposed by Kahraman et al [56].

reduction, with almost no uptake in putamen or caudate nucleus, (b) *egg shape*, bilateral uptake reduction in both putamina and normal or borderline normal uptake from caudate nucleus, creating an oval shape, (c) *mixed type*, asymmetrical ioflupane uptake, with reduced uptake in the putamen of one side, (d) *eagle wing*, borderline normal, symmetrical ioflupane uptake, with only discrete reduction in one or both putamina, creating the shape of a wing, and (e) *normal*, symmetrical ioflupane uptake in putamen and caudate nucleus bilaterally (Figure 4). In their cohort, the “burst striatum” degeneration pattern was predominantly associated with APS, whereas the “egg shape” pattern was associated with PD.

The sensitivity of DaTSCAN® SPECT in distinguishing PD patients from non-PD patients has been previously compared to that of a basic smell test [57]. The main finding of that study indicates that these two modalities are equally sensitive in detecting PD, without, though, being disease-specific. However, olfactory examination has the advantage of being widely available and considerably cheaper compared to DaTSCAN® SPECT. An emerging question is whether the combination of ioflupane imaging and smell test can successfully distinguish PD from APS.

Diffusion Tensor Imaging

Diffusion tensor imaging (DTI) is a magnetic resonance imaging (MRI) technique that allows noninvasive characterization of white matter and is highly sensitive to microstructural tissue changes [58]. Diffusion MRI is based on measuring the random motion (i.e. diffusion) of water molecules within a medium. The diffusion of water in brain tissue is modulated by the spacing, orientation, and density of barriers within this tissue, such as cell membranes and myelin. Additionally, water diffusion will be altered by changes in tissue microstructure and organization. The fibrous tissue structure of white matter hinders water diffusion mostly perpendicularly to the white matter tracts. Thus, the apparent diffusivity is much higher in the direction parallel to

Figure 5: Illustration of the diffusion tensor ellipsoid representing anisotropic diffusion in a white matter bundle.

the white matter fibers than in the perpendicular orientation. In contrast, the diffusion of water in grey matter and cerebrospinal fluid is more isotropic (i.e. without strong directional preference).

Water diffusion in white matter can be represented by a 3D ellipsoid, where eigenvectors ($\epsilon_1, \epsilon_2, \epsilon_3$) represent the orientations of the ellipsoid directions (Figure 5). The length of each eigenvector is described by the eigenvalues ($\lambda_1, \lambda_2, \lambda_3$), in descending order of magnitude. Common DTI measures that are derived from these eigenvalues include fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD). Mean diffusivity and fractional anisotropy describe complementary information about water diffusion. Mean diffusivity is the average of the three eigenvalues:

$$MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$$

Fractional anisotropy is defined as a normalized standard deviation of the diffusion tensor eigenvalues:

$$FA = \sqrt{\frac{3(\lambda_1 - MD)^2 + (\lambda_2 - MD)^2 + (\lambda_3 - MD)^2}{2(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}}$$

Fractional anisotropy ranges from 0 to 1: isotropic tissues (e.g. grey matter) are characterized by small fractional anisotropy, whereas white matter is associated with higher fractional anisotropy (typically greater than 0.2). In other words, grey matter is characterized by a more

spherical ellipsoid, with more even diffusion, whereas white matter is characterized by an elongated ellipsoid, with more pronounced diffusion parallel to the tracts. Fractional anisotropy is commonly referred to as a measure of microstructural integrity since it is highly sensitive to microstructural changes or differences in white matter, including myelination and axonal density. Despite that, it cannot characterize the type of structural changes [59]. Mean diffusivity, being the average of the three eigenvalues, is sensitive to the density of tissue barriers in all directions. It is very similar for both grey and white matter, and sensitive to the effects of tissue cellularity, edema and necrosis. Axial and radial diffusivity represent microstructural directions in parallel and perpendicularly to the white matter tracts and, thus, provide more direct measures of microstructural dimensions:

$$AD = \lambda_1 \quad \text{and} \quad RD = \frac{\lambda_2 + \lambda_3}{2}$$

Elevated radial diffusivity may indicate demyelination, according to studies in animal models, whereas decreased axial diffusivity reflects axonal injury [60, 61]. In general, it is strongly recommended that all DTI measures should be investigated in order to better interpret the underlying changes in tissue microstructure [62].

Techniques for analyzing DTI data include voxelwise whole brain analysis and region-of-interest (ROI)-based analysis. In voxelwise analysis, brain images are spatially coregistered across subjects and aligned (normalized) in a common space, followed by statistical testing at each voxel within the brain. The outcome of voxel-based analysis consists of statistical maps, exhibiting spatial “blobs” of significance on the brain map. Possible pitfalls of this method include poor coregistration and normalization, as well as decreased statistical power from multiple comparisons (since statistical significance is tested for every voxel). Conversely, ROI analysis is only performed in specific *a priori* defined ROIs, thus limiting the number of tested voxels and the number of multiple comparisons, which results in higher statistical power. However, ROIs are often defined manually, which is prone to user variability. Automated regional segmentation can overcome this issue, but it is instead prone to the same potential pitfalls of voxelwise analysis (e.g. poor coregistration and normalization). Tractography is another analytical approach for DTI, but it was not employed in this project and, therefore, it will not be further discussed.

Few studies have investigated the ability of DTI to detect structural changes in the white matter adjacent to the olfactory cortex and its main projections in PD patients. All of them have mainly focused on fractional anisotropy and only some on mean diffusivity [63-67]. Their

analytical approaches vary significantly: some have performed both voxelwise and ROI analysis of the olfactory cortex, others have focused only on fiber tractography of the olfactory tract or ROI analysis of the olfactory bulb and the substantia nigra.

Magnetization Transfer

Magnetization transfer (MT) is an MRI contrast mechanism, first proposed by Wolff and Balaban in 1989 [68]. Like DTI, it is used to detect myelin abnormalities, but MT is sensitive to macromolecular content, whereas DTI reflects the structural integrity of white matter. MT is based on the exchange of energy between highly bound protons within semi-solid macromolecules (e.g. myelin) and the mobile protons of free water [58]. The MT contrast is induced by applying a strong off-resonance, radiofrequency pulse, at a frequency far from the free water resonance frequency. This pulse selectively saturates the magnetization of macromolecule-bound protons, which have a very broad frequency spectrum, while leaving the mobile protons of free water (narrow frequency spectrum) relatively unaffected. Thereafter, a fast exchange of magnetization between the pools occurs, leading to partial saturation of free water protons, which in its turn cause a decrease of the observed MRI signal in tissues with natural abundance of macromolecules (e.g. myelin).

The most common measure of the MT effect is the MT ratio, which is traditionally calculated as the relative change in intensity of images acquired with (I_{MT}) and without (I_0) the MT pulse:

$$MT \text{ ratio} = \frac{I_0 - I_{MT}}{I_0}$$

Figure 6 illustrates examples of source images and their corresponding MT ratio map. However, it should be noted that the MT ratio depends on the sequence parameters and is influenced by T1 relaxation and flip angle inhomogeneities. To overcome these limitations, Helms et al. proposed an improved acquisition protocol, where the MT ratio is estimated by three datasets: and MT-weighted, a proton density weighted and a T1 weighted dataset [69].

Increased MT ratio reflects increased macromolecular content. In white matter, the higher

Figure 6: Example of source images and the corresponding MT ratio map. At first, images without the MT pulse are acquired, with little contrast between white and grey matter. After applying the MT pulse, the collected images demonstrate reduction of the MR signal in tissues with natural abundance in myelin (e.g. white matter). Such tissues have higher values in the corresponding MT ratio map.

MT ratio is associated with the presence of myelin. Therefore, MT imaging has been extensively used to investigate white matter disorders (especially demyelinating disorders, such as multiple sclerosis) and to somewhat lesser extent in the examination of grey matter pathologies, as well as brain development and aging [70]. In PD, several studies have shown a decreased MT ratio in the basal ganglia, but so far, no study has employed MT to detect abnormalities in olfactory brain areas of PD patients [71-74].

Functional MRI

The invention of the functional MRI (fMRI) technique in 1990 had a real impact on the development of cognitive science. The application of fMRI in research has blossomed since its inception: from only four fMRI publications in 1992, the publication rate has increased to roughly 6000 fMRI papers per year since 2014. Main advantages of fMRI include: a) the technique's noninvasive nature, b) its high availability, and c) its ability to identify entire networks of brain regions that are activated by specific tasks.

