Sniffing out Parkinson's disease

Psychophysical and neurophysiological studies of impaired olfactory information processing in Parkinson's disease

Sanne Boesveldt

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Sanne Boesveldt

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“To explain the mind, we have to show how minds are built from mindless stuff, from parts that are much smaller and simpler than anything we'd consider smart.”

Marvin Minsky – The Society of Mind, 1985

“Everything will be okay in the end. If it's not okay, then it's not the end.”

Anonymous

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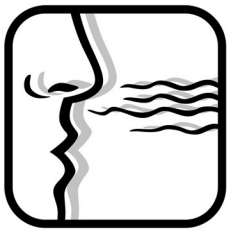
General introduction



PARKINSON'S DISEASE

Parkinson's disease (PD) is a neurodegenerative movement disorder, first (officially) described by James Parkinson in 'An Essay on the Shaking Palsy' published in 1817.[1] This essay was based on merely six cases he had observed in his own practice as well as on walks around his London neighbourhood. Parkinson coined the term *paralysis agitans* (shaking palsy) and described the affected individuals as "having involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forwards, and to pass from a walking to a running pace: *The senses and intellect being uninjured"*. The French neurologist Jean-Martin Charcot recognized the importance of Parkinson's work four decades later. He emphasized that tremor need not be present in the disorder, added a fourth symptom, muscular rigidity, to the clinical picture and suggested that the disease be named 'Parkinson's disease'.[2] In current clinical practice, tremor, rigidity, brady/hypokinesia and loss of postural reflexes are still regarded as the four cardinal motor symptoms of PD. This symptom complex is commonly known under the name of parkinsonism.

It was many years after Parkinson's essay before the basal ganglia were first recognized by Meynert in 1871 as being involved in disorders of abnormal movement.[3] In 1913, the German neurologist Lewy reported specific abnormalities in the brains of individuals with PD.[4] At autopsy, he found cytoplasmic inclusions, now widely recognized as the pathological hallmark of the disorder and referred to as Lewy bodies. Soon thereafter, the Russian pathologist Tretiakoff was the first to emphasize the importance of the substantia nigra when he reported a loss of pigmented cells in this midbrain nucleus in PD patients.[5] Although involvement of other brain stem nuclei such as the locus coeruleus was reported in studies in the ensuing decades,[6;7] pathology in the substantia nigra was regarded to be most constant and severe.[8] In 1958, Carlsson and colleagues observed high dopamine concentrations in the basal ganglia, and suggested that PD might be associated with a dopamine deficiency in the striatum.[9;10] This speculation was confirmed by the studies of Hornykiewicz [11] and Sourkes [12] in PD patients, and in the mid-1960s considerable evidence was gathering in favour of the existence of a nigrostriatal dopaminergic pathway, involved in the regulation of motor behaviour. Up to then, pharmacological treatment was largely limited to the administration of anticholinergic agents, but now introduction of the first exceptionally successful pharmacological treatment of PD followed closely; Birkmayer and Hornykiewicz,[13] and almost concurrently Sourkes and Barbeau,[12] conceived the idea of administering levodopa, a precursor of dopamine, to patients with PD, with spectacular results. The treatment of PD evolved in the following years, with optimization of administration regimens by Cotzias et al.,[14] as well as with the introduction of decarboxylase inhibitors. By then, the concept of dopamine as a neurotransmitter had

reached mainstream status and the nigrostriatal pathway had even become a model for the study of central synapses.

The prevailing view of PD over the rest of the 20th century was that of a movement disorder of unknown aetiology, principally associated with the degeneration of dopaminergic neurons in the substantia nigra and resulting in low levels of dopamine in its projection areas in the basal ganglia. Yet, in the meantime, evidence was accumulating that PD is actually a *multisystem* disorder, clinically characterized not only by motor deficits, but also by a wide range of non-motor disturbances such as autonomic dysfunction, sleep disturbances, cognitive deficits and olfactory dysfunction.[15]

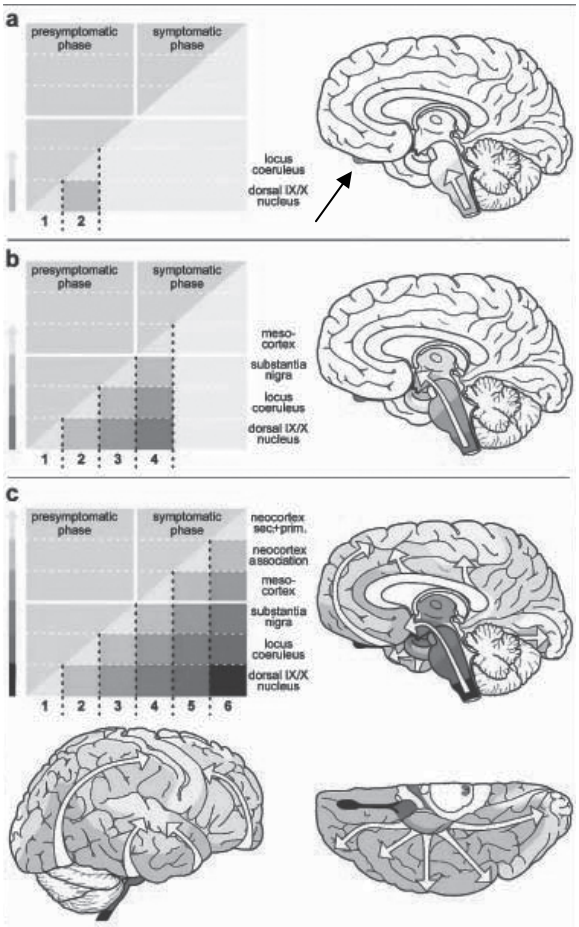
Neuropathology of Parkinson's disease

The concept of PD as a multisystem disorder was strongly driven by novel neuropathological insights gained from a meticulous study by Braak and co-workers (see Figure 1). According to their neuropathological PD staging system,[16] brain pathology evolves following a predictable topographical sequence over the course of the disease. The neuropathology of PD is characterized by Lewy bodies and Lewy neuritis,[17] which are protein aggregates containing (among other substances) alpha-synuclein.[18] PD pathology starts with Lewy body inclusions and Lewy neurites within the projection neurons of the dorsal IX (glossopharyngeal) and X (vagal) motor nuclei, the medulla oblongata, and the anterior olfactory nucleus (stages 1 and 2). During stages 3-4 the severity of the lesions increases and neuropathology spreads to the midbrain (in particular the substantia nigra, pars compacta, and locus coeruleus) and to the temporal mesocortex and allocortex. Besides the progressive damage in subcortical and mesocortical structures at stage 5, the olfactory areas are severely affected, and at stage 6, involvement of nearly the entire neocortex can be seen, in association with clinical cognitive dysfunction.[16;19]

During the first pathological stages, clinical motor symptoms are not apparent yet, but sensory dysfunction or autonomic failure can be present.[20-24] Postmortem studies have reported alpha-synuclein pathology in the olfactory bulb of up to 20% of 'healthy' subjects over 55 years,[25;26] suggesting that the presence of these changes may mark the preclinical phase of PD. By the time motor symptomatology arises, as many as 60% of the dopaminergic neurons projecting to the putamen already have been lost.[27;28]

The most striking observation here is that the olfactory system may be among the induction sites of the neuropathological process in PD disease, and not the substantia nigra. Consequently, the olfactory system is an interesting research focus in PD, e.g. as a tool for future early diagnosis as well as to gain insight in the pathophysiology of PD.

Figure 1. Ascending stages of brain pathology in idiopathic Parkinson's disease (PD).



A. In stage 1, first lesions appear in the *olfactory bulb, anterior olfactory nucleus* (see arrow), and dorsal motor nucleus of the vagal nerve. From stage 2, lesions are present in the so-called gain setting nuclei: the locus coeruleus, gigantocellular reticular nucleus, and caudal raphe nuclei.
B. In stage 3, pathological changes reach the amgygdala, the cholinergic nuclei of the basal forebrain, and the pars compacta of the substantia nigra. In stage 4, the anteromedial temporal mesocortex is the first cerebral cortical area to become involved. At this juncture, the presymptomatic phase probably yields to the symptomatic (i.e., clinically evident) phase of PD.
C. Higher order cortical association areas become involved in stage 5, followed by first-order association areas and primary fields in stage 6.
Increasing degrees of grey shading indicate growing severity of the lesions.

(Reproduced with permission from Braak et al. J Neural Transm 2003;110:517-536.)

THE OLFACTORY SYSTEM

Human olfaction, although a unique sense, is still poorly understood. This could be due to the lack of a widely accepted odour classification or to inadequate instrumentation for measuring olfactory function, but also to the low value people give to smelling. Nevertheless, olfactory (dys)function plays an important role in safety and quality of life, adequate nutritional intake and social pleasures.
Odour perception begins with stimulation of dendritic neurons in the olfactory mucosa of the nasal cavity. Odorant molecules bind to receptors on these neurons, which have axons passing through the cribriform plate of the ethmoid bone to synapse in the olfactory bulb. The olfactory bulb projects via the olfactory tract to five separate areas of the cerebral

hemisphere. The first three, the anterior olfactory nucleus, the olfactory tubercle and the amygdala, are part of the limbic system, and are thought to be involved in odour memory and the emotional processing of odours. The projection to the pyriform cortex (the primary olfactory cortex), may be important for olfactory perception and odour discrimination. Unlike other primary sensory cortical areas, the input to the pyriform cortex is not relayed through the thalamus. The last area to receive input from the olfactory bulb is the entorhinal cortex, which projects to the hippocampal formation, a structure important in memory, and to the orbitofrontal cortex, which might play a role in the conscious perception of smell and odour discrimination.[29;30]

The identification of a large family of odorant receptor genes by Linda Buck and Richard Axel in 1991,[31] for which they were awarded with the Nobel prize in Physiology or Medicine in 2004, led to a revival of scientific interest in what has often been referred to as the neglected sense. Since then, giant leaps have been made in the understanding of olfactory transduction mechanisms and the organization of the olfactory system. In parallel, assessment techniques of olfactory function in humans have rapidly become more sophisticated.

Psychophysical testing of olfactory function

In order to reliably assess olfactory function in clinical practice, many psychophysical tests have been developed that provide a quantitative measure of olfactory function. The University of Pennsylvania Smell Identification Test (UPSIT) and the "Sniffin' Sticks" are the most widely used. The UPSIT is a 40-item, forced-choice odour identification test, originally developed for the US population.[32] The "Sniffin' Sticks" is a multimodal olfactory test battery that can be used to assess three different aspects of olfactory function: Odour identification, discrimination and detection,[33] each consisting of 16 items.

Odour detection threshold testing measures the lowest concentration of an odorant that can be perceived by a subject. It is generally determined by the administration of increasing dilutions of *n*-butanol or phenylethyl alcohol (PEA) in a single staircase design. Odour identification testing involves the perception and naming of an odour presented, most often in a forced-choice format. An odour discrimination task measures the ability to differentiate between a set of odorants, generally by selecting the odd odour out of a series of odorants, all of which are identical except for one, without the need to name or recognize the odour. Odour recognition memory can be tested by presenting subjects with a set of odours and then, after an interval, requesting the subject to pick the target odorant from a series of odours presented. So far, it remains unclear whether these aspects are truly separable or represent a common mechanism. In a few studies a principal component analysis has been performed, which yielded a primary component on which both odour identification and discrimination (and detection thresholds) loaded.[34;35]

When measuring olfactory function, there are a number of confounding factors and selection criteria that should be taken into account. Firstly, confounding of olfactory data by non-specific cognitive factors is a risk that can vary considerably. For instance, working memory and attention can be critical when assessing olfactory discrimination or odour recognition memory, whereas language capacity is involved during identification testing (for review see [36]). Cognitive deficits should therefore be ruled out before testing, or taken into account when interpreting olfactory data. Furthermore, the type of odours employed should preferably activate only the first (olfactory) cranial nerve, and not also the fifth (trigeminal) nerve, as this could cloud the interpretation of acuity data. Reduced olfactory acuity may, at least theoretically, affect performance on other olfactory tasks and thus lead to an underestimation of the actual performance on the olfactory task in question. It has been argued that olfactory detection thresholds should therefore always be assessed in addition to the specific olfactory modality under consideration and used in appropriate statistical analyses to correct for impairments in odour detection.[37]

A few environmental and demographic factors that can influence olfactory test scores are (a history of) smoking, medication, sex, age and exposure to toxic environmental agents. Decrements in smell are known to increase with age; especially after the 6th decade there is an age-related decline in olfactory function that is superior to the effects of sex or smoking.[33;38-40] Smoking is reported to have a negative effect on odour identification in a dose-related manner. However, return of function will occur when smoking is given up, corresponding to the amount of prior smoking and duration of cessation.[38] Findings on the relationship between sex and olfactory function indicate that women in general have a better sense of smell then men.[38;39]

Functional brain imaging of the olfactory system

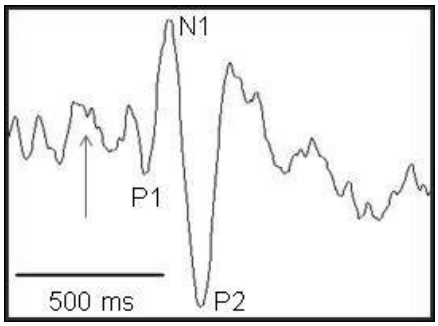
Functional imaging techniques could provide more objective methods to assess olfactory function. Olfactory information processing has been studied with electroencephalography (EEG), magnetoencephalography (MEG), positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) techniques.