Nevertheless, it is important to note that fMRI does not measure neuronal activity directly. The most common fMRI technique employs the Blood Oxygen Level Dependent (BOLD) contrast in order to make inferences about neuronal activity [75, 76]. Active neurons have higher metabolic demands, causing the release of oxygen from oxyhemoglobin, which then becomes

Figure 7: BOLD Hemodynamic Response Function (HRF) following a brief stimulation (occurring at 0 seconds).

deoxyhemoglobin. Oxyhemoglobin is diamagnetic, whereas deoxyhemoglobin is strongly paramagnetic. Therefore, there are local magnetic field distortions (i.e. signal loss) in and around the blood vessels containing deoxyhemoglobin. The BOLD contrast is related to the regional blood concentrations of deoxy- and oxyhemoglobin. The regional BOLD response to a brief stimulus is known as the Hemodynamic Response Function (HRF). An initial dip in BOLD response is commonly observed, but its exact physiological origin is still controversial. It may reflect an early increase in deoxyhemoglobin or increased local cerebral blood volume. After the initial dip comes an overcompensation in blood flow, resulting in an increased ratio of oxyhemoglobin to deoxyhemoglobin and, thus, increased BOLD response and MRI signal. Interestingly, the peak is commonly delayed, often occurring 4-6 seconds after stimulation. After reaching its peak, the BOLD signal decreases to an amplitude below baseline (post-stimulus undershoot), which is considered to represent a combination of decreased blood flow and increased blood volume. Figure 7 illustrates the typically observed parts of the HRF. However, the exact shape of the BOLD response varies across subjects and brain regions.

Task-based fMRI has been extensively used in studies of attention, perception, imagery, language, working memory, semantic memory retrieval, episodic memory encoding, episodic memory retrieval, priming, and procedural memory [77]. Furthermore, there has been an increased interest in applying this technique at rest. Resting-state fMRI emerged in 1995 when Biswal et al. observed spontaneous, slow temporal fluctuations in the BOLD signal at rest,

which were correlated between functionally related areas [78]. The application of resting-state fMRI has led to the identification of several resting-state brain networks, with the default mode and the salience network probably being the most extensively investigated ones in several neuropsychiatric diseases, including PD [79-82]. Anatomically, the default mode network includes the medial prefrontal cortex and the posterior cingulate cortex, whereas the salience network consists of the insula and the anterior cingulate cortex. In healthy individuals the activity within the salience network increases during cognitive tasks that require attention to external and internal stimuli, whereas the default mode network is suppressed.

The analysis of fMRI data starts with several preprocessing steps, aiming at identifying and removing artifacts and standardizing the location of brain regions across subjects. Brain images are first corrected for motion, then spatially coregistered across subjects and aligned (normalized) in a common space. Thereafter, there are two main, but not exclusive, approaches for the statistical analysis of task-based fMRI data: a) the general linear model (GLM) analysis, and b) the independent component analysis (ICA). Analysis of resting-state fMRI is commonly performed with: a) seed-based functional connectivity, or b) ICA.

In GLM analysis, data are treated as a linear combination of model functions and noise: $Y = X\beta + \varepsilon$, where Y is the acquired fMRI data, X is the design matrix, β is the calculated weighting factors and ε is the error (noise). The design matrix X is defined by the investigator and reflects specific factors (“regressors”) of the tested paradigm. Each regressor is a set of idealized predictions of what the HRF should look like, if a voxel was activated. In other words, GLM is a task-driven analysis, where the HRF needs to be predefined.

On the other hand, ICA is a task-free analytical approach, allowing blind signal separation, without predefining the HRF. ICA allows a model-free decomposition of the variance in the signal, into different activation and artefactual components, including their spatial maps and time courses [83, 84]. Important advantages of ICA compared to GLM include: a) the possible detection of unexpected responses to stimuli, and b) effective denoising, both in terms of random noise, but also in terms of confounding signals (heart pulsation, breathing). However, ICA results depend highly on the number of chosen components (which is defined manually by the investigator). Additionally, deciding which components reflect noise and which components are relevant to the tested paradigm also depends on the investigator, introducing potential bias.

Seed-based analysis requires the *a priori* definition of a ROI, from which the BOLD time course is extracted [85]. Thereafter, this extracted BOLD signal is compared to time courses

from other brain voxels in search of potential temporal correlation. Seed-based connectivity has been popular due to its simplicity and reproducibility. However, this method depends on the *a priori* definition of a seed, which is potentially not the most representative node of a functional network. Furthermore, simultaneous investigation of multiple networks is not possible.

Several studies have employed fMRI in order to identify the neural basis of olfaction in both health and disease [86-89]. Resting-state fMRI has demonstrated that cognitive impairment in PD is associated with altered functional connectivity in the default mode network and dysfunctional coupling between the default mode and the salience networks [81, 90-92]. However, results from previous task-based fMRI studies on PD patients are inconsistent: two studies show declined activity in the olfactory cortex in PD, whereas one study reports hyperactivation of the olfactory cortex in early stages of PD [93-95]. This inconsistency can to certain extent be attributed to methodological diversity, both in the experimental designs and in the analytical approaches.

Collecting reliable olfactory fMRI data can be affected by various factors, both physiological and methodological, such as the participant's respiration, magnetic susceptibility artifacts due to air-tissue interfaces at the skull base, the length of odorous stimulation, and the sampling rate (repetition time, TR) of the fMRI sequence [96-99]. Prolonged odorous stimulation has long been associated with rapid adaptation (decreased BOLD signal) of olfactory brain regions, especially the piriform cortex and the orbitofrontal cortex [97, 100, 101]. To avoid adaptation, most olfactory fMRI studies rely on either event-related designs with short odorous stimulation or block designs with short odorous pulses incorporated in each block of stimulation. Furthermore, fast sampling rate results in more accurate temporal resolution of the BOLD response, which has led to the development of novel acquisition techniques that achieve high temporal resolution by reducing the TR to <1 s [98, 102-106]. Therefore, several factors should be considered when designing an fMRI study for the investigation of neural activity within the olfactory network.

Despite the advances in both olfactory and resting-state fMRI, it is still not fully understood how olfactory dysfunction in PD is reflected in the activation pattern of the olfactory network. Likewise, it is not fully investigated if olfactory dysfunction in PD is associated with potential changes in the functional connectivity within the olfactory, default mode and salience networks.

AIMS

The overall aim of this thesis was to study the structural and functional neural basis of olfactory dysfunction in PD with different imaging methods and to evaluate whether olfactory examination can increase diagnostic accuracy. The specific aim for each of the included studies are presented below.

- I. To compare DaTSCAN imaging and simple olfactory examination in terms of accuracy and efficacy in diagnosing parkinsonian syndromes. To assess the diagnostic contribution of the combined results from DaTSCAN imaging and olfactory examination in separating PD from APS.
- II. To assess whether DTI (including all four DTI measures) and MT can detect changes both in integrity and content of the white matter adjacent to the olfactory cortex and the orbitofrontal cortex.
- III. To establish a robust and reliable fMRI paradigm that can be employed in olfactory fMRI studies in healthy individuals and in patients with neurodegenerative disorders, by investigating how the length of odorous stimulation and the sampling rate influence the activation pattern of the olfactory cortex.
- IV. To apply the methodological findings of Study III in PD in order to elucidate potentially altered activation patterns and functional connectivity within olfactory brain areas.

MATERIALS AND METHODS

All studies were conducted in accordance with the current revision of the Declaration of Helsinki and were approved by the Regional Ethical Review Board in Linköping, Sweden (registration number 2011/415-31 with amendments 2014/392-32 and 2018/144-32). Written consent was obtained by all participants. Study I was conducted at the Department of Nuclear Medicine, Linköping University Hospital. Studies II-IV were conducted at CMIV, Linköping University Hospital.

PARTICIPANTS

Study I

In total, 60 patients were included in data analysis. They were divided into four groups, according to their diagnosis: PD (24 patients), APS (16 patients), SP (5 patients), Non-parkinsonism (15 patients). The latter group consisted of patients with essential tremor, postural tremor or other pathology that was not related to the basal ganglia. Among the patients with APS, 6 were diagnosed with MSA, 4 with PSP, 1 with DLB and 5 with unspecified form of APS. Among the patients with SP, 3 were diagnosed with vascular parkinsonism and 2 with drug-induced parkinsonism. The SP group was too small to be included in the statistical comparisons among groups; it was however included in the comparisons between parkinsonism (all PD, APS and SP patients) and Non-parkinsonism.

Study II

A total of 22 PD patients and 13 healthy controls were included in the study. Two PD patients were excluded from the MT analysis due to motion artifacts. Prior to inclusion in the study, all PD patients underwent the following clinical evaluation: Mini-Mental State Examination (MMSE), Unified Parkinson's Disease Rating Scale (UPDRS), Hoehn and Yahr Staging (H&Y) and Schwab and England activities of daily living scale (S&E). Healthy controls were clinically evaluated only with the MMSE. Inclusion criteria included age less than 79 years and MMSE score above 25. Subjects with history of severe head injury, intracranial surgery,

surgery in the nasal cavity, seasonal allergies, sinusitis or other current respiratory infection, as well as current smoking were excluded from the study.

Study III

A total of 25 healthy participants were recruited in this study. All participants underwent olfactory evaluation prior to fMRI examination; 3 participants were excluded from data analysis due to mildly impaired olfaction.

Study IV

A total of 20 PD patients were recruited from the register of the Department of Nuclear Medicine at the University Hospital of Linköping, Sweden, where they had previously undergone DaTSCAN SPECT examination. All PD patients demonstrated abnormal uptake of ioflupane. 20 age-matched healthy controls were recruited among patients' spouses or friends or through advertisements at the University Hospital of Linköping and at local non-political service organizations. All participants underwent olfactory evaluation prior to fMRI examination. All PD patients underwent clinical evaluation prior to fMRI with MMSE, UPDRS (part III, motor examination) and H&Y.

OLFACTORY EVALUATION

Study I

Prior to DaTSCAN SPECT, all participants were examined with the Brief 12-item Smell Identification Test (B-SIT, Sensonics, Inc., New Jersey, USA). The performance of each patient is presented as B-SIT score, which has a range from 0 to 12. The norms provided by the manufacturer were used in order to classify the patients as normosmic, hyposmic or anosmic in accordance to their sex and age. Prior to the B-SIT examination, all participants were asked to self-evaluate their olfactory ability as impaired or normal.

Study II

Prior to the MRI examination, all participants underwent an evaluation of their olfactory performance with the "Sniffin' Sticks" discrimination test (Burghart Messtechnik GmbH, Wedel,

Germany). Each participant was presented with three odorants, two of which were identical, while the third one was different. The task was to identify the divergent odorant. The test consisted of sixteen triplets of odorants, contained in felt-tip pens (maximal possible score was, therefore, 16).

Studies III and IV

Prior to fMRI, all participants underwent self-administered olfactory examination with the University of Pennsylvania Smell Identification Test (UPSIT, Sensonics, Inc., New Jersey, USA). This test consists of 40 different odorants. The performance of each patient is presented as UPSIT score, which has a range from 0 to 40.

IMAGE ACQUISITION

DaTSCAN SPECT (Studies I and IV)

Imaging was performed in accordance with the recommendations of GE Healthcare, manufacturer of DaTSCAN. Before isotope administration, all patients received 120 mg of sodium perchlorate *per os* to block uptake of iodine in the thyroid. One hour later 185 MBq DaTSCAN was given intravenously; SPECT was performed 3–4 hours after DaTSCAN administration with a dual-head, multi-geometry gamma camera (Millennium VG, GE Healthcare). The two camera heads were positioned as close as possible to patient's head.

Diffusion Tensor Imaging and Magnetization Transfer (Study II)

All MR images were acquired on a 3 Tesla Philips Ingenia MR scanner (Philips Healthcare, Eindhoven, the Netherlands), using a 32-channel head coil. T1-weighted images were acquired and reviewed by a radiologist to ensure that no subject had any morphological abnormalities. The total scan time for the diffusion sequence was 10 minutes and 35 seconds. MT imaging was performed using a method described by Helms et al. [69]. It consisted of three consecutive 3D gradient echo acquisitions: one acquisition was T1-weighted, one was proton-density-weighted, and one intermediate, which contained the off-resonance MT saturation pulse. The total scan time was 6 minutes.

fMRI acquisition (Studies III and IV)

fMRI was performed with a 3 Tesla scanner (Siemens MAGNETOM Prisma, Siemens AG, Erlangen, Germany) using a 20-channel head-neck coil. In Study III, two different multiplex echo planar imaging sequences were used in order to test the effect of TR: a short TR (901 ms, total scan time = 635.205 seconds) and a long TR (1340 ms, total scan time = 635.16 seconds). Additionally, high-resolution 3D T1-weighted and T2-weighted Fluid Attenuated Inversion Recovery (FLAIR) structural scans were acquired in all subjects. T1-weighted images were later coregistered with the functional images. T2-weighted FLAIR images were acquired to ensure that the participants did not have any obvious pathological changes in the brain. The above-mentioned sequence with the short TR was also employed in Study IV.

Olfactory fMRI design (Studies III and IV)

In Study III, natural coffee oil extract (Sigma-Aldrich, St. Louis, USA), diluted (50% v/v) in odorless diethyl phthalate (Sigma-Aldrich) was used as odorant. In Study IV, two odorants were employed: natural coffee oil extract and vanillin (Sigma-Aldrich, St. Louis, USA). Both coffee oil extract (40% v/v) and vanillin (10% w/v) were also diluted in odorless diethyl phthalate. In both studies, stimulation was administered in blocks of events. In Study III, two different stimuli lengths, 6 seconds and 15 seconds, were randomly embedded in one session, which included 10 blocks for each of the abovementioned stimulation lengths; the same task design was repeated for each of the tested TRs (901 ms and 1340 ms). In Study IV, only short stimulation (6 seconds) was employed. In both studies, a 20 seconds long resting period, consisting of odorless air, separated the stimulation blocks from each other. To avoid habituation, each stimulation block consisted of 1 second long odorous pulses, followed by 2 seconds of odorless air. The odorant was delivered simultaneously to both nostrils, using the OG001 Multistimulator (Burghart Messtechnik GmbH, Wedel, Germany), embedded in medical air stream (2.5 l airflow per nostril), through Teflon-tubing (4 mm inner diameter). All subjects were instructed to breathe normally through the nose and avoid sniffing. Figure 8 illustrates the employed stimulation paradigm in both studies. In both studies, all participants were asked to click a button with their index finger every time they could sense the smell of coffee or vanillin in order to objectively confirm odor sensation.

Figure 8: Schematic representation of the fMRI experimental design for Studies III and IV.

IMAGE ANALYSIS

DaTSCAN SPECT

Visual and semiquantitative evaluation of the reconstructed transaxial slices were performed, using the software EXINI dat™ (EXINI Diagnostics AB, Lund, Sweden). The specific ligand binding to putamen and caudate nucleus was calculated, with the occipital lobe serving as reference for non-specific binding. Two ratios were calculated: the ratio between the striatum uptake and the uptake in the occipital lobe, hereinafter mentioned as striatum ratio, and the ratio between the putamen uptake and the caudate nucleus uptake, hereinafter mentioned as putamen/caudate ratio. Additionally, all transaxial SPECT images were visually assessed and the uptake pattern of ioflupane was classified in five different grades, as previously illustrated in

Figure 4: Grade 1 – Burst striatum, Grade 2 – Egg shape, Grade 3 – Mixed type, Grade 4 – Eagle wing, Grade 5 – Normal.

Diffusion Tensor Imaging

Voxelwise, whole brain, statistical analysis of the diffusion data was performed using TBSS (Tract-Based Spatial Statistics), part of FSL 5.0 (FMRIB Software Library, University of Oxford, UK). The voxelwise statistical analysis compared the fractional anisotropy, mean diffusivity, radial diffusivity and axial diffusivity between PD patients and healthy controls, with age, gender and olfactory score serving as covariates. Additionally, a correlation analysis between each DTI measure and the score of the olfactory evaluation was performed, focusing only on PD patients in order to examine if lower olfactory score is associated with diffusivity changes.

Furthermore, ROI analysis of fractional anisotropy, mean diffusivity, radial diffusivity and axial diffusivity between healthy controls and PD patients was performed. ROI analysis was performed on the TBSS-normalized data, focusing on the: (1) anterior piriform cortex, (2) posterior piriform cortex, (3) entorhinal cortex, and (4) orbitofrontal cortex. In order to isolate the tracts of white matter adjacent to these regions, these anatomical ROIs were multiplied with the mean fractional anisotropy skeleton, using the FSL toolbox.