Both fMRI and PET are imaging techniques that measure neuronal activity indirectly by means of changes in cerebral blood flow or metabolism, with a high spatial resolution. Olfactory brain regions identified by these techniques are highly correlated to known anatomical data, such as the pyriform cortex, orbitofrontal cortex, insular cortex, the amygdala and other parts of the limbic system that are involved in olfactory information processing.[41-49]

EEG measures electrical potential differences across the scalp that reflect the underlying neuronal activity of the brain, in particular synaptic activity (excitatory and inhibitory postsynaptic potentials) of cortical pyramidal neurons. It is a non-invasive technique with a high temporal, but limited spatial resolution, and easy to employ in a clinical setting.

When the brain processes a stimulus, two types of changes in the EEG may occur: Evoked activities, which are exactly time-locked to the stimulus, and induced activities, which are changes in the EEG that are not phase-locked to the stimulus. The most basic approach to study the induced effects of olfactory stimulation was taken by Moncrieff in 1962.[50] He presented healthy subjects with different odours while recording their EEG, and found that several odours reduced alpha activity, although probably not as a result of an olfactory-specific response but more likely due to arousal effects. Subsequent studies using EEG have found both increases and decreases of spectral power in almost all frequency bands upon olfactory stimulation.[51-59] Much of the variation in these studies can probably be attributed to differences in EEG recording techniques and conditions, and in the type and quality of odours presented. Measurement of olfactory event-related potentials (OERPs) involves averaging of brain activity recorded from EEG electrodes following the presentation of odours using a so-called constant-flow olfactometer,[60] avoiding trigeminal nerve stimulation and assuring a steep stimulus onset that is not detectable for the subject. In 1966, Finkenzeller, and in 1967, Allison and Goff first described cerebral potentials, which they assumed to be of olfactory origin.[61;62] Measurement of OERPs has since become a useful method to quantify olfactory function in a manner relatively independent of subjective biases (for review see [63]). When analyzing the averaged, stimulus-locked EEG signal, a number of waveforms can be identified (see Figure 2): The N1/P2 component of the event-related potential is generally used to assess sensory function,[63;64] whereas the later P3 component is considered a cognitive component.[65]

Figure 2. Typical example of an olfactory event-related potential, averaged over 160 trials in a single subject in response to H_2S, recorded from midline position Cz.



Arrow is stimulus onset; N1P2 amplitude is approximately 20μV.

The largest amplitudes after olfactory stimulation are found between 250-750 ms at positions Cz and Pz (parietocentral recording sites), and the trigeminal activation is mainly located at position Cz. Upon trigeminal stimulation, latencies are shorter, and amplitudes

larger than those evoked by pure odorants.[66] Analyzing these two types of chemosensory responses separately is crucial, since they are recorded from similar areas on the skull and might therefore interfere with each other. Murphy et al.[67] provided normative data for the OERP across the human life span: OERPs showed smaller N1/P2 and P3 amplitudes and longer latencies with increasing age. Furthermore, interindividual differences in OERP latencies should be taken into account.[63;68] When evaluating the clinical significance of OERPs, the presence of an OERP indicates the presence of olfactory function, whereas absence of an OERP in subjects with intact olfactory function, as determined by psychophysical testing, has no diagnostic value.[69]

MEG measures the magnetic field generated by electrical currents from active neurons in the brain, allowing for functional imaging of the brain's electrophysiology at a millisecond temporal resolution. In contrast to EEG, it is a reference-free method and is not impaired by distortions created by the conductivity of the scalp.[70] A recent MEG study using frequency analysis combined with a beamforming technique (a method to improve spatial resolution by reducing noise), reported olfactory event-related desynchronization in the beta and gamma band in the right precentral gyrus, frontal gyri, and the superior parietal lobe gyrus.[71] Olfactory evoked magnetic fields have been found at latencies comparable to OERPs, bilaterally in the anterior-central parts of the insula, the parainsular cortex, the superior temporal sulcus,[72-75] and near the orbitofrontal sulcus.[76] These findings are to some extent supported by anatomical regions known to be involved in olfactory processing.[30;48;77]

OLFACTORY DYSFUNCTION IN PARKINSON'S DISEASE

Olfactory deficits in Parkinson's disease were first empirically documented in 1975 by Ansari and Johnson,[78] who found higher odour detection thresholds in 45% of PD patients. Over the ensuing years it has become clear that most PD patients have olfactory disturbances that are not restricted to a single functional modality but include impairments of odour detection, discrimination and identification.[79-85] The reported prevalence of the odour identification deficit in PD patients ranges between 50-90%.[80;85;86] Nevertheless, only a third of PD patients have a subjective impairment of the sense of smell.[85] The olfactory impairment is generally considered to be bilateral, and unrelated to disease stage and duration or the use of dopaminergic medication.[79;83-85;87]

At present, it is quite firmly established that olfactory dysfunction is already present in early stages of the disease,[79;81] and also occurs in first-degree relatives of PD patients, possibly as a first sign of incipient PD.[24;88] The results of a prospective study in asymptomatic first-degree relatives of PD patients using a combination of smell testing and single-photon emission computed tomography (SPECT) scanning to assess

nigrostriatal dopaminergic function indicate that otherwise unexplained olfactory deficits in this population are associated with a 10% risk of developing PD within two years.[23;89] Further support for this finding comes from a German study in which 7% of a group of subjects with idiopathic hyposmia had newly developed clinical PD symptoms four years from baseline.[90] These findings in selected populations were recently confirmed in the Honolulu Asia Aging Study, a large epidemiological cohort study involving 8006 men. In this population, deficits in olfactory function were associated with both an increased risk of future PD and an increased likelihood of incidental Lewy bodies at autopsy.[91]

Olfactory (dys)function in other neurodegenerative disorders

In contrast with the severe olfactory impairments in PD, olfactory function in most other degenerative movement disorders is either spared or only mildly affected. In progressive supranuclear palsy and corticobasal degeneration, for example, olfactory acuity and odour identification are intact,[86;92] whereas multiple system atrophy patients show mild to moderate hyposmia.[93;94] The observed differences in olfactory function between the various neurodegenerative movement disorders suggest that smell testing might be applied to discriminate between PD and other parkinsonian syndromes in routine clinical practice.

Also in Alzheimer's disease (AD), the presence of olfactory impairments is a well-established feature, and in individuals with mild cognitive impairment, odour identification deficits have also been reported and constitute a risk factor for later conversion to AD.[95;96] The olfactory deficits in AD appear to be similar in frequency and severity to those observed in PD.[97;98] Nevertheless, there appear to be slight differences in the development of the olfactory impairments over time.[99] These differences in olfactory function could be of assistance in differentiating between PD and AD in an early stage.

PATHOPHYSIOLOGY OF OLFACTORY IMPAIRMENTS IN PARKINSON'S DISEASE

The pathophysiology underlying the olfactory deficits in PD is far from being elucidated. According to the Braak staging system, the olfactory bulb, tract and anterior olfactory nucleus may be among the induction sites of PD pathology.[16;19] In later pathological stages, the olfactory bulb and tract are among the brain regions where Lewy bodies and Lewy neurites are particularly abundant.[16] In prior pathological studies, neuronal loss had been observed in the anterior olfactory nucleus of PD patients.[100] Considering that loss of dopaminergic neurons in the substantia nigra is an important pathological feature of PD, the possibility of a relationship between the olfactory impairments and the dopaminergic deficit in PD may seem likely. However, there is little evidence to support this hypothesis. For instance, patients with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced

parkinsonism show dopamine deficiencies, however without a decreased sense of smell.[101] Furthermore, the use of dopaminergic medication does not appear to influence the olfactory deficit in PD.[79] Most striking, however, is a recent study showing that the number of dopaminergic neurons in the olfactory bulb in PD is not reduced but, instead, doubled in comparison to age-matched controls.[102] The authors suggest that this remarkable increase in the number of dopaminergic neurons may be a factor in the pathophysiology of the olfactory deficit in PD, because of the known inhibitory effect of activation of dopamine D2 receptors in the olfactory bulb on synaptic input to the bulb.[103]
Clearly, the above-described pathological data point in different directions and leave many questions regarding the pathophysiology of olfactory impairments in PD unanswered. Ultimately, we need to know how the known pathological changes contribute to the clinical olfactory deficits observed in PD. Therefore, olfactory imaging studies, structural as well as functional, are necessary to provide additional information.
Structural imaging studies have revealed a disruption of the olfactory tract,[104] but no abnormalities of olfactory bulb volume in PD patients.[105] A recent functional MRI study pointed to yet other brain areas that may be involved in PD-related olfactory dysfunction: After olfactory stimulation, neuronal activity in the amygdala and hippocampus was lower in PD patients when compared to control subjects.[106] Also, impaired sniffing may contribute to the olfactory deficit in patients with PD, although actively increasing sniff vigour only slightly improved their odour identification scores.[107] When applying electrophysiological techniques to measure olfactory function, OERP latencies, but not amplitudes, were found to be prolonged in PD patients when compared to controls, whereas the trigeminal system was not affected by the neuronal degeneration.[80;108]
So far, MEG studies of olfactory information processing have not been performed in PD patients. However, a number of recent studies have used advanced time-series analysis techniques of resting-state MEG data to show changes in both local neural synchrony, as measured using calculations of spectral power, and functional connectivity between brain areas in PD.[109-111] Furthermore, these advanced analysis techniques have proven their use in the analysis of resting-state data in a number of other neurological conditions.[112-115] Similar techniques can also be applied to study task-related MEG data,[115;116] and might be promising to gain more insight in the neurophysiological parameters of olfactory information processing in both healthy subjects and PD patients.

RESEARCH QUESTIONS AND THESIS OUTLINE

In the previous sections, it was emphasized that olfactory dysfunction is a common symptom of PD, that may be among the earliest clinical manifestations of this disorder. However, still little is known about the (relative) involvement of the various specific

olfactory modalities, the optimal test to use for diagnostic purposes or the underlying neurophysiological basis of the olfactory dysfunction in PD.
In this thesis the following research questions are addressed:

- What is the prevalence and nature of impairments in the different specific olfactory modalities in PD and how do they relate to other (motor and non-motor) disease characteristics?
- Which (combination of) olfactory test(s) is best in discriminating PD patients from control subjects?
- Is it possible to explore the neurophysiological basis of olfactory (dys)function by means of MEG in healthy controls and PD patients?

To reliably determine the prevalence of olfactory dysfunction in PD patients, normative olfactory values for a matched control population are necessary. Normative values for the "Sniffin' Sticks" test battery, are available for the German population, based on over 3000 subjects.[117] However, two of the three elements in this test battery are culture-dependent. In **section I**, we provided normative values for the Dutch population over 45 years for the two culture-dependent components of the "Sniffin' Sticks" test battery: Odour identification and odour discrimination.
In **section II**, we describe the results of a number of studies aimed at elucidating the nature and prevalence of impairments in the various olfactory modalities, comparing PD patients with two different control populations: Healthy young subjects, and healthy age-matched subjects. In addition, we studied the relationship between performance on odour identification and odour discrimination tasks, and other disease characteristics, including motor function, cognition, autonomic function, depressive symptoms, sleep, and psychiatric complications, in order to determine whether olfactory (dys)function might contribute to the phenotypic characterization of PD patients. The last chapter of this section focuses on determining what the best (combination of) olfactory test(s) is to distinguish between PD patients and control subjects.
Section III describes the results of two neurophysiological studies. The initial aim was to explore the potential of recording olfactory event-related brain activity to serve both as a biological marker of impaired olfactory function in PD and as a means to study the pathophysiology of these olfactory deficits. In the first study, we determined the number of chemosensory stimuli needed to obtain an optimal signal-to-noise ratio by means of EEG. A subsequent pilot study applying these results to MEG showed that olfactory event-related magnetic fields could not be obtained very consistently in all individuals, not even in healthy subjects (*unpublished observations*). Therefore, we chose to focus on time-series analyses of MEG data instead, in particular on functional interactions between brain areas, as a means to gain more insight in the neurophysiological basis of olfactory information processing deficits in PD. We describe the results of the first MEG study to

report on the effects of olfactory stimulation on spectral power and functional connectivity in both healthy subjects and PD patients.