Magnetization Transfer

As with DTI, two analytical approaches were employed in the analysis of MT data: a) voxelwise, whole brain analysis, and b) ROI analysis. Each subject's MT map was aligned into the MNI common space, by using the spatial normalization tool, implemented in SPM12 (Wellcome Trust Centre for Neuroimaging, University College London, London, UK). Further MT processing was performed using the FSL software, including intensity normalization and nonparametric permutation inference. The MT ratios between PD patients and healthy controls were compared with a cluster significance level of $p < 0.05$, corrected with threshold-free cluster enhancement. ROI analysis was performed, using the abovementioned original anatomical brain areas.

Olfactory fMRI (Study III)

Extracting the event related time course of brain activation in four olfactory brain areas was the main analytical approach. Task-driven GLM analysis and model-free tensorial ICA were also performed as supportive analytical tools.

GLM analysis was carried out with SPM12, with the default preprocessing pipeline. Separate GLM analyses were performed for each TR. In both cases, the stimulation blocks of the fMRI paradigm were modeled as regressors of interest, one for each stimulation length (6 seconds and 15 seconds).

ROI analysis was performed on normalized data from the GLM analysis and it included plotting the Finite Impulse Response (FIR) event related time courses for four different olfactory brain areas: the anterior piriform cortex, the posterior piriform cortex, the orbitofrontal cortex and the anterior insula. ROI analysis was carried out with MarsBaR 0.44 toolbox for SPM. Prior to ROI analysis, the point with the peak activity was identified for each participant in each ROI, separately for each combination of TR and stimuli length. The coordinates from all these points were averaged out to identify an average peak point for each ROI for the whole sample. This average point was then used as the center of eight new spherical ROIs (5 mm diameter for anterior and posterior piriform cortex, 10 mm diameter for orbitofrontal cortex and insula). The following three components of all the time courses were calculated separately for each ROI: maximal signal change, time to peak and estimate of oscillations.

Finally, to further confirm the GLM- and FIR-based results, tensorial ICA was carried out with MELODIC version 3.14, part of FSL 5.0. Tensorial ICA allows a model-free decomposition of the variance in the signal, into different activation and artefactual components [83]. The signal was separated into 15 independent components. Each independent component consists of a thresholded spatial map, a time course (temporal mode) and an s-mode (measure of the effect size of each independent component for each participant).

Olfactory fMRI (Study IV)

Three analytical approaches were employed for the analysis of olfactory fMRI: a) model-free tensorial ICA, b) task-driven GLM analysis, and c) extracting the event related time course of brain activation in four olfactory brain areas.

With tensorial ICA, the signal was separated into 20 independent components. Comparison of the s-modes of the two groups was performed to test for differences in the effect size between

PD patients and healthy controls. GLM analysis was carried out with SPM12, with the same preprocessing steps as in Study III. The stimulation blocks were modeled as regressors of interest, one for each odorant (coffee and vanillin). At second level analysis, healthy controls and PD patients were compared separately for each contrast (coffee, vanillin) with a 2-samples t-test, but also with ANOVA for the combination of both contrasts.

Subsequently, the FIR event time courses were plotted for the anterior piriform cortex, the posterior piriform cortex, the orbitofrontal cortex and the anterior insula. Firstly, the point with the peak activity was identified for each participant in each ROI, separately for each odorant, in both the right and the left hemisphere. The coordinates from all these points were used as the center of a small spherical cluster with 2 mm radius, so that a unique sphere was designed for each participant, each odorant and each ROI. At last, the following three components of all the time courses were calculated separately for each ROI: maximal signal change, time-to-peak and area under the curve (only the area and the first 10 seconds of the curve were included).

Resting-state fMRI (Study IV)

Two analytical approaches were employed for the analysis of resting-state fMRI: a) functional connectivity analysis among the four abovementioned olfactory brain ROIs, and b) ICA in order to identify the default mode and the salience network.

For functional connectivity analysis, the CONN toolbox was used (<http://www.nitrc.org/projects/conn>) [107]. An ROI-to-ROI analysis was conducted to examine whether differences in connectivity strength between PD patients and healthy controls were present, with the UPSIT score serving as covariate.

ICA, as implemented in MELODIC version 3.14, part of FSL 6.0, was carried out in an effort to separate the signal into resting-state networks and, then, identify the default and the salience network in this cohort. As opposed to tensorial ICA, which was used in the olfactory fMRI since the stimulus paradigm was consistent among subjects, for the resting-state fMRI the temporal concatenation was employed in order to look for common spatial patterns, without assuming that the temporal response is consistent among subjects. FSL's randomize permutation-testing tool was employed to test for differences between PD patients and healthy controls, with the UPSIT score serving as covariate. Additionally, we tested for potential correlation between the UPSIT score and the recruitment of the default mode and the salience networks.

STATISTICS

All results are presented in the form median value with lower and upper 95% confidence intervals (CI).

Study I

Kruskal-Wallis one-way ANOVA, with Mann-Whitney U Test as *post hoc* test for pairwise comparisons, were employed to investigate potential differences among groups in terms of age, illness duration, B-SIT score and semiquantitative analysis. The analysis of the B-SIT score was cross-examined for possible confounders with ANCOVA, by using age as covariate and sex as additional factor. Fisher's exact test was used to investigate potential differences in sex among groups. Pearson's Chi-square test was used to compare the results of visual assessment of DaTSCAN. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated for the B-SIT score and the visual evaluation. Correlation was assessed by Pearson coefficient (r). Binary logistic regression was employed in order to estimate the predictive value of B-SIT and of the visual assessment of DaTSCAN. Significance was defined at $p < 0.05$.

Study II

Potential differences between groups regarding age, olfactory examination and ROI analysis were investigated with Mann-Whitney U test. Fisher's exact test was employed to investigate potential differences in sex and MMSE score. For the demographic and ROI analysis, significance was defined at $p < 0.05$. For the voxelwise whole brain analysis of DTI and MT, significance was defined at $p < 0.05$, corrected with threshold-free cluster enhancement.

Study III

Repeated measures ANOVA with Bonferroni correction was employed separately for each ROI in order to estimate the effect of stimulation length and TR on the abovementioned components of these time courses: maximal signal change, time to peak (in seconds) and estimate of oscillations. Statistical significance for ROI analysis was set at $p < 0.05$. For the GLM analysis, whole brain analysis was assessed at $p < 0.001$. The default threshold level of $p > 0.5$ was used for ICA.

Study IV

Potential differences between groups regarding age, olfactory examination, response monitoring, the FIR event related time courses and the s-modes of ICA were investigated with Mann-Whitney U test. Fisher's exact test was employed to investigate potential differences in sex and MMSE score. Statistical significance for the analysis of FIR event related time courses was set at $p < 0.05$. For the GLM analysis, whole brain analysis was assessed at $p < 0.001$. The default threshold level of $p > 0.5$ was used for ICA.

RESULTS

STUDY I

There were no significant differences regarding age, sex and illness duration among groups. The median value of B-SIT score in the PD group was 4.0 (95% CI: 3 and 4.5) vs. 6.0 (95% CI: 4 and 9) in the APS group and 8.0 (95% CI: 7 and 9) in the Non-parkinsonism group. There was a significant difference between PD and Non-parkinsonism ($p < 0.01$), but no significant difference between PD and APS or APS and Non-parkinsonism. Age and sex were not significant confounding factors. Based on the B-SIT norms provided with the test, 71% of PD patients were anosmic and 21% were hyposmic vs. 31% anosmic and 38% hyposmic APS patients. There were significantly more anosmic PD patients than anosmic APS patients ($p = 0.016$).

Patients with impaired olfaction (anosmic and hyposmic) were grouped together and were compared to normosmic patients in terms of sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy for B-SIT. The sensitivity of B-SIT in distinguishing parkinsonism (all PD, APS and SP patients included) from Non-parkinsonism was 80%, the specificity 40%, the positive predictive value 80%, the negative predictive value 40% and the diagnostic accuracy 70%.

The distribution of PD and APS patients in the five grades of DaTSCAN uptake is illustrated in Figure 9. All PD patients had abnormal striatal uptake pattern (Grades 1-3), while one APS patient had normal striatal uptake pattern (Grade 5). There were significantly more APS patients with Grade 1 uptake pattern compared to PD patients ($p = 0.025$). In converse, there were significantly more PD patients with Grade 2 uptake compared to APS patients ($p = 0.001$). The majority (80%) of Non-parkinsonism patients were classified as normal (Grade 5). However, 13% were classified as Grade 4 and 7% as Grade 3.

Patients with abnormal striatal uptake of DaTSCAN (Grades 1-4) were grouped together and were compared to patients with normal striatal uptake (Grade 5) in terms of sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy. The sensitivity of DaTSCAN in distinguishing parkinsonism (all PD, APS and SP patients included) from Non-parkinsonism was 96%, the specificity 80%, the positive predictive value 93%, the negative predictive value 86% and the diagnostic accuracy 92%.

Figure 9: Distribution of PD and APS patients in the five grades of DaTSCAN uptake.

Figure 10: Results from the semiquantitative analysis of DaTSCAN. The horizontal upper and lower limits represent the 75th and 25th percentiles. The horizontal line within each box represents the median

The results from the semiquantitative analysis of DaTSCAN are illustrated in Figure 10. There was no significant difference in the striatum uptake ratio between PD and APS. However, there was a significant difference ($p < 0.001$) between PD and Non-parkinsonism as well as between APS and Non-parkinsonism ($p < 0.001$). The putamen/caudate uptake ratio in PD was significantly lower compared to APS ($p = 0.006$) and to Non-parkinsonism ($p < 0.001$).

There was a strong positive correlation (Pearson correlation $r = 0.697$, $p < 0.01$) between B-

SIT and the striatum uptake ratio for the PD group. A weak positive correlation between B-SIT and the striatum uptake ratio was shown even for the APS (Pearson correlation $r = 0.352$, $p = 0.181$) and Non-parkinsonism groups (Pearson correlation $r = 0.408$, $p = 0.131$). There was no correlation between B-SIT and the putamen/caudate ratio for any of the diagnostic groups.

Binary logistic regression was employed to evaluate if the combination of B-SIT and Grade 1 or Grade 2 uptake patterns can increase the predictive value for PD and APS. The rate of overall correctly classified patients was improved by the combination of B-SIT and Grade 1 or Grade 2, as shown in the table below:

Model	Model comparison	Overall correctly classified
1 B-SIT		70%
2 Grade 1 uptake pattern of DaTSCAN		70%
3 Grade 2 uptake pattern of DaTSCAN		77.5%
4 Combination of B-SIT and Grade 1 uptake pattern	$p < 0.02^a$ $p < 0.001^b$	72.5%
5 Combination of B-SIT and Grade 2 uptake pattern	$p < 0.002^a$ $p > 0.05^c$	82.5%

^a Comparison with Model 1

^b Comparison with Model 2

^c Comparison with Model 3

STUDY II

There were no significant differences regarding gender, age or MMSE score between PD patients and healthy controls. PD patients had significantly lower ($p = 0.007$) olfactory score (8 out of 16, 95% CI 7 and 10) compared to healthy controls (11 out of 16, 95% CI 10 and 11).

Voxelwise whole brain statistical comparison between healthy controls and PD patients did not identify microstructural changes in the white matter adjacent to olfactory brain areas. However, PD patients had significantly lower mean diffusivity in the left corticospinal tract and the posterior limb of the right internal capsule. Additionally, PD patients had lower axial diffusivity in the body and the splenium of corpus callosum, in both the anterior and the posterior limb of the internal capsule bilaterally, the frontal component of the left uncinate fasciculus, as

Figure 11: Results from the ROI analysis of the DTI measures. Box-and-whisker diagram, where the whiskers represent the minimum and the maximum values. *: $p < 0.05$

well as the white matter of left gyrus rectus. There were no significant differences between the two groups regarding fractional anisotropy and radial diffusivity. There was no significant correlation between the olfactory evaluation score and any of the four DTI measures within PD patients.

ROI analysis for all DTI measures included the white matter adjacent to four olfactory brain areas: anterior and posterior piriform cortex, entorhinal cortex and orbitofrontal cortex. The white matter adjacent to the left entorhinal cortex had significantly lower mean diffusivity ($p = 0.045$) and axial diffusivity ($p = 0.013$) in PD patients (Figure 11A and 11B). The white matter adjacent to the right orbitofrontal cortex had significantly lower axial diffusivity ($p = 0.024$) in PD patients (Figure 11C). There were no significant differences in the white matter adjacent to the anterior or the posterior piriform cortex for any DTI measure.

As for MT, no voxel survived the threshold of $p < 0.05$ during the voxelwise statistical comparison of the MT maps between PD patients and healthy controls. Similarly, the ROI analysis revealed no significant differences in the MT ratios between the two groups, for any of the abovementioned olfactory ROIs.

STUDY III

During fMRI acquisition, all subjects were asked to verify the presence of odor by pressing a specific button with their index finger. The average of registered responses for the 6 seconds and the 15 seconds stimulations, from both TR sessions, was calculated for each participant. The identification rates were 83% and 97% respectively for the 6 seconds and 15 seconds stimulation.

The FIR event related time courses for the ROIs of the right hemisphere are illustrated in Figure 12. The ROIs of the left hemisphere had similar patterns and are, therefore, not presented here. The short stimulation resulted in an almost typical BOLD response in all four ROIs,

Figure 12: Finite Impulse Response (FIR) event related time courses for the four olfactory brain areas of interest: anterior piriform cortex (A), posterior piriform cortex (B), insula (C), and orbitofrontal cortex (D). The time courses represent percent signal change. Data from the short TR (901 ms) are plotted in blue. Data from the long TR (1340 ms) are plotted in red.

regardless of TR. However, the long stimulation resulted in an oscillating BOLD response: after reaching a peak, the BOLD signal continued with several lower peaks thereafter, without a distinct undershoot below the baseline in most ROIs.

TR had statistically significant effect on maximal signal change for all four ROIs bilaterally, with long TR resulting in lower maximal signal change in all ROIs. Stimulus length only had a statistically significant effect on maximal signal change in the left anterior piriform cortex and right orbitofrontal cortex, with the shorter stimulus length producing a higher peak signal.

Stimulus length had significant effect in time to peak in the anterior piriform cortex and the insula bilaterally as well as in the right orbitofrontal cortex, with short stimulation being generally associated with faster time to peak. TR only affected time to peak in left anterior piriform cortex, with the long TR resulting in shorter time to peak.

TR had significant effect on the number of oscillations for all four ROIs bilaterally, with short TR being able to detect more oscillations compared to long TR. Similarly, the effect of stimulus length on oscillations was significant for most ROIs, except for the orbitofrontal cortex, with long stimulation resulting in a larger number of oscillations.

The GLM analysis demonstrated that the combination of short TR (901 ms) and short stimulation (6 seconds) resulted in visually more extensive activation in olfactory brain areas bilaterally, namely the posterior piriform cortex, the orbitofrontal cortex and the insula. The combination of long TR and long stimulation activated the insula bilaterally and partly the right orbitofrontal cortex, but not the piriform cortex. The left motor cortex was activated in all cases, due to the response-tracking task.

Model free ICA was performed separately for each TR. For the short TR (901 ms), one independent component was associated with a functionally connected network that included the anterior and the posterior piriform cortex, as well as insula and parts of the orbitofrontal cortex (Figure 13). The temporal representation of this component consisted of an oscillation with twenty distinct peaks, coinciding with the olfactory stimulation of the tested fMRI task design. Likewise, for the long TR (1340 ms) one independent component showed a very similar spatial and temporal representation, interconnecting the abovementioned olfactory brain areas (not illustrated here).

Figure 13: Results of the Independent Component Analysis (ICA) for the short TR (901 ms). Color bars are given in terms of T-statistic. IC: Independent Component

STUDY IV

There were no significant differences regarding gender or age between PD patients and healthy controls. PD patients had significantly lower ($p < 0.001$) UPSIT score (median 18.5 out of 40, 95% CI 14 and 23) than healthy controls (median 33 out of 40, 95% CI 32 and 35). As for response tracking, PD patients gave significantly fewer responses for the combination of both odors ($p = 0.028$) and for coffee ($p = 0.041$). There were no significant differences in the number of responses for vanillin or in the mean reaction time for each odor.