Lastly, the general discussion combines the data presented in the various chapters of this thesis and provides a consideration of the potential implications as well as future research perspectives.

Section I

Psychophysical testing in healthy subjects

Chapter 1

Odour identification and discrimination in Dutch adults over 45 years

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ABSTRACT

Aim The aim of the study was to establish normative values for the two culture dependent components (odour identification and odour discrimination) of the "Sniffin' Sticks" test battery in the Dutch population over 45 years of age, and to assess the influence of age and sex on olfactory function in this population.

Methods This study was performed in 150 healthy Dutch subjects (87 male and 63 female, mean age 59.2 years, range 45-78 years). Olfactory performance was assessed using the odour identification and discrimination parts of the "Sniffin' Sticks" test battery.

Results In women, odour discrimination scores declined significantly with age, whereas there was no effect of age on odour discrimination performance in men. For odour identification, there were no effects of age or sex in this population. A moderate correlation was found between identification and discrimination test scores.

Conclusion Provisional population-specific normative data for olfactory testing using the identification and discrimination parts of the "Sniffin' Sticks" olfactory test battery have been established for the Dutch population over 45 years of age. The current data are applicable to the clinical evaluation of patients with olfactory disorders.

INTRODUCTION

The prevalence of olfactory dysfunction in the general population depends on how it is defined. Subjective impairments of the sense of smell are present in 1.4% of US adults.[118] When using psychophysical tests of olfactory function, approximately 15% of the population can be classified as having mild to moderate hyposmia and around 5% as being anosmic.[119;120] With increasing age, the prevalence of hyposmia increases.[117;121;122] However, it is important to realize that there may be a difference between physiological age-related loss ("presbyosmia") and excessive or unexplained loss of olfactory function in older age. A recent study [123] suggests that true presbyosmia is only a minor component of age-related olfactory impairments. In this study, much of the commonly observed age-related decline in olfactory function appeared to be associated with other age-related factors such as use of medication.

Olfactory dysfunction can also be an early sign of a neurodegenerative disorder, in particular Parkinson's disease [81;85] or Alzheimer's disease.[99] In Parkinson's disease, hyposmia may even be a prodromal sign, preceding the development of the characteristic motor features such as tremor and slowness of movement.[89;124] Assessment of olfactory function in the elderly using validated tests is therefore bound to become an important element of early diagnostic strategies in neurodegenerative disorders.[125]

In order to reliably assess olfactory function, many psychophysical tests have been developed that provide a quantitative measure of olfactory function (for review see [126]). The University of Pennsylvania Smell Identification Test (UPSIT) and the "Sniffin' Sticks" are the most widely used. The UPSIT is a 40-item, forced-choice odour identification test, developed for the US population.[32] The "Sniffin' Sticks" is an olfactory test battery that can be used to assess three different aspects of olfactory function: Odour identification, discrimination and detection.[33] Normative values for the "Sniffin' Sticks" have been established in various populations.[117;127;128] While odour threshold values are not culture dependent,[129] performance on odour identification (and discrimination) tests relies on prior exposure to and familiarity with the odours.[130] This could severely limit the tests' validity in other cultures or populations. For instance, recently published normative values for the "Sniffin' Sticks" in a Greek population [128] were clearly different from those in a previously published German study.[117]

The present study was initiated to establish normative values for the two culture dependent components (odour identification and odour discrimination) of the "Sniffin' Sticks" in the Dutch population over 45 years of age, and to assess the influence of age and sex on olfactory function in this population.

METHODS

Subjects

This study was performed in 150 Dutch subjects (87 male and 63 female, mean age 59.2 years, range 45-78 years), who did not have a history of major olfactory or neurological disorders. The age range was chosen to enable evaluation of olfactory function in (mostly) elderly patients with (suspected) neurodegenerative disorders. All participants were volunteers recruited among employees and partners of patients from the outpatient clinics of the Departments of Neurology of the VU University Medical Center (VUMC; n = 70) and the Leiden University Medical Center (LUMC; n = 80). All subjects provided written informed consent. The study was approved by the Medical Ethics Committees of the VUMC and the LUMC.

Olfactory function testing

The "Sniffin' Sticks" test battery (Burghart, Wedel, Germany) is an olfactory test battery comprising reusable felt-tip pens ('sticks') containing odorants dissolved in propylene glycol which the subject has to sniff. Olfactory tests were administered birhinally in a quiet, well-ventilated room to avoid any background smell interfering with the test odours.

Odour identification was measured by presenting 16 odorants in suprathreshold intensity, in a multiple (4)-forced choice format with verbal descriptions. Each stick was held approximately 2 cm in front of the nostrils for 2-3 sec, with an interval of 20-30 sec between each stick. In the odour discrimination test, subjects were blindfolded and presented with 16 odour-triplets, with an interval of 30 sec between each triplet. Each triplet consisted of two identical and one aberrant odorant. Subjects were asked to select the odd odour out of the three odorants presented, without the need to recognize or name the odours.

In both tests, olfactory scores were defined as the number of correct responses (0-16). The test odours and their response choices are listed in Table I a and I b.

Data analysis

To verify that there were no differences in olfactory test scores between the two sites of recruitment (VUMC, LUMC), data from the two centres were compared using the univariate general linear model UNIANOVA, with 'recruitment centre' as factor, and corrected for 'age' (covariate) and 'sex' (factor).

To explore the influence of sex and age on olfactory function, olfactory test scores were submitted to linear regression analysis by means of a GLM UNIANOVA with 'sex' as factor, 'age' as covariate and the interaction 'age*sex'. Analyses were performed for odour identification and odour discrimination separately.

Subsequently, the 95% lower bound of the individual prediction interval of the linear regression lines for each of the olfactory tests plotted against age was used to determine cut-off values for men and women separately in six age-groups (45-49 years, 50-54 years, 55-59 years, 60-64 years, 65-69 years, ≥ 70 years). When the regression lines for men and women coincided, the combined regression line was used to calculate cut-off values. The 95% prediction interval used indicates that 95% of the population with a specific age will have a test score within the computed interval.

Pearson correlation coefficients were computed to determine the correlation between identification and discrimination scores, both overall and for men and women separately.

To determine which items were best identified, the percentage of subjects that had responded correctly was calculated for each item of the identification and discrimination tasks.

Data were analyzed using SPSS 15.0 for Windows (Chicago, IL, USA).

Table I a. Odour identification items.

	Target	Alternative response choices	% correct responses
Odour 1	Orange	Blueberry, Strawberry, Pineapple	85.3
Odour 2	Leather	Smoke, Glue, Grass	88.7
Odour 3	Cinnamon	Honey, Vanilla, Chocolate	71.3
Odour 4	Peppermint	Chives, Fir, Onion	96.0
Odour 5	Banana	Coconut, Walnut, Cherry	94.7
Odour 6	Lemon	Peach, Apple, Grapefruit	58.0
Odour 7	Liquorice	Caramel, Chewing gum, Biscuit	75.3
Odour 8	Turpentine	Mustard, Rubber, Menthol	38.7
Odour 9	Garlic	Onion, Sauerkraut, Carrot	83.3
Odour 10	Coffee	Cigarette, Wine, Candle smoke	84.7
Odour 11	Apple	Melon, Peach, Orange	48.7
Odour 12	Cloves	Pepper, Cinnamon, Mustard	91.3
Odour 13	Pineapple	Pear, Plum, Peach	70.7
Odour 14	Rose	Chamomile, Raspberry, Cherry	81.3
Odour 15	Aniseed	Rum, Honey, Fir	88.7
Odour 16	Fish	Bread, Cheese, Ham	99.3

RESULTS

Odour identification scores were not significantly different between centres (VUMC mean identification score = 12.5; LUMC = 12.6; F [1,146] = 0.24, p = 0.625). The same was true for the discrimination scores of subjects tested at the different centres (VUMC mean discrimination score = 11.2; LUMC = 11.7; F [1,146] = 1.37, p = 0.244). Furthermore, there was no significant age difference between men (mean age 59.3 years) and women (mean age 59.1 years; t = 0.23, p = 0.822).

Table I b. Odour discrimination items.

	Target	Distracter	% correct responses
Odour 1	Octylacetate	Cinnamonaldehyde	81.3
Odour 2	*n*-Butanol	2-Phenylethanol	68.0
Odour 3	Isoamylacetate	Anethole	76.0
Odour 4	Anethole	Eugenol	78.7
Odour 5	Geraniol	Octylacetate	74.7
Odour 6	2-Phenylethanol	Isoamylacetate	87.3
Odour 7	(+)-Limonene	(+)-Fenchone	80.7
Odour 8	(-)-Carvone	(+)-Carvone	44.0
Odour 9	(-)-Limonene	Citronellal	62.7
Odour 10	2-Phenylethanol	(+)-Menthol	72.0
Odour 11	(+)-Carvone	Geraniol	70.0
Odour 12	*n*-Butanol	(-)-Limonene	85.3
Odour 13	Citronellal	Linalool	54.7
Odour 14	Pyridine	(-)-Limonene	68.7
Odour 15	Eugenol	Cinnamonaldehyde	70.7
Odour 16	Eucalyptol	α-Ionone	67.3

Odour identification

The mean identification score (± SD) of men and women combined was 12.6 ± 2.3; for men only this was 12.5 ± 2.3, and for women 12.7 ± 2.2 (Table II). There was no significant interaction effect between age and sex (F [1,146] = 1.59, p = 0.209), nor was there a main effect of age (F [1,147] = 0.50, p = 0.480) or sex (F [1,147] = 0.29, p = 0.590).

Table II. Descriptives and parameter estimates of the regression lines for identification and discrimination scores plotted against age (in years) of men and women.

	Identification			Discrimination		
	Male	Female	All	Male	Female	All
Mean	12.5	12.7	12.6	11.4	11.5	11.4
SD	2.3	2.2	2.3	2.2	2.5	2.3
Intercept	11.97	15.99	13.62	11.46	22.11	15.80
b coefficient	0.008	-0.056	-0.018	-0.001	-0.179	-0.074
R^2	< 0.001	0.033	0.003	< 0.001	0.273	0.055
p value	ns	ns	ns	ns	< 0.001	0.004

ns = non-significant.

Regression analysis revealed no significant decline in identification scores with increasing age in men (regression coefficient b = 0.008, p = 0.798) or women (b = -0.056, p = 0.157) (Table II). Furthermore, the regression lines for men and women were not significantly different from each other (F [2,146] = 0.94, p = 0.392). No age effects were found when data of all subjects were pooled (b = -0.018, p = 0.473), therefore a horizontal line through the overall mean identification score was used to determine the 95% lower bound of the

individual prediction interval in order to calculate cut-off values for hyposmia. The 95% cut-off value for hyposmia based upon all subjects was 8.81 (see Table III). Ten subjects (6.7%; three women, seven men) scored below the 95% lower bound of the individual prediction interval for identification scores (Figure 3).

Items that were best identified by the Dutch subjects were 'fish' (99.3% correct) and 'peppermint' (96.0% correct). 'Turpentine' was least often identified correctly (38.7% correct identification), followed by 'apple' (48.7% correct) (Table I a).

Table III. Cut-off values for hyposmia based upon the 95% lower bound of the individual prediction interval of the linear regression lines for identification (ID) or discrimination (DIS) scores plotted against age, for both sexes.

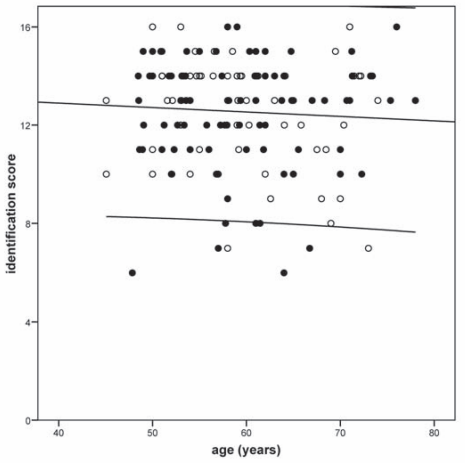
	Cut-off value 95% ID male	Cut-off value 95% ID female	Cut-off value 95% DIS male	Cut-off value 95% DIS female
45-49 years	8.81	8.81	7.76	9.99
50-54 years	8.81	8.81	7.76	9.15
55-59 years	8.81	8.81	7.76	8.28
60-64 years	8.81	8.81	7.76	7.38
65-59 years	8.81	8.81	7.76	6.46
≥ 70 years	8.81	8.81	7.76	5.11

Odour discrimination

The mean odour discrimination score of men and women combined was 11.4 ± 2.3; for men only this was 11.4 ± 2.2, and for women 11.5 ± 2.5 (Table II). There was a significant interaction effect between age and sex ($F [1,146] = 12.98$, $p < 0.001$): A decrease in discrimination scores with increasing age was found for women ($b = -0.179$, $p < 0.001$), but not for men ($b = -0.001$, $p = 0.962$) (Table II). Furthermore, the regression lines for men and women were significantly different from each other ($F [2,146] = 6.56$, $p = 0.002$). For men, a horizontal line through their mean discrimination score was used to determine the 95% lower bound of the individual prediction interval in order to calculate cut-off values for hyposmia. The 95% cut-off value for hyposmia for men was 7.76 (Table III). For women, the 95% lower bound of the individual prediction interval of the regression line was used to calculate the cut-off values for hyposmia (Table III).

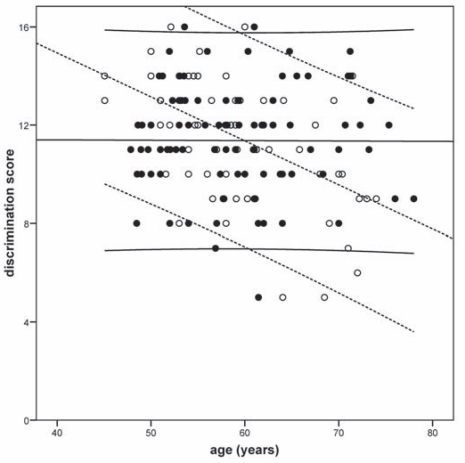
A total of six subjects (4.0%; four women, two men) scored below the 95% lower bound of the individual prediction interval of the regression lines for discrimination scores (Figure 4).

Figure 3. Identification scores plotted against age.



● Male subjects; ○ Female subjects; the solid lines are the combined regression line and 95% limits of the prediction interval for all subjects.

Figure 4. Discrimination scores plotted against age.



● Male subjects; ○ Female subjects; the solid lines are the regression line and 95% limits of the prediction interval for male subjects; dotted lines are the regression line and 95% limits of the prediction interval for female subjects.

Odour combinations that were best discriminated by the Dutch subjects were 2-phenyl ethanol with distracter isoamyl acetate (87.3% correct), and *n*-butanol with distracter (-)-limonene (85.3% correct). The odour combination with target (-)-carvone and distracter (+)-carvone was least often discriminated correctly (44.0% correct), followed by citronellal with linalool as distracter (54.7% correct) (Table I b).

Correlation between identification and discrimination scores

Identification and discrimination scores were only moderately correlated (Pearson correlation coefficient r = 0.30, p < 0.001). When analyzed separately for men and women, a moderate correlation was found in men (r = 0.35, p = 0.001) but not in women (r = 0.23, p = 0.071). At the individual level, two subjects (1.3%, one male: identification score = 8, discrimination score = 5; one female: identification score = 7, discrimination score = 8) had a deviant score on both tests.

DISCUSSION

The present study provides normative data for routine clinical use of the identification and discrimination parts of the "Sniffin' Sticks" olfactory test battery in the Dutch population over 45 years of age. Effects of age and sex were observed for discrimination scores, but

not for identification scores. Furthermore, only in men a moderate correlation between identification and discrimination task performance was found.

The normative data and cut-off values established for the Dutch population in the present study are comparable to the German normative data for subjects over 55 years,[117] but lower than the values recently reported for the Greek population.[128] Although Katotomichelakis et al. suggested that climatological differences would be the most likely explanation for the differences between the Greek and German populations, there are no clear data to support their hypothesis.[131] Since performance on olfactory tasks is dependent on familiarity with the odours [130] and eating habits,[132] the differences in odour discrimination and identification performance between the Greek population on the one hand and the German and Dutch populations on the other hand might alternatively be explained by a more important role of odours in the Greek cuisine. The odour discrimination and identification tasks of the "Sniffin' Sticks" test battery mainly make use of odours related to food and spices, and could therefore give the Greek population an advantage over the Dutch and German populations.

In the present study, there was no influence of sex on odour identification scores in healthy controls aged between 45-78 years. Although women have previously been shown to outperform men on tests of olfactory function,[121] data from two recent studies using the "Sniffin' Sticks" [117;133] indicate that the influence of sex on identification performance may not necessarily be a consistent finding. Hummel et al. found the sex difference to be age-related, and only present in subjects under 55 years of age.[117] The present data confirm that there is no sex-effect on odour identification scores in older adult subjects, at least when using the "Sniffin' Sticks".

In the present population of subjects over 45 years of age, we were unable to confirm the age-related decline in identification scores that has been reported previously.[117;121] The current results are in agreement with the results of two recent studies in which there were no significant age-related differences between subgroups of older subjects.[133;134] In the latter study, using "Sniffin' Sticks", an age-related difference in odour identification scores could only be demonstrated when comparing younger age groups (under 36 years) with older age groups (36 years and up).[133] Apparently, the age-related decline in identification scores measured using the "Sniffin' Sticks" per decade is small, and can therefore only be demonstrated in samples with a broad representation of all ages.[117;121;128;133;135] Another factor that may explain the discrepancy with earlier studies, is the difference in sample size between the present study and some of the previous studies.[117;121]

Odour discrimination performance in the present study was related to age in women, but not in men. In a very large sample, Hummel et al. found odour discrimination performance to decline more rapidly with increasing age than identification performance.[117] In addition, women's discrimination scores tended to decline more than those of men in the age

groups 36-55 years and > 55 years. In those over 55 years of age, there was no difference in mean odour discrimination score between men and women. The present data obtained in a smaller sample are largely in accordance with these findings.

Previously, Doty et al. found a correlation between identification and discrimination test scores of 0.59,[34] and proposed that both olfactory modalities load on a primary component. In the present study, only in men a moderate correlation (0.35) between identification and discrimination scores was found, suggesting that the odour discrimination task assesses a different aspect of olfactory function than the identification task. The differences with respect to the effects of age and sex on the two olfactory test scores in the current study seem to strengthen this notion. Several imaging studies provide additional anatomical evidence for this concept, demonstrating that olfactory functions are mediated by common as well as task-specific regions in the brain.[45] Specifically, a PET study showed distinct areas to be active during odour discrimination (hippocampus) and identification (Broca's area and left inferior frontal lobe).[42] Combining all of these data, we hypothesize that odour identification and odour discrimination tests involve at least partly differential components of olfactory information processing.

Cognitive status is an important factor in olfactory function; odour identification may be considered a semantic memory task, whereas odour discrimination draws more on working memory (for review see [36]). Variations in cognitive function are inevitable in the general population and may therefore influence olfactory function. The aim of the present study was to establish normative values applicable to the general population. Therefore we did not correct for variations in cognitive function, but did exclude individuals suffering from a neurological disorder. It is therefore unlikely that the presence of disease-related cognitive dysfunction could have negatively influenced olfactory test scores.

Data obtained in a recent study [123] suggest that the actual physiological age-related decline in olfactory function (presbyosmia) is probably smaller and more gradual than previously assumed. The authors argue that the commonly observed age-related decline in olfactory function results to a large degree from age-related factors, such as use of medication or (a history of) nasal disease, that each independently affect olfactory function.[123] Furthermore, smoking is generally reported to be adversely associated with olfactory function in a dose-related manner.[136;137] Clearly, 'pure' normative values based upon selected healthy, non-smoking subjects are valuable in showing the true effect of aging on olfactory function. However, when olfactory testing is used in a clinical setting, e.g. to screen for neurodegenerative diseases, it is important to avoid unnecessarily high proportions of false positives (subjects with impaired olfactory ability from other causes). In this situation, normative values based upon a non-selected heterogeneous population as established in this study are more appropriate.

Conclusion

In conclusion, provisional normative values for the identification and discrimination parts of the "Sniffin' Sticks", as well as cut-off scores for hyposmia, are now available for the Dutch population over 45 years of age. Although normative values for younger subjects are also recommended, the current results are applicable to the clinical evaluation of patients with olfactory disorders, including those with olfactory dysfunction after head trauma or (sino)nasal surgery. They can also be used to quantify olfactory function for medico-legal purposes. Future applications may include the incorporation of olfactory testing into screening strategies for incipient neurodegenerative disorders.

Section II

Psychophysical testing in Parkinson's disease

Chapter 2

Prevalence of smell loss in Parkinson´s disease – a multicenter study

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ABSTRACT

Aim Previous data on the prevalence of olfactory dysfunction in Parkinson´s disease (PD) range from 45% to 90%. The present multicentre study aimed to provide data on the prevalence of smell loss in a large sample of PD patients from three independent populations.

Methods Olfactory sensitivity was tested in 400 patients from Australia, Germany, and the Netherlands by means of a psychophysical olfactory test, the "Sniffin' Sticks", which is comprised of three subtests of olfactory function.

Results Out of the total number of patients 45.0% presented as functionally anosmic, 51.7% were hyposmic, whereas only 3.3% were normosmic. This indicates that 96.7% of PD patients present with significant olfactory loss when compared to young normosmic subjects. This figure falls to 74.5%, however, when adjusted to age-related norms.

Conclusion Thus, olfactory dysfunction should be considered as a reliable marker of the disease.

INTRODUCTION

There is convincing evidence from numerous studies using both psychophysical [85;138] and electrophysiological approaches [108;139;140] that olfaction is markedly reduced in Parkinson´s disease (PD). Data on the prevalence of olfactory dysfunction in PD however, range from 45% and 49% in the pioneering studies of Ansari and Johnson,[78] and Ward,[84] respectively, up to 74% in the work of Hawkes et al.[80], or as high as 90% in a study published by Doty et al.[85] These differences may be due to the type of olfactory test used, sample size, normative data used, and age distribution which varied between these investigations. The aim of the present study was to more accurately estimate prevalence of olfactory loss in PD using a large sample of PD patients from three independent populations. Olfactory function should not only be investigated with an odour identification test, therefore other tests of olfactory function were used additionally, namely odour threshold measurements and the (non-verbal) subjects' ability to discriminate odours at suprathreshold concentrations.

METHODS

Subjects

A total of 400 patients with PD were included in the study (mean age, 64.3 years; range, 33-85 years; 137 women and 263 men) for retrospective analysis. Disease duration ranged from 6 months to 30 years (mean 6.6 years). Patients presented with a mean "Unified Parkinson's Disease Rating Scale III" (UPDRS III) [141] score of 22.7 (range, 5-63; mean Hoehn and Yahr (H&Y) stage, 2.25; range I-IV).

This study population comprised patients from public and private movement disorders clinics in Brisbane, Australia (n = 164; mean age, 67.0 years; mean duration of the disease, 7.1 years; mean UPDRS-III 25.2), the Department of Neurology at the University of Dresden Medical School, Germany (n = 161; mean age, 62.8 years; mean duration of the disease, 6.4 years; mean UPDRS-III 28.7), and the Department of Neurology, VU University Medical Center, Amsterdam, The Netherlands (n = 75; mean age, 61.5 years; mean duration of the disease, 6.8 years; mean UPDRS-III 19.3). Consecutive patients were included in the study. Apart from a few newly diagnosed cases United Kingdom Parkinson's Disease Society Brain Bank Diagnostic Criteria for PD [142] were applied for all patients. Alternatively, DATScan or F-DOPA-PET imaging was performed. Previous studies [143] suggest that olfactory function may differ between subgroups of PD patients depending on their dominant movement symptoms. For this reason, where possible, we classified patients as either akinetic-rigid, tremor dominant or mixed depending on the presence of a dominating symptomatology.