All PD patients underwent DaTSCAN SPECT examination prior to inclusion in the study and they all demonstrated abnormal ioflupane uptake. Thirteen PD patients were visually classified as “egg shape”, i.e. bilateral uptake reduction in both putamina and normal or borderline normal uptake from caudate nucleus, whereas seven PD patients were visually classified as “mixed type”, i.e. asymmetrical ioflupane uptake, with reduced uptake in the putamen of one side.

ICA of the olfactory fMRI isolated an independent component that was associated with a functionally connected olfactory network, including the posterior piriform cortex, the insula and the thalamus bilaterally, as well as the right orbitofrontal cortex (Figure 14a). The hand motor area of the left precentral gyrus was also part of this network, due to the response monitoring task. The time course of this component consisted of an oscillation with 21 distinct peaks, coinciding with the olfactory stimulation of the tested fMRI task design (Figure 14b); the first peak occurred prior to olfactory stimulation and was probably associated with the sensation of odorless air at the beginning of the fMRI design. The s-mode values of all participants were

Figure 14: The olfactory network as isolated with Independent Component Analysis (ICA) of the olfactory fMRI. Color bars are given in terms of T-statistic. R: right hemisphere.

compared to test differences in the effect size of this component between healthy controls and PD patients; healthy controls had significantly higher s-mode values compared to PD patients ($p = 0.014$, Figure 14c).

Additionally, ICA isolated a cerebellar functional network, consisting of the posterior and lateral parts of cerebellum bilaterally (Figure 15a). The time course of this component consisted of an oscillation with 21 distinct peaks, coinciding with the olfactory stimulation of the tested fMRI task design (Figure 15b). Comparison of the s-mode values showed that healthy controls recruited this cerebellar network significantly more compared to PD patients ($p = 0.026$, Figure 15c).

Olfactory fMRI was also analyzed with GLM, using two different contrasts: coffee and vanillin. For vanillin, healthy controls showed higher activation in parts of right insula ($p < 0.001$, uncorrected). For coffee, healthy controls showed higher activation in parts of right insula and right orbitofrontal cortex ($p < 0.001$, uncorrected). For the combination of both coffee and vanillin, healthy controls showed higher activation in parts of insula bilaterally and for parts of the right orbitofrontal cortex ($p < 0.001$, uncorrected). There were no significant differences

Figure 15: The cerebellar network as isolated with Independent Component Analysis (ICA) of the olfactory fMRI. Color bars are given in terms of T-statistic. R: right hemisphere.

between healthy controls and PD patients in any of the piriform cortices for any of the contrasts. In all cases (vanillin, coffee and the combination of them) healthy controls showed higher activation in the hand motor area of the left precentral gyrus, as well as the posterior limb of the left internal capsule, due to the response monitoring task.

Lastly, the FIR event related time courses were extracted from the normalized fMRI data, for the following four olfactory brain areas: the anterior and the posterior piriform cortex, the orbitofrontal cortex and the insula. In order to compare the time courses between the two groups, three parameters were assessed statistically: maximal signal change, time-to-peak and area under the curve (only for the first 10 seconds). Healthy controls demonstrated higher maximal signal change compared to PD patients in the right orbitofrontal cortex ($p = 0.007$), the right insula ($p = 0.008$) and the left insula ($p = 0.045$). Healthy controls also demonstrated broader area under the curve compared to PD patients in the right orbitofrontal cortex ($p = 0.044$), the right insula ($p = 0.045$) and the left insula ($p = 0.041$). There were no significant differences for the time-to-peak between subjects. Additionally, potential differences between the two odors were tested for the same three parameters, without yielding any significant differences between coffee and vanillin.

Resting-state fMRI data was analyzed with a functional connectivity analysis and ICA. There were no significant differences between healthy controls and PD patients in terms of functional connectivity among the anterior and posterior piriform cortex, orbitofrontal cortex and insula. The temporal concatenation approach of ICA yielded two independent components, corresponding to the salience and the default mode networks of this cohort. Dual regression analysis, with the UPSIT score as covariate, demonstrated no significant differences between healthy controls and PD patients for any of these networks. There was a tendency for positive correlation ($p = 0.07$) between the UPSIT score and the recruitment of the salience network, however not statistically significant.

DISCUSSION

STUDY I

The results of this study demonstrate that DaTSCAN is more efficient in discriminating parkinsonism from Non-parkinsonism, compared to a simple olfactory test. Moreover, DaTSCAN offers prospect of differentiating PD from APS. However, the combination of DaTSCAN and smell identification testing can increase the rate of correctly classified patients.

Olfactory test

According to this study, a simple identification olfactory test can successfully distinguish PD patients from Non-parkinsonism, which is in line with previous studies [57, 108]. However, this test fails in distinguishing PD from APS or APS from Non-parkinsonism. The different disorders that comprise APS, cannot be regarded as a uniform entity in terms of olfaction, as olfaction in APS varies significantly, from preserved (PSP) to differentially impaired (DLB, MSA). Therefore, a simple olfactory test cannot differentiate APS from PD or Non-parkinsonism.

The B-SIT score had a sensitivity of 80% for discriminating parkinsonism from Non-parkinsonism and an even higher sensitivity of 92% for discriminating PD from Non-parkinsonism. However, its specificity was poor (40%) in both cases. These results are comparable to Deeb et al. (who, however, used UPSIT instead of B-SIT), but not in accordance with other studies where sensitivity and specificity were nearly on the same level [57, 108, 109]. This divergence can to certain extent be explained by cultural biases and odor familiarity. Even though the B-SIT test is a cross-cultural brief smell test, the Swedish population is not familiar with some of the included odors (i.e. wintergreen and lime in this study), resulting in a lower score even for normosmic patients. None of the participants of this study managed to get a perfect score (12 out of 12).

DaTSCAN SPECT

Most PD patients had Grade 2 (egg-shape) uptake pattern, suggesting that dopamine depletion in most PD patients is prominent in putamen, while caudate nucleus retains normal or almost normal function. On the other hand, most APS patients showed Grade 1 (burst striatum) uptake

pattern, suggesting a more severe dopamine depletion both in putamen and in caudate nucleus in APS. Compared to the B-SIT, the visual assessment of DaTSCAN was more efficient in discriminating parkinsonism from Non-parkinsonism in terms of sensitivity (96%), specificity (80%) and diagnostic accuracy (92%). These results contradict Deeb et al., who suggested that a basic smell test is as sensitive as DaTSCAN, but are in accordance with Hong et al. who, by comparing the Cross-Cultural Smell Identification Test and dopamine transporter Positron Emission Tomography, proved that the olfactory test alone has little power in detecting dopaminergic depletion [57, 110].

The semiquantitative analysis of DaTSCAN showed that the striatum ratio is accurate to differentiate PD from Non-parkinsonism, as well as APS from Non-parkinsonism, but it cannot differentiate PD from APS. These results are in accordance with one meta-analysis on the diagnostic accuracy of DaTSCAN and two previously published studies on the same topic [53, 54, 111]. However, the putamen/caudate ratio is greater in APS than in PD, suggesting a more uniform dopamine depletion both in putamen and in caudate nucleus in APS patients; this result concurs with the previously discussed visual assessment. Two more studies have verified a more symmetric loss of dopaminergic nerve terminals in MSA and in PSP compared to PD [112, 113]. This finding could depend on higher severity of clinical symptoms in APS patients at the time of the first clinical evaluation.

There was a strong correlation between B-SIT and the striatum ratio in PD patients, which was line with previous studies, indicating that a lower striatum ratio in a PD patient signals a more severely impaired olfaction [57, 110]. Regression analysis showed that the combination of B-SIT and DaTSCAN had better predictive value, with a higher overall rate of correctly classified PD and APS patients, compared to when these two methods are used separately. The combination of Grade 1 uptake pattern and B-SIT was a significantly better diagnostic model than Grade 1 alone. The addition of B-SIT in Grade 2 uptake pattern also led to a higher rate of correctly classified patients, but this improvement was not statistically significant. In conclusion, B-SIT could be a useful supplementary diagnostic tool, despite its lower diagnostic accuracy compared to DaTSCAN, especially for Grade 1 uptake pattern, which was dominated by APS patients.

Discordant results

Two normosmic PD patients had abnormal DaTSCAN, which is in line with the low specificity of the smell test in detecting parkinsonism. According to a previous study, 26% of PD patients had an olfactory test score within the normal range [26]. One APS patient with clearly impaired olfaction had normal DaTSCAN and can, therefore, be classified as SWEDD. This patient was later diagnosed with cerebellar ataxia (after the study was concluded). Also, one normosmic patient with vascular parkinsonism can be classified as SWEDD; after the publication of the study he was diagnosed with polyneuropathy. These findings support previous indications that SWEDD patients are unlikely of having parkinsonism [52].