Patients with a history of major sinonasal disease or known cognitive impairment were excluded from the study. Dementia screening was not performed.

Participants underwent a standardized psychophysical olfactory test, the "Sniffin' Sticks".[33;144;145] Odorants were presented in pen-like odour dispensing devices. For odour presentation the pen's cap was removed by the experimenter for approximately 3 sec; then the pen's tip was placed approximately 2 cm in front of both nostrils.

Olfactory function testing

Testing was performed bilaterally. It involved tests for odour threshold, discrimination, and identification (duration of testing was approximately 30 min). *Odour thresholds* for butanol or phenylethyl alcohol were assessed using a single-staircase, 3-alternative forced-choice procedure. Sixteen dilutions were prepared in a geometric series starting from pure 4% butanol, or phenylethyl alcohol (dilution ratio 1:2 in aqua conservans), respectively. This approach was used as previous research had shown that odour thresholds obtained with either odour are comparable.[146] Three pens were presented in a randomized order, with two containing the solvent and the third the odorant at a certain dilution. The subject's task was to identify the odour-containing pen. Reversal of the staircase was triggered when the odour was correctly identified in two successive trials for a total of 7 reversals. Threshold was defined as the mean of the last four staircase reversal points. Subjects' scores ranged between 0 and 16. In the *odour discrimination* task, triplets of pens were presented in a randomized order, with two containing the same odorant and the third, a different odorant. Using a 3-alternative forced choice technique, subjects had to determine which of three odour-containing pens smelled differently. A total of 16 triplets were tested. When measuring odour thresholds and odour discrimination, subjects were blindfolded to prevent visual identification of some of the odorant-containing pens. *Odour identification* was assessed by means of 16 common odours. Using a multiple forced choice design, identification of individual odours was performed using a list of four descriptors. Again, the subjects' scores ranged from 0 to 16 (for details see [33;144;145]).

Results of the three subtests were presented as a composite "TDI score" (range 0-48) which was the sum of the results obtained for threshold, discrimination, and identification measures.[40;147] Using this measure, olfactory abilities can be classified in terms of functional anosmia (< 16), hyposmia, and normosmia.[40;123] Apart from an absolute definition of the presence of hyposmia with a TDI < 30.5 (based upon healthy subjects aged between 18-35 years), in the present study definitions of hyposmia were also used in relation to the subjects´ age. For example, for male subjects aged over 55 years the definition of hyposmia applies to a TDI score ≤ 19.75. These definitions of hyposmia are based on the results of a previous multicenter study in over 3000 healthy subjects.[117] In contrast, the range of TDI scores which characterize functional anosmia have been

established in patients with proven anosmia.[40] Thus, the TDI score of 15.5 represents an age-independent cut-off score that separates functional anosmia from hyposmia.

Data analysis

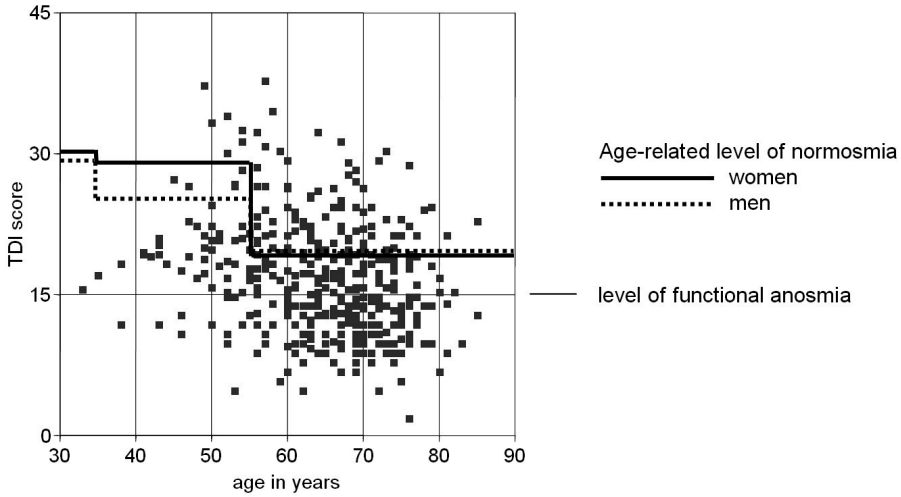
Statistical analyses were performed by means of SPSS 14.0 (Chicago, IL, USA). Differences between groups (e.g., akinetic-rigid, tremor-dominant, or mixed) were assessed by means of ANOVAs (between subject factor 'group') with Bonferroni post-hoc testing. The level of significance was set at 0.05. The studies had been approved by the local ethics committees at the three sites and were performed according to the Guidelines for Biomedical Studies Involving Human Subjects (Helsinki Declaration).

RESULTS

Olfactory dysfunction in relation to age-independent definitions of hyposmia

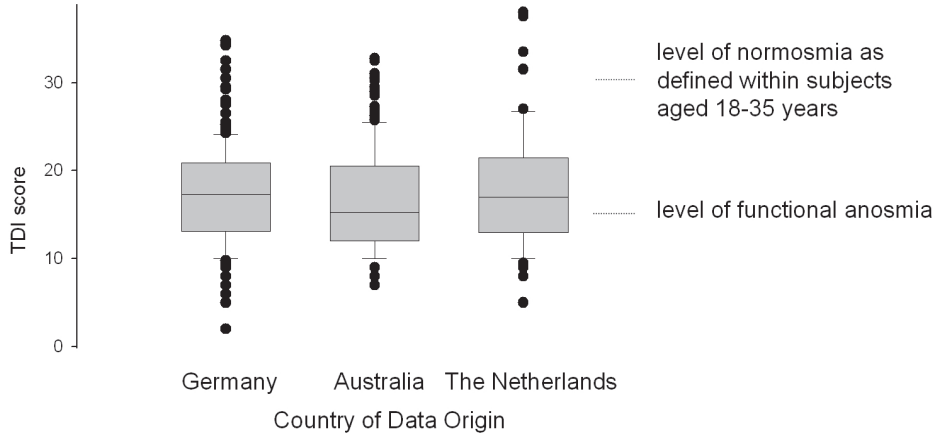
In this analysis we applied age-independent criteria for hyposmia, which have been derived from olfactory function in a group of 18-35 year old, healthy subjects, considered to be the standard population in terms of normal olfactory sensitivity.[117] Out of the total number of 400 patients 180 (45%) presented as functionally anosmic, whereas only 13 (3.3%) were normosmic as identified by means of the composite TDI score (mean $_{n=400}$ 17.1; range 2-38) (Figure 5).

Figure 5. Olfactory loss in the different groups of Parkinson's disease patients.
Results are shown as a composite TDI score (sum of odour threshold, odour discrimination, and odour identification score)



Separate analysis of olfactory loss for the groups of patients showed that only 3.1% of the German PD patients, 2.4% of the Australian, and 5.3% of the Dutch patients presented with normosmia, i.e., overall, 96.7% of PD patients suffered from olfactory loss as described by the TDI score (Figure 6).

Figure 6. Olfactory function of the total number of 400 Parkinson's disease patients.
Results are shown as a composite TDI score (sum of odour threshold, odour discrimination, and odour identification score)



When the diagnosis was based on the results from individual olfactory tests a slightly different picture emerged. Specifically, 17 and 13% of all patients tested had normal odour threshold and odour discrimination scores, whereas 4.3% had normal odour identification scores (Table IV). When using the combined TDI score 3.3% of the patients were classified as normosmic. This clearly indicates that the diagnosis depends on the test used to establish this diagnosis, with odour identification tests providing the highest portion of olfactory loss.

Table IV. Number/percentage of patients with normosmia and hyposmia/anosmia when assessed with different olfactory tests.

olfactory test	diagnosis	n	%
TDI score	hyposmia / anosmia	387	96.7
	normosmia	13	3.3
odour thresholds	hyposmia / anosmia	332	83.0
	normosmia	68	17.0
odour discrimination	hyposmia / anosmia	349	87.3
	normosmia	51	12.8
odour identification	hyposmia / anosmia	383	95.8
	normosmia	17	4.3

Olfactory dysfunction in relation to age- and sex-dependent definitions of hyposmia

When the diagnosis was based on normative data in relation to the subjects' age and sex,[117] we found that, for the TDI score, 25.5% of the investigated population were normosmic whereas 74.5% had hyposmia or were functionally anosmic.

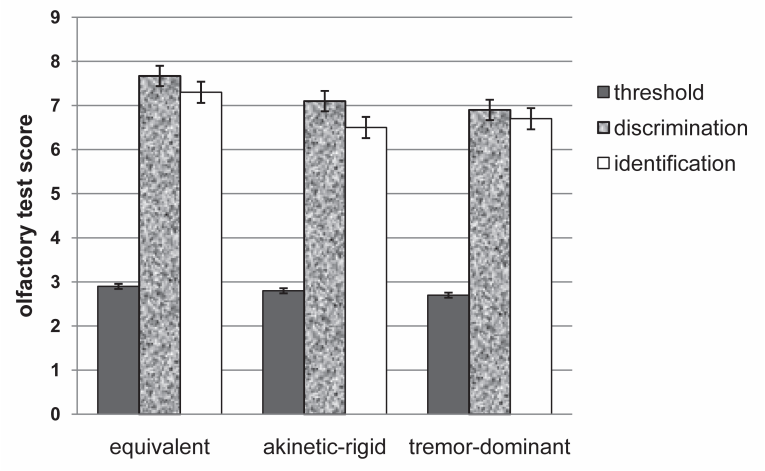
Sex-related differences in olfactory function

Furthermore, when comparing olfactory sensitivity between male and female PD patients, women exhibited significantly higher TDI scores ($t = 2.7$, $p = 0.008$), and threshold scores ($t = 3.1$, $p = 0.001$), indicating better olfactory function. This difference was not seen for discrimination ($t = 1.6$, $p = 0.11$), and identification scores ($t = 1.5$, $p = 0.14$). Men and women did not, however, differ significantly in terms of age, duration of the disease, or UPDRS score.

Differences between PD subtypes

With regard to the TDI score there were no significant differences between patients with different disease subtypes (tremor dominant type [n = 49], akinetic-rigid type [n = 68] or mixed type [n = 114]; $p = 0.24$; Figure 7). This was also the case when individual tests of olfactory function were considered (odour thresholds, odour discrimination, and odour identification).

Figure 7. Olfactory function separately for three subtypes of Parkinson's disease.
Results are shown separately for odour thresholds, odour discrimination, and odour identification (means, standard error of means).



Relationship between olfactory function and severity/duration of disease

Correlational analyses between olfactory sensitivity in relation to the severity of PD were made across all patients and, separately, for hyposmic patients only. However, there were no significant correlations (Pearson) between the TDI score, duration of disease (r = -0.09, p = 0.20), the Hoehn and Yahr scale (r = -0.12, p = 0.18), and the UPDRS-III score (r = -0.09, p = 0.19), respectively. Duration of the disease correlated significantly with both UPDRS-III score (r = 0.23, p = 0.015) and Hoehn and Yahr score (r = 0.48, p = 0.001).

DISCUSSION

The main outcomes of the present study are A) that over 96% of PD patients present with olfactory dysfunction - compared to young and healthy subjects. B) More than 80% of PD patients with smell loss are functionally anosmic or severely hyposmic, respectively, regardless of the olfactory test being used for diagnosis. Additional findings were: C) With regard to olfactory function we did not observe major differences between subtypes of PD, namely tremor-dominant PD, akinetic-rigid PD, and equivalent-type PD. D) No correlation was found between olfactory loss and duration of disease, Hoehn and Yahr stage and disease severity as measured by means of the UPDRS III score.

In three independent populations, the prevalence of olfactory dysfunction in people with PD is greater than previously reported with regard to norms obtained in healthy young subjects.[78;80;84;85] There are several differences in methodologies that may contribute to differences in estimates of prevalence. The present study used a comprehensive psychophysical olfactory function test comprised of three subtests of olfactory function,[33] whereas previous work was mostly focused on odour identification tests. We demonstrate here that some tests, used alone, provide different estimates of the prevalence of olfactory dysfunction (Table IV). With regard to the identification of olfactory deficits it appears noteworthy that smell identification appears to be the most sensitive component of the TDI index. The TDI index identified only a further 4/400 patients compared to the results from odour identification alone. Thus, it appears that in this instance a test of odour identification alone might be simpler and equally informative compared to more extensive tests – this needs to be explored further.

By using age-independent criteria for hyposmia, which have been derived from olfactory function in a group of 18-35 year old subjects, a total olfactory loss of 96% in the study population was identified – compared to an established olfactory loss of at least 25% in the normal population over 52 years of age.[122] When normative data in relation to the subjects' age and sex were applied however, 74.5% of this study population was diagnosed with olfactory loss. Thus, olfactory loss needs to be qualified in terms of the olfactory test used and the normative data being applied

The present study used a population-based sampling design with large sample size, with olfactory function scores compared to normative values based on very large samples,[117] whereas other studies have used smaller patient groups matched with control groups [78;85] even though case-control designs are less robust for estimating prevalence. In the present study great care was taken to establish a precise diagnosis according to the United Kingdom Parkinson's Disease Society Brain Bank Diagnostic Criteria for Parkinson's Disease whereas in other studies the criteria for inclusion are not always specified.

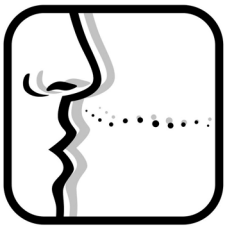
The present data also confirmed numerous previous studies with regard to the missing correlation between olfactory loss and both duration of disease [85;139;143] and the clinical severity of PD as measured by means of the Hoehn and Yahr scale and the UPDRS-III (compare [141]) - although some studies found a correlation between the severity of PD and certain measures of olfactory function, namely latencies of olfactory event-related potentials [148] or results from an odour discrimination task.[81] Overall, this is in line with the idea that olfactory dysfunction is an early sign of PD [149] which can already be detected at the moment when motor symptoms appear. Recent investigations (e.g. [89;90]) demonstrate that olfactory loss is a symptom that is present at the earliest stages of the disease, which is also compatible with predictions made on the basis of neuropathological investigations.[16] With regard to pathophysiology of olfactory loss, Huisman et al.[102] found an increase of (inhibitory) dopaminergic neurons in the olfactory bulb in PD patients. They interpreted their finding within the context of a possible compensatory mechanism in response to the loss of dopaminergic neurons in the basal ganglia.[150]

With regard to olfactory function we did not find major differences between subtypes of PD, namely tremor-dominant PD, akinetic-rigid PD, and mixed-type PD. While this confirms previous observations in a small sample size of 37 patients [93] the present findings are in contrast to reports by Stern and colleagues [143] who reported significantly better odour identification scores in patients with tremor-predominant PD (n = 40) than in cases with postural instability-gait disorder-predominant PD (n = 23). However, because Stern et al. investigated a relatively small group of patients and their finding was not confirmed in the present study, it may be hypothesized that such differences in olfactory function between subgroups of PD are relatively subtle.

Conclusion

In conclusion, considering the current data on smell loss in over 95% of PD patients, olfactory dysfunction has to be seen as a significant marker of the disease which is even more frequent than the classical symptom tremor.[151] Consequently, structured and validated tests of olfactory function should be a mandatory part in the diagnosis of PD. Of course, it does not make sense to tell individuals with olfactory loss that they are likely to end up with a diagnosis of PD, simply because there are so many reasons for olfactory

loss.[152] However, it appears to be valid to question a diagnosis of PD in patients with a normal sense of smell.[153]



Chapter 3

A comparative study of odour identification and odour discrimination deficits in Parkinson's disease

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ABSTRACT

Aim To compare the characteristics of odour discrimination and odour identification deficits in a large population of Parkinson's disease (PD) patients, and to determine which of these olfactory tests best distinguishes between PD patients and controls.

Methods Olfactory performance was assessed in 404 PD patients and 150 controls, using the odour identification and discrimination parts of the Sniffin' Sticks battery.

Results Mean identification and discrimination scores in PD patients were significantly lower than in controls. Linear regression analysis using a 95% confidence interval revealed that, relative to the performance of controls, 65.0% of PD patients had an impairment in odour identification, whereas 42.1% of patients were impaired on the odour discrimination task. ROC curves revealed a higher sensitivity and specificity for odour identification than for odour discrimination in separating patients from controls. In PD patients, odour discrimination performance decreased with increasing disease duration, whereas odour identification was not correlated with disease stage or duration.

Conclusion In PD, odour identification is more frequently impaired than odour discrimination and allows a better discrimination between patients and controls. Although an odour identification deficit is generally believed to be independent of disease progression, the impairment in odour discrimination appears to increase with disease duration.

INTRODUCTION

Olfactory deficits in Parkinson's disease (PD) were first empirically documented in 1975 by Ansari and Johnson.[78] Over the ensuing years it has become clear that most PD patients have olfactory disturbances that are not restricted to a single functional modality but include impairments of odour detection, discrimination and identification.[79-85] At present, it is quite firmly established that olfactory dysfunction is one of the first and most prevalent clinical manifestations of this disorder. Clinical deficits in the sense of smell may even precede the development of overt motor symptoms.[23;89;90] Pathological studies support these observations by demonstrating that the olfactory bulb, tract and anterior olfactory nucleus may be among the induction sites of PD pathology.[16;19]

Most studies on olfactory dysfunction in PD have focused on odour identification performance using the University of Pennsylvania Smell Identification Test (UPSIT [32]), or on composite scores based on multiple olfactory tests (e.g. the "Sniffin' Sticks" test battery [33]). The reported prevalence of the odour identification deficit in PD patients ranges between 50-90%,[80;85;86] and appears to be unrelated to disease stage and duration or the use of dopaminergic medication.[79;83-85;87]

Few studies have reported odour discrimination performance separately in PD. There are some indications to suggest that, contrary to odour identification, odour discrimination is related to clinical measures of disease progression. In a small sample of PD patients, we found that odour discrimination scores correlate with disease stage and severity.[81] In addition, odour discrimination performance in PD patients improves after stereotactic neurosurgical treatment using deep brain stimulation,[154] concurrent with clinical motor improvement. In spite of these interesting observations, little is known about the prevalence of odour discrimination deficits in PD. In a small sample of PD patients we studied previously, only 34% of PD patients scored below two standard deviations of the mean of control subjects.[81]

The present study was initiated to directly compare odour discrimination and odour identification deficits, and their relationship with disease stage and duration, in a large population of PD patients, and to determine which of these olfactory tests best distinguishes between PD patients and control subjects.

METHODS

Subjects

This study was performed in 404 PD patients (253 males; mean age 61.5 years, range 40-90 years, Hoehn and Yahr (H&Y) stages I-V, disease duration 0-44 years), and 150 control subjects without a history of major olfactory or (other) neurological disorders (87 males;

mean age 59.2 years, range 45-78 years). PD patients were recruited from the outpatient clinics of the departments of Neurology of the VU University Medical Center (VUMC; n = 72) and the Leiden University Medical Center (LUMC; n = 332). PD was diagnosed according to the United Kingdom Parkinson's Disease Society Brain Bank criteria.[155] Patients were tested 'ON' medication, and rated for disease stage by means of the H&Y scale.[151] All control subjects were volunteers recruited among hospital employees and partners of patients (VUMC (n = 70) and LUMC (n = 80)). All subjects provided written informed consent. The study was approved by the Medical Ethics Committees of the VUMC and the LUMC.

Olfactory function testing

The "Sniffin' Sticks" test battery (Burghart, Wedel, Germany) is an olfactory test battery comprising reusable felt-tip pens ('sticks') containing odorants dissolved in propylene glycol which the subject has to sniff. Olfactory tests were administered birhinally in a quiet, well-ventilated room to avoid any background smell interfering with the test odours.

Odour identification was measured by presenting 16 odorants in suprathreshold intensity in a multiple (4)-forced choice format with verbal descriptions. Each stick was held approximately 2 cm in front of the nostrils for 2-3 sec, with an interval of 20-30 sec between each stick. In the odour discrimination test, subjects were blindfolded and presented with 16 odour-triplets, with an interval of 30 sec between each triplet. Each triplet consisted of two identical and one aberrant odorant. Subjects were asked to select the odd odour out of the three odorants presented, without the need to recognize or name the odours.

In both tests, olfactory scores were defined as the number of correct responses (0-16).

Data analysis

To determine the prevalence of odour identification and odour discrimination deficits in Parkinson patients, we compared olfactory test scores between patients and control subjects using the general linear model UNIANOVA in SPSS for both sexes separately, with factor 'group' (PD patients or control subjects), and adjusted for age. For each of the olfactory tests, and for men and women separately, we then modelled the 95% lower bound of the individual prediction interval of the linear regression lines for control subjects plotted against age and used those as a cut-off for hyposmia. When the regression lines for men and women coincided, the combined regression line was used as a cut-off for hyposmia.[156] Both sexes were analyzed separately, since olfactory function is generally considered to be sex-dependent, also in PD.[157;158]

To establish criteria to best distinguish between PD patients and control subjects, we calculated ROC curves for each of the olfactory tests separately, and for both tests combined.

To explore the influence of sex, age, disease duration (in years, based on first symptoms perceived by the patient) and disease stage (H&Y stage) on olfactory function in PD patients, olfactory test scores were submitted to a linear regression analysis by means of an UNIANOVA with factors 'sex', 'age', 'disease duration', 'disease stage' and relevant interactions. Non-significant interactions were excluded from the analyses. Analyses were performed for odour identification and odour discrimination separately.

For each item in the identification and discrimination tasks, the percentage of subjects in each group (patients and control subjects) that had responded correctly was calculated. To determine which items best separated patients from control subjects, we subtracted the percentage of patients that responded correctly from the percentage of control subjects that responded correctly, for each item separately.

Pearson correlation coefficients were computed to determine the correlation between identification and discrimination scores, both overall and for men and women separately.

Data were analyzed using SPSS 15.0 for Windows (Chicago, IL, USA).

RESULTS

Odour identification

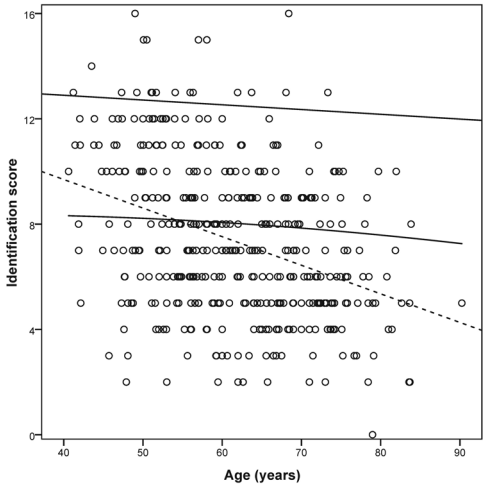
A total of 400 PD patients (250 males) and 150 control subjects (87 males) completed the identification task. Both male and female PD patients had lower mean identification scores, compared to the control subjects, when adjusted for age (mean identification score PD males = 6.9; control males = 12.4; F [1,334] = 305.14, $p < 0.001$. Mean identification score PD females = 8.3; control females = 12.5; F [1,210] = 99.70, $p < 0.001$).

260 PD patients (65.0%; 183 males) scored below the 95% lower bound of the individual prediction interval for identification scores of the control subjects (Figure 8).

A cut-off value of 10.5 for the identification task best discriminated between PD patients and control subjects, with a sensitivity of 0.83 and a specificity of 0.82 (Table V, Figure 9).

There were main effects of sex (women performed better than men; F [1,397] = 25.99, $p < 0.001$), and age (F [1,370] = 61.75, b = -0.108, $p < 0.001$) on odour identification scores of PD patients. No relationship was found between identification performance and disease stage (H&Y stage; F [1,392] = 1.06, $p = 0.303$) or disease duration (F [1,392] = 0.07, $p = 0.785$).

Figure 8. Identification scores of Parkinson's disease (PD) patients plotted against age.



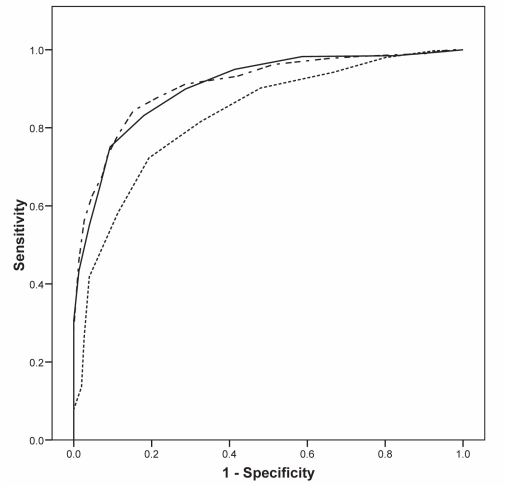
Solid lines are the regression line and 95% limits of the prediction interval for healthy subjects; the dotted line is the regression line for PD patients.