Three patients received a diagnosis different than parkinsonism, despite moderately abnormal DaTSCAN uptake (Grade 4) and impaired olfaction. One of them has been since diagnosed with essential tremor. Two of them have not received a certain diagnosis yet, but parkinsonism has been excluded. Dopaminergic deficit has been previously documented in some patients with essential tremor, suggesting a possible predisposition to developing PD [114].

STUDY II

This study investigated the ability of DTI and MT to detect microstructural changes in central olfactory areas of PD patients. To our knowledge, this is the first study in this field including all four DTI measures. ROI analysis of DTI could detect alterations in the white matter adjacent to central olfactory areas in PD, whereas MT could not, suggesting that DTI is more sensitive for this purpose.

Diffusion Transfer Imaging

According to previous studies, PD patients with impaired olfaction present lower fractional anisotropy in the white matter adjacent to gyrus rectus and in the white matter surrounding olfactory areas of the medial temporal lobe [63, 64]. Moreover, the values of fractional anisotropy in the aforementioned areas correlate with the score of the odor identification test [63]. Decreased fractional anisotropy has also been found in the olfactory nerve and in the olfactory bulb of PD patients [65, 67]. However, the skull base is susceptible to artifacts, which could potentially explain why the present cohort did not present significant differences in the fractional anisotropy of olfactory areas. Besides, Skorpil et al. examined their cohort with two

identical DTI series, getting significant differences for their two chosen ROIs only in one of them [67]. Additionally, it is important to highlight that estimating fractional anisotropy is reliable in homogeneous tissues with high orientation coherence, but only 10% of the brain is estimated to consist of such tissues [115, 116]. DTI is, therefore, more accurate when analyzing large, homogeneous fiber bundles, whereas the olfactory cortex consists mainly of small neural tracts.

Whole brain voxelwise analysis demonstrated lower axial diffusivity in the left uncinate fasciculus and in the left gyrus rectus of PD patients. The uncinate fasciculus connects the lateral orbitofrontal cortex with anterior temporal areas, playing a suggested role in decision making, which in its turn is mediated by dopaminergic mechanisms [117, 118]. The function of gyrus rectus remains unclear; it appears to be associated with executive function [119]. This analytical method did not show significant diffusivity changes in any component of the olfactory cortex, which mainly comprises small neural tracts. Furthermore, this analytical approach did not reveal any significant correlation between the olfactory evaluation score and any of the four DTI measures within PD patients.

ROI analysis showed microstructural changes in two olfactory areas, the left entorhinal cortex and the right orbitofrontal cortex. The changes in axial diffusivity can be attributed to potential axonal degeneration in the aforementioned olfactory areas. The interpretation of decreased mean diffusivity in the left entorhinal cortex is more complicated since mean diffusivity is the average of axial and radial diffusivity. ROI analysis did not demonstrate any significant diffusivity changes in the piriform cortex, which can be attributed to the fact that it mainly consists of grey matter.

Magnetization transfer

In the present study, neither voxelwise randomized permutation nor olfactory ROI analysis revealed significant differences in the MT ratio between PD patients and healthy controls, implying a similar number of macromolecules. This in its turn suggests that the amount of myelin does not appear to differ between PD and healthy subjects. It could also indicate a lower sensitivity of the acquisition method. According to previous studies, PD patients demonstrate lower MT ratios in substantia nigra, putamen, and thalamus [71-74]. None of these studies used normalization and whole brain voxelwise morphometry for data analysis; they instead performed ROI localization and extraction on single-subject level. This could be an indication that whole

brain voxelwise morphometry is inappropriate for MT analysis.

STUDY III

The main findings of this study showed that fast sampling rate is associated with more pronounced relative signal increase, compared to slow sampling rate. Long stimulation was associated with longer time to peak signal and oscillatory BOLD response. On the other hand, fast sampling rate and short stimulation resulted in a more typical BOLD response.

Sobel et al. demonstrated that odorous stimulation results in an early and sharp activation within the piriform cortex, followed by a rapid decrease of signal after continuous stimulation, explaining why early fMRI studies yielded small or no activation in the olfactory cortex [100]. Poellinger et al. tried to evaluate the effects of short (nine seconds) and long (sixty seconds) continuous olfactory stimulation in olfactory related brain areas [97]. Similar to the findings presented here, nine seconds stimulation consistently activated the piriform cortex, the entorhinal cortex and the amygdala, whereas sixty seconds stimulation resulted in an oscillating response in these brain areas, followed by a prolonged decline below baseline. However, in Poellinger's experiment the orbitofrontal cortex showed a sustained increase in activation after long stimulation. Our fMRI paradigm differs in terms of both stimulus length and interstimulus interval, which can to certain extent explain the discrepancies between Poellinger's experiment and our study. A more recent study by Li et al. confirmed the findings of Sobel and Poellinger and additionally demonstrated habituating activity (decreased BOLD signal) in the left orbitofrontal cortex [101]. To bypass the negative effects of long, continuous, olfactory stimulation, a broad range of olfactory fMRI studies employ stimulation in blocks of events, lasting twenty seconds or longer. Nevertheless, our study indicates that odor administration in blocks of events may still cause an oscillating response, when the blocks last for 15 seconds.

The effects of sampling rate on the temporal properties of the HRF have long been studied, showing that the accuracy of HRF peak time determination increases with short TR [98]. Several novel acquisition techniques have, therefore, been developed, aiming to increase the sampling rate of the BOLD signal and indicating significant benefits of collecting fMRI data with $TR < 1$ s [102-106]. In particular, faster TRs achieved with multi-slice echo planar imaging sequences (as the sequence used in this study) can capture more information per time unit, allowing more accurate representation of the BOLD response [120]. In contrast, with longer TR

the response is averaged over longer time, which could lead to missing the actual peak and thereby lower signal increase and longer time to peak [106]. This study confirms that a short TR value (901 ms) is associated with more pronounced signal increase in all four olfactory brain regions, by giving more densely sampled information of the BOLD response.

An inherent limitation of the GLM approach is the employment of the canonical HRF for all brain regions. However, the activation pattern of several brain regions may vary significantly from this *a priori* specified pattern of signal change, and this could also be applicable to the four olfactory brain regions studied here. This confounding factor can partly explain the variability in olfactory fMRI literature. To mediate this problem, we opted for a sequence with short TR, allowing fast sampling rate and, hence, more accurate estimation of the BOLD response.

STUDY IV

ICA of olfactory fMRI isolated an olfactory and a cerebellar network, with significantly lower recruitment in PD patients compared to healthy controls. According to the task-driven GLM analysis, PD patients demonstrated decreased activation of the insula bilaterally and the right orbitofrontal cortex, but not in the olfactory cortex itself. The analysis of resting-state fMRI did not yield any significant differences in the connectivity within the olfactory, the salience and the default mode networks, between the two groups.

Olfactory fMRI

As most odorants differentially stimulate both the olfactory and the trigeminal nerve, choosing stimulants for olfactory fMRI needs to be thorough. Vanillin is generally considered as a purely olfactory stimulant [121]. Coffee has both olfactory and trigeminal components. Both vanillin and coffee were considered as familiar and pleasant odorants by the participants of this study. The analysis of the FIR event related time courses showed no significant differences between these two odors in the olfactory brain areas studied here, despite the fact that these odors have different evocative properties.

Response monitoring during the olfactory fMRI was employed as an objective measure of chemosensation during the experiment. PD patients gave significantly fewer responses compared to healthy participants during the whole session (for both coffee and vanillin), as well as for coffee alone. There was no significant difference for vanillin, which could possibly be

explained by two factors: a) the purely olfactory nature of this odorant, b) the lower concentration of vanillin compared to that of coffee, making the detection of vanillin difficult even for healthy participants. The absence of significant differences in the mean reaction time between the two groups indicates that the significantly different number of responses cannot be attributed to PD patients' motor deficit.