Table V. Receiver Operating Characteristics (ROC).
Optimal cut-off values, associated sensitivity and specificity estimates, and area under the curve (AUC) for each olfactory test, determined from ROC curves (Figure 9).

	Cut-off value	Sensitivity	Specificity	AUC
ID	10.5	0.83	0.82	0.91
DIS	9.5	0.72	0.81	0.83
ID plus DIS	20.5	0.84	0.85	0.91

ID = identification task; DIS = discrimination task; ID plus DIS = identification task plus discrimination task

Figure 9. Receiver Operating Characteristic (ROC) curves.



Relating sensitivity and specificity for olfactory identification, discrimination, and the combination of identification and discrimination scores.
Solid line represents the odour identification test; dotted line represents the odour discrimination test; striped line represents the combination of the odour identification and discrimination tests

Odorants that were identified least often by the PD patients were 'apple' (15.3% correct identification), 'cinnamon' (28.3% correct) and 'liquorice' (34.0% correct) (Table VI). Items that best separated patients from control subjects were 'aniseed' (38.0% vs. 88.7% correctly identified), 'cinnamon' (28.3% vs. 71.3%), and 'liquorice' (34.0% vs. 75.3%). Items that least separated patients from control subjects were 'turpentine' (36.8% vs. 38.7% correctly identified), 'garlic' (64.3% vs. 83.3%), and 'lemon' (34.3% vs. 58.0%) (Table VI).

Table VI. Identification items.

	Target	Alternative response choices	% correct responses PD patients	% correct responses controls	% controls - % PD patients
Item 1	Orange	Blueberry, Strawberry, Pineapple	54.5	85.3	30.8
Item 2	Leather	Smoke, Glue, Grass	55.8	88.7	32.9
Item 3	Cinnamon	Honey, Vanilla, Chocolate	28.3	71.3	43.1
Item 4	Peppermint	Chives, Fir, Onion	57.3	96.0	38.8
Item 5	Banana	Coconut, Walnut, Cherry	58.0	94.7	36.7
Item 6	Lemon	Peach, Apple, Grapefruit	34.3	58.0	23.8
Item 7	Liquorice	Caramel, Chewing gum, Biscuit	34.0	75.3	41.3
Item 8	Turpentine	Mustard, Rubber, Menthol	36.8	38.7	1.9
Item 9	Garlic	Onion, Sauerkraut, Carrot	64.3	83.3	19.1
Item 10	Coffee	Cigarette, Wine, Candle smoke	45.8	84.7	38.9
Item 11	Apple	Melon, Peach, Orange	15.3	48.7	33.4
Item 12	Clove	Pepper, Cinnamon, Mustard	60.3	91.3	31.1
Item 13	Pineapple	Pear, Plum, Peach	37.5	70.7	33.2
Item 14	Rose	Chamomile, Raspberry, Cherry	48.3	81.3	33.1
Item 15	Aniseed	Rum, Honey, Fir	38.0	88.7	50.7
Item 16	Fish	Bread, Cheese, Ham	69.0	99.3	30.3

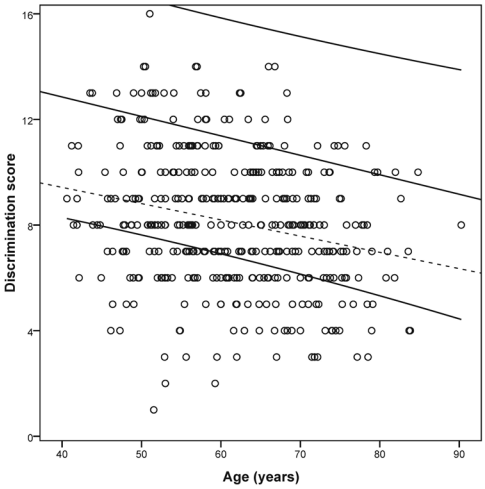
Odour discrimination

A total of 401 PD patients (251 males) and 150 control subjects (87 males) completed the discrimination task. Both male and female patients had lower mean discrimination scores, compared to the control subjects, when adjusted for age (mean discrimination score PD males = 7.8; control males = 11.3; $F\ [1,335] = 155.05$, $p < 0.001$. Mean discrimination score PD females = 8.8; control females = 11.4; $F\ [1,210] = 45.29$, $p < 0.001$).

169 PD patients (42.1%; 118 males) scored below the 95% lower bound of the individual prediction interval for discrimination scores of the control subjects (Figure 10).

A cut-off value of 9.5 for the discrimination task best differentiated between PD and control subjects, with a sensitivity of 0.72 and a specificity of 0.81 (Table V, Figure 9). Combining odour identification and odour discrimination did not provide a better differentiation between patients and control subjects than identification testing alone (Table V, Figure 9).

Figure 10. Discrimination scores of Parkinson's disease (PD) patients plotted against age.



Solid lines represent the regression line and 95% limits of the prediction interval for healthy subjects; the dotted line represents the regression line for PD patients.

In the group of PD patients, a significant interaction was found between sex and age: A decrease in discrimination performance with increasing age was found for women (F [1,396] = 3.39, b = -0.081, p < 0.001), and men (b = -0.032, p = 0.050). In addition, we found a main effect of disease duration on odour discrimination scores (F [1,396] = 13.90, b = -0.070, p < 0.001). No relationship was found between discrimination scores and disease stage (H&Y stage; F [1,392] = 1.05, p = 0.306).

Odour combinations that were discriminated least often by the PD patients were target (-)-carvone and distracter (+)-carvone (38.9% correct), (+)-carvone with distracter geraniol (41.9% correct), and (-)-limonene with distracter citronellal (44.4% correct) (Table VII). Odour combinations that best separated patients from control subjects were anethole/eugenol (47.4% vs. 78.7% correctly discriminated), octylacetate/cinnamonaldehyde (51.6% vs. 81.3%), (+)-limonene/(+)-fenchone (51.6% vs. 80.7%). Odour combinations that least discriminated between patients and control subjects were (-)-carvone/(+)-carvone (38.9% vs. 44.0% correctly discriminated), followed by citronellal/linalool (48.9% vs. 54.7%), and eucalyptol/α-ionone (51.4% vs. 67.3%) (Table VII).

Relationship between odour identification and discrimination test scores

In PD patients, a moderate correlation was found between odour identification and discrimination scores (Pearson correlation coefficient r = 0.50, p <0 .001), also when analyzed separately for men (r = 0.43, p < 0.001) and women (r = 0.55, p < 0.001).

296 PD patients (73.3%; 202 males) scored below the 95% lower bound of the individual prediction interval of the control subjects for either identification or discrimination scores.

Only 133 PD patients out of 397 PD patients (33.5%; 99 males) had a deviant score on both olfactory tests.

Table VII. Discrimination items.

	Target	Distracter	% correct responses PD patients	% correct responses controls	% controls - % PD patients
Item 1	Octylacetate	Cinnamonaldehyde	51.6	81.3	29.7
Item 2	*n*-Butanol	2-Phenylethanol	45.1	68.0	22.9
Item 3	Isoamylacetate	Anethole	57.4	76.0	18.6
Item 4	Anethole	Eugenol	47.4	78.7	31.3
Item 5	Geraniol	Octylacetate	54.1	74.7	20.6
Item 6	2-Phenylethanol	Isoamylacetate	66.6	87.3	20.7
Item 7	(+)-Limonene	(+)-Fenchone	51.6	80.7	29.0
Item 8	(-)-Carvone	(+)-Carvone	38.9	44.0	5.1
Item 9	(-)-Limonene	Citronellal	44.4	62.7	18.3
Item 10	2-Phenylethanol	(+)-Menthol	54.1	72.0	17.9
Item 11	(+)-Carvone	Geraniol	41.9	70.0	28.1
Item 12	*n*-Butanol	(-)-Limonene	59.9	85.3	25.5
Item 13	Citronellal	Linalool	48.9	54.7	5.8
Item 14	Pyridine	(-)-Limonene	47.1	68.7	21.5
Item 15	Eugenol	Cinnamonaldehyde	50.9	70.7	19.8
Item 16	Eucalyptol	α-Ionone	51.4	67.3	16.0

DISCUSSION

The present study confirms that olfactory dysfunction in PD includes impairments in both odour identification and discrimination performance. In the present population of PD patients, impaired odour identification performance was more prevalent than a deficit in odour discrimination. Moreover, odour identification testing differentiated better between patients and control subjects than odour discrimination. Odour discrimination performance worsened with increasing disease duration, whereas odour identification scores were not correlated with measures of disease progression.

The prevalence of an olfactory deficit in PD patients on any of the two tasks in this study was 73%. A deficit on the odour identification task was present in 65% of PD patients. Although this figure is lower than that reported in a previous study by Doty, who found a prevalence of 90%,[85] our data correspond with other studies reporting an odour identification deficit in 50-74% of PD patients.[80;86] The present study is the first to directly compare odour identification and odour discrimination in a large population of PD patients. Our study shows a prevalence of impaired odour discrimination performance of 42%, which is clearly lower than the prevalence of odour identification deficits in the same

population and confirms our previous observations using odour discrimination testing in a much smaller sample.[81]

Using the 16-item odour identification part of the "Sniffin' Sticks" test battery, we found a sensitivity of 0.83 and a specificity of 0.82 in discriminating between PD patients and control subjects at a cut-off value of 10.5. These sensitivity/specificity values are similar to the estimates in previous studies by Doty et al. using the 40-item UPSIT [138] and Daum et al. using the same 16-item identification subtest of the "Sniffin' Sticks".[159] Combining odour discrimination and identification testing did not improve sensitivity or specificity over odour identification testing alone. It appears that odour identification can stand alone as a reliable and valid measure to discriminate between PD patients and control subjects. Lötsch et al. also recently suggested that testing of olfactory function in PD patients could be reduced to a single test,[35] in their case odour detection thresholds. In the present study, we did not measure odour detection thresholds, and therefore cannot exclude that this modality differentiates better between PD patients and control subjects.

In the present study, we found a moderate correlation between odour identification and discrimination scores in PD patients. Interestingly, this correlation was higher in PD patients than in control subjects. This phenomenon may be related to a lower overall variance in olfactory test scores in control subjects, leading to a lower correlation between the individual tasks than in PD patients. Alternatively, the higher correlation between odour identification and discrimination scores in PD patients may reflect a common underlying olfactory deficit, such as an increased odour detection threshold (see also [37]). The latter explanation finds support in studies by Doty et al. and Lötsch et al. who found a primary component on which both odour identification and discrimination (and detection thresholds) loaded.[34;35] Even though odour identification and odour discrimination may share a certain aspect of olfactory function, this does not imply that odour identification and discrimination are fully equivalent olfactory modalities. Several imaging studies provide additional anatomical evidence for this notion, demonstrating that olfactory functions are mediated by common as well as task-specific regions in the brain.[42;45]

The present study confirms previous findings that the impairment of odour identification in PD is independent of disease stage or severity.[85] For odour discrimination performance, however, we found an inverse correlation with disease duration. This result partly relates to the observations in a smaller sample of patients, that disease stage and severity accounted for part of the variance in discrimination scores of PD patients.[81] Longitudinal follow-up of a small group of five *de novo* PD patients indicated that olfactory function (based on composite scores of multiple olfactory tests) decreased in relation to the duration of disease, at least during the first phases of PD.[160] Further support for a possible relationship between olfactory dysfunction and clinical disease variables comes from electrophysiological [108] and neuropathological studies.[16;100] Clearly, this relation needs to be addressed more thoroughly in future, preferably in longitudinal studies.

A selective hyposmia for identifying specific odorants in PD has been suggested by some authors. The very first study, using the UPSIT, reported this phenomenon for the odours 'pizza' and 'wintergreen'.[161] Since then, several studies have suggested a 'selective hyposmia' in PD for a variety of other odorants, including liquorice, pineapple, aniseed, banana, dill pickle, gasoline, smoke, cinnamon and mint.[159;162-164] In the present study, odorants that best separated patients from control subjects, based on identification scores, were aniseed, cinnamon and liquorice. Considering the sheer number of different odorants over the various studies, none of which has consistently been implicated in the alleged 'selective hyposmia' in PD, we believe that there is no convincing evidence for this concept.

Odours that have related molecular structures might have overlapping chemical and psychophysical properties. It is likely that such odours will be recognized by very similar sets of odorant receptors, which will make them more difficult to differentiate from one another than odorants with highly divergent structures.[165;166] In this study, the odour combination that was least often discriminated correctly was indeed a combination of stereo isomers, (-)-carvone and (+)-carvone, that have identical molecular structures. However, since this was the most difficult combination in the discrimination test for both patients and control subjects, there does not seem to be a selective deficit in distinguishing between structurally strongly related odorants in PD.

Conclusion

Olfactory dysfunction is a consistent feature of PD and includes impairments in odour identification and odour discrimination. Odour identification is more frequently impaired in PD patients than odour discrimination and allows a better discrimination between PD patients and control subjects. The present findings further indicate that, contrary to the odour identification deficit, which is independent of disease progression, the impairment in odour discrimination increases with disease duration.

Chapter 4

Is olfactory impairment in Parkinson's disease related to phenotypic or genotypic characteristics?

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Submitted

ABSTRACT

Aim To evaluate the relation between olfactory impairment (OI) and other impairment domains in Parkinson's disease (PD) and the characteristics of OI in patients with certain genotypic characteristics.

Methods In 295 non-demented PD patients and 150 controls with a similar overall age and sex distribution, olfactory function was evaluated with the identification (ID) and discrimination (DIS) tests of the "Sniffin' Sticks". In patients, demographic and clinical characteristics were evaluated, and genetic analyses were carried out.

Results Of all patients, 61% had an impaired ID and 43% had an impaired DIS. Age and sex contributed significantly to the explained variance in the ID score regression model (total explained variance 22%), whereas age, sex, and disease duration contributed significantly to the explained variance in the DIS score regression model (total explained variance 15%). Parkin and DJ-1 mutation carriers (homozygous or heterozygous compound, n = 6) had normal ID scores. Carriers of apolipoprotein E (APOE) ε2- or APOEε4 allele(s) had no significantly different olfactory scores compared to non-carriers. The distribution of the alleles of the alpha-synuclein (SNCA)-REP1 polymorphism in groups with a normal or impaired ID or DIS was comparable.

Conclusion OI in PD is not related to other impairment domains. This may indicate that olfaction is an independent feature of the disease. Parkin and DJ-1 mutation carriers had normal ID scores but the number of patients with mutations is too small to draw conclusions. The APOE genotype (APOEε2 or APOEε4 alleles) and SNCA-REP1 polymorphism do not seem to influence olfaction in PD.

INTRODUCTION

Olfactory impairment (OI) is one of the many non-motor features of Parkinson's disease (PD), which may also include cognitive impairment, autonomic dysfunction, depression, nighttime sleep problems, daytime sleepiness, and psychiatric complications.[15] In PD, OI is very common and may consist of impairments in the detection, identification (ID), or discrimination (DIS) of odours.[80;81] OI may occur early in the disease course [81] and even antedate the onset of motor symptoms in PD,[90] which is in line with pathological findings in PD showing that neurodegenerative changes may start in the lower brainstem and olfactory bulb, and extend gradually onto the rostral brainstem and cerebral cortex.[16]
The relation between OI and cognitive impairment in PD patients has been assessed, but no associations were found.[84;167] As far as we know, no other non-motor domains have been evaluated with respect to their relation with OI, although relations between OI and other premotor manifestations in PD,[22] such as depression and autonomic symptoms, could be expected. Furthermore, only two genotype-phenotype studies evaluated olfaction in PD patients with mutations and found that ID scores of patients carrying Parkin and LRRK2 mutations and controls were comparable.[168;169] Therefore, the aims of this study were to assess the relations between OI and other impairment domains in PD and to evaluate characteristics of OI in patients with certain genotypic characteristics.

METHODS

Design

The study is part of the "PROfiling Parkinson's disease" (PROPARK) study, a longitudinal cohort study of patients with PD (n = 420), who are profiled on phenotype, genotype, disability, and global outcomes of health, using valid and reliable assessment instruments for PD. Patients from this longitudinal cohort with their annual appointment between November 2005 and August 2006 (n = 337) were tested with regard to olfactory function.

Subjects

All patients fulfilled the United Kingdom Parkinson's Disease Society Brain Bank criteria for idiopathic PD.[155] Recruitment of patients in the PROPARK study was based on age at onset and disease duration, which are important determinants of disease course in PD.[170] The recruitment procedure has been described elsewhere.[171] For this particular study, patients with Mini Mental State Examination (MMSE) scores < 24 were excluded. No other selection criteria were applied. Most patients were assessed at the Leiden University Medical Center (LUMC). To avoid bias towards recruiting less severely affected patients, patients who were unable to come to the hospital were assessed at home. Controls (n =

150) were volunteers recruited among employees and partners of patients from the outpatient clinics of the Departments of Neurology of the LUMC (n = 80) and the VU University Medical Center (VUMC; n = 70). Controls had no history of major olfactory or neurological disorders and were selected to match the overall age and sex distribution of the patients. Characteristics of the controls have been published elsewhere.[156] This study was approved by the medical ethical committees of the LUMC and VUMC and all participants gave informed consent.

Olfactory function testing

For the "Sniffin' Sticks" ID test,[33] 16 odorants in suprathreshold intensity were presented, in a multiple-forced choice format with four descriptions (written and verbal). Each stick was held approximately 2 cm in front of the nostrils for 2-3 sec, with an interval of 20-30 sec between each stick.

For the "Sniffin' Sticks" DIS test,[33] subjects were blindfolded and presented with 16 odour-triplets, with an interval of 30 sec between each triplet. Each triplet consisted of two identical and one deviant odorant. Subjects were asked to select the odd odour out of three odorants presented, without the need to recognize or name the odours.

The tests were administered birhinally in a well-ventilated room to avoid any background smell interfering with the test odours. In both tests, olfactory scores were defined as the number of correct responses (0-16). Both olfactory tests have been proven to be reliable and valid in controls.[33] To determine if patients had an impaired ID or DIS, "Sniffin' Sticks" cut-off points of ID and DIS for age and sex groups which were based on control values, were used as described previously.[156]

SCOPA/PROPARK

Within PROPARK, all patients received a standardized assessment, including evaluation of demographic and clinical characteristics, family history of PD, and medication use. Measurement instruments for the different clinical domains of PD were derived from a prior project (SCales for Outcomes in Parkinson's disease: SCOPA).[172]

For the current study, data obtained for disease severity (Hoehn & Yahr (H&Y)),[151] motor function (SPES/SCOPA-motor, range 0-42),[173] cognition (SCOPA-COG, range 0-43),[174] autonomic function (SCOPA-AUT, range 0-69),[175] depressive symptoms (Beck Depression Inventory (BDI), range 0-63),[176] nighttime sleep (SCOPA-SLEEP NS, range 0-15) and daytime sleepiness (SCOPA-SLEEP DS, range 0-18),[177] and psychiatric complications (first six items of the SCOPA-PC, range 0-18) [178] were used. Except for the SCOPA-COG, higher scores indicate more severe impairment. Motor phenotype (tremor-dominant, postural instability gait difficulty (PIGD), or indeterminate) was determined for every patient with a method that has been described earlier.[179]

All instruments were either self-administered (SCOPA-AUT, BDI, SCOPA-SLEEP) or administered by trained research associates (H&Y, SPES/SCOPA-motor, SCOPA-COG, and SCOPA-PC). For reasons of comparability, all patients who used levodopa or a dopamine-agonist and experienced motor fluctuations, were assessed during 'ON'-state. For each patient, a total levodopa equivalent (LDE) for the dose of levodopa and dopamine agonists was calculated.[180]

Genetic testing

Peripheral blood was collected and genomic DNA was isolated according to standard procedures.

Mutation screening

DNA from patients was screened for the most frequent LRRK2 mutations which occur at the exons 19, 31, 35, 38, 41 and 48, whereas only DNA from patients with an age at onset < 50 years was screened for Parkin, DJ-1 and PINK1 mutations by direct sequencing of all exons. Additionally, DNA from patients with an age at onset < 50 years was screened for the A30P missense mutation in the alpha-synuclein (SNCA) gene and analyzed for genomic rearrangements (including deletions and duplications) for all exons of SNCA, Parkin, DJ-1, and PINK1 genes except for exons 2 and 4 of the DJ-1 gene with the multiplex ligation-dependent probe amplification method.

APOE genotyping

For APOE allelic discrimination, two non-synonymous coding single nucleotide polymorphisms (SNPs), rs429358 (R130C) and rs7412 (R176C), were genotyped. A validated TaqMan assay was used for detection of these SNPs, catalog numbers C_3084793_20 and C_904973_10 (Applied Biosystems, Foster City, CA). For double heterozygotes a direct PCR-based restricted fragment length polymorphism method was used. In brief, this method consisted of PCR amplification of a APOE fragment (244b) containing the gene region encoding the amino acids 130 and 176 (primers AAACGCGGGCACGGCTGTCCAAG and AAAAAAAAAGCCCCGGCCTGGTACACTG) followed by cleavage with Hhal Fastdigest Enzyme (Fermentas) and electrophoresis in a 15% non-denaturing polyacrylamide gel.[181]

SNCA genotyping

PCR was performed with 20 ng of DNA and the following conditions: An initial denaturation of 95°C for 12 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 30 sec. The final extension was at 72°C for 45 min.

The sequence of PCR primers are: Fluorescent-labelled Forward, 5'-CCTGGCATATTTGATTGCAA-3', and Reverse, 5'-GACTGGCCCAAGATTAACCA-3'. Two µl of

50X diluted PCR product was mixed with 10 µl of the following mixture, which was prepared with 10 µl of 500 Liz size standard (ABI) in 1000 µl of formamide (ABI). The mixture consisting of diluted PCR product and size standard-formamide was denaturated at 95°C for 5 min and cooled on ice for 10 min. Fluorescent labelled PCR fragments were resolved by the capillary electrophoresis on an ABI 3730 and allelic sizes assessed using GeneMapper® software version 4.0 provided by ABI.

Statistical Analysis

If 25% or more of the data from a questionnaire or scale was missing, data from this scale for this patient was excluded from statistical analyses. Differences between groups were analyzed with Chi-square tests (X^2), student's T-tests for independent samples, or analysis of covariance. Pearson's correlation coefficient or Spearman's rho were used to assess relations between ID or DIS scores and other demographic and clinical variables. Multiple forward linear regression analyses were used to explore the contribution of different variables to the ID and DIS score. A p-value < 0.05 was considered significant. All analyses were performed with SPSS 14.0 Software (Chicago, IL, USA).

RESULTS

Of the 337 patients, four patients had too many missing values on both the ID and DIS test and were therefore excluded from the study. Furthermore, 35 patients had MMSE scores < 24 and three patients had a missing MMSE score, and were also excluded. In total, 295 patients (65% men) with a mean (SD) age of 60.2 (10.6) years participated in the study (Table VIII). Two patients had too many missing values on the ID test and were therefore excluded from analyses for that particular test.

Olfaction in patients

The patients had a mean (SD) ID score of 7.6 (3.0), and a mean (SD) DIS score of 8.3 (2.6). Women had higher scores than men on both tests (ID mean scores: 8.7 versus 7.1 (mean difference 1.6, 95% CI 0.9 to 2.3); DIS mean scores: 9.2 versus 7.9 (mean difference 1.3, 95% CI 0.6 to 1.9)). Current smokers (n = 19) were younger than non-smokers (n = 260) (mean difference -8.5, 95% CI -13.4 to -3.6). After correction for age, olfactory scores of smokers and non-smokers were comparable (ID: F = 0.88, p = 0.350; DIS: F = 2.81, p = 0.095).

Table VIII. Characteristics of patients with Parkinson's disease.

Characteristics	Patients
No. of patients	295
Sex (M/F)	192/103
Age, in years; mean (SD)	60.2 (10.6)
Disease duration, in years; mean (SD)	11.8 (6.3)
Age at onset, in years; mean (SD)	48.4 (11.2)
Hoehn and Yahr stage (%; 1/2/3/4/5/missing)	4/42/37/14/2/1
Motor phenotype (%)	
Tremor-dominant	37
PIGD	50
Indeterminate	12
Missing	1
Total LDE, mg/day; mean (SD)	683.5 (513.5)
Levodopa therapy, no. of patients	214
Dopamine-agonist therapy, no. of patients	211

PIGD: postural instability gait difficulty; LDE: levodopa dosage equivalent

Patients had lower scores on both tests compared to controls (ID