ICA is considered to be superior of GLM when there is uncertainty about the position and the timing of activity, due to condition-dependent, brain region-dependent, or subject-dependent variations [122]. In this study, ICA was able to detect a functional network that consisted of both the olfactory cortex (posterior piriform cortex) and its main projections (insula and thalamus bilaterally, right orbitofrontal cortex), and it coincided temporally with the design of the olfactory fMRI. Additionally, PD patients demonstrated significantly lower recruitment of this network (lower s-mode values) compared to healthy controls. GLM and FIR event related time course analysis showed decreased activation in main olfactory projections (insula bilaterally and right orbitofrontal cortex), but no significant differences in the olfactory cortex itself. Hence, ICA was more effective for detecting olfactory impairment in this cohort, in both the olfactory cortex and its main projections.

Cerebellar activation due to olfactory stimulation, as well as olfactory impairment in patients with cerebellar lesions have been previously described [123-126]. Cerebellum is considered to be involved in the control of sniffing, and thereby it may also play a role in higher-order olfactory processing [127]. Sobel et al. have demonstrated odor-induced activation posteriorly and laterally in the cerebellar hemispheres, independently of sniffing, supporting the argument that sniffing is more than a merely stimulus carrier [123]. Additionally, a recent study in hyposmic and anosmic patients shows correlation between the recruitment of the cerebellar network and the olfactory function scores of the patients [128]. Interestingly, PD patients demonstrate both sniffing and olfactory impairment [129]. This study verifies the recruitment of a cerebellar network during olfactory fMRI, and indicates that its recruitment is weaker in PD patients.

There is currently a limited number of task-induced fMRI studies focusing on olfactory dysfunction in PD. Their task-designs differ considerably and their results are to some extent contradictory. Two studies have shown reduced activity in the amygdalo-hippocampal complex in PD patients, both with pleasant and unpleasant odors [93, 94]. However, Moessnang et al. have shown a profound hyperactivation of the piriform and the orbitofrontal cortices in early stages of PD [95]. These three studies employed solely GLM analysis. Both ICA and GLM

analysis in this study indicate that olfactory impairment in PD is in line with decreased activation in the olfactory cortex and its main projections in the brain.

Resting-state fMRI

There has been evidence that olfactory processing deactivates the default mode network in task-induced fMRI, but there is no previous resting-state fMRI study on whether the olfactory impairment in PD correlates to decreased recruitment of the default and the salience network [130]. ICA of the resting-state data from this cohort identified two networks that corresponded with the salience network and the dorsal default mode network. Dual regression analysis, with the UPSIT score serving as covariate, did not reveal any significant differences between PD patients and healthy controls in recruiting these two networks. There was, however, a tendency for positive correlation between the UPSIT score and the recruitment of the salience network. It is important to highlight that PD patients of this study were examined with the MMSE and they did not present any cognitive impairment. Potentially co-morbid depression was not explicitly examined.

ICA of the resting-state fMRI did not identify an olfactory network. The analysis of functional connectivity between the olfactory cortex (anterior and posterior piriform cortex) and two of its main projections (insula and right orbitofrontal cortex) was tested with a different toolbox, which did not yield any significant differences between the two groups. However, PD patients demonstrated impaired olfaction, which was verified both from UPSIT and from the results of olfactory fMRI. Therefore, one could assume that the functional connectivity within the olfactory network should also be impaired. However, potential changes in the connectivity within the olfactory network appeared to be subtle, at least in this cohort, and resting-state fMRI could not detect them.

LIMITATIONS

In Studies I, II and IV, potentially incorrect classification of patients cannot be totally ruled out since the only gold standard for the diagnosis of parkinsonism is *post mortem* examination of the brain. In fact, one patient with APS and one with SP from Study I received different diagnosis (other than parkinsonism) after the conclusion and publication of the study. Notably, both these patients had normal DaTSCAN uptake.

In Study I information about UPDRS and Hoehn and Yahr scale was not documented in the medical journals of all patients since it is not standard practice; analysis of covariance based on this classification was consequently not possible. However, the results of Study I are in line with other previously published studies.

Studies II and IV were conducted on a relatively homogeneous group of patients, with a clinical diagnosis of PD, verified olfactory dysfunction and absence of cognitive impairment. As with most MRI studies in this field, the cohorts of Studies II – IV are small and the results should be interpreted cautiously.

Standardization and reproducibility of the study protocol were of high importance, in all MRI studies (Studies II – IV). Therefore, standardized analytical methods were employed, including the default pipeline of SPM12 and FSL. Additionally, the ROIs were chosen from open-access anatomical atlas or in accordance with a previously reported statistical localization of the human olfactory cortex [15]. These two factors can to a certain extent explain the inconsistencies among previous studies.

Respiration-triggered event-related fMRI designs are proven to yield a stronger activation of the olfactory cortex compared to fixed-timing odor delivery [131]. Nevertheless, Studies III-IV were conducted with a less complex experimental design, with fixed-timing odor delivery. In Study IV, the lack of significant differences between PD patients and healthy controls in the activation of olfactory cortex, when analyzed with the GLM, could be partially attributed to the fixed-timing odor delivery; a combination with visual cuing prior to odor presentation could potentially be beneficial in future studies [95].

CONCLUSIONS

Overall, the studies presented here show evidence of disease-related structural and functional changes in olfactory brain areas of patients with PD. Moreover, olfactory tests can be a beneficial supplementary tool in the clinical examination of patients with parkinsonism.

Study I

The semiquantitative analysis of DaTSCAN SPECT is an accurate diagnostic tool to differentiate parkinsonism from Non-parkinsonism. Additionally, the visual assessment of DaTSCAN together with the uptake ratio between putamen and caudate nucleus can offer valuable aid in differentiating PD from APS. A smell identification test has lower diagnostic value than DaTSCAN in differentiating parkinsonism from Non-parkinsonism. Olfactory identification test is efficient in detecting PD, but it cannot successfully detect APS, and its specificity is low. Nonetheless, the combination of smell identification test and DaTSCAN imaging has a higher predictive value than these two methods separately.

Study II

By employing ROI analysis on normalized DTI data, decreased axial and mean diffusivity was found in olfactory areas of the temporal and frontal lobes, specifically the left entorhinal cortex and the right orbitofrontal cortex. DTI analysis did not reveal any significant alterations of the diffusivity of the white matter adjacent to the piriform cortex in PD patients. MT analysis was not able to show any significant differences between PD patients and healthy controls. Hence, DTI with ROI-focused analytical approach, including all four DTI scalars, appears to be more suitable in detecting disease-related microstructural changes in the white matter adjacent to central olfactory areas in PD.

Study III

The findings of this study support the choice of fast sampling rate and short stimulation length in order to achieve maximum signal increase and short time to peak in olfactory fMRI. These

two parameters should be taken into consideration during the design of olfactory fMRI studies, both in healthy subjects and in patients with impaired olfaction.

Study IV

ICA of olfactory fMRI shows evidence that olfactory impairment in PD is associated with significantly lower recruitment of the olfactory network. GLM analysis revealed significant differences between PD patients and healthy controls in the activation pattern of olfactory projections (insula and right orbitofrontal cortex), but not in the olfactory cortex itself. Hence, ICA was more effective than GLM for studying the differences in the olfactory activation pattern between PD and healthy controls in this cohort, which could be attributed to brain region-dependent, subject-dependent or thresholding variations. Resting-state fMRI did not detect any significant changes in the functional connectivity within the olfactory network of PD patients without cognitive impairment.

FUTURE PERSPECTIVES

Olfactory dysfunction, not being a unique feature of PD, is common in a broad spectrum of disorders, such as dementia and depression, apart from being attributed to purely organic reasons related to the nasal cavity. The olfactory fMRI paradigm that has been established for this thesis, could be applied on patients with such disorders, in an effort to identify functional alterations in the olfactory networks in different cohorts, which in its turn would allow a comparison among PD, dementia and depression in terms of olfactory recruitment. A more ambitious approach would be to evaluate olfactory dysfunction as a screening tool for parkinsonism, dementia and depression. To achieve this, patients with objective olfactory loss could be screened and followed in a period of 2 to 5 years in order to evaluate how many of them will develop any of these disorders.

On a different note, other novel MRI sequences could be applied on PD, in an effort to visualize disease-related structural changes in the central nervous system. Such sequences with, for the time being, limited application on PD, can measure tissue intrinsic properties and quantify the blood-brain barrier permeability. The latter would serve as an interesting evaluation of the Braak hypothesis, due to its potential to verify blood-brain barrier breakdown in PD.

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APPENDIX

Publications

The publications associated with this thesis have been removed for copyright reasons. For more details about these see:

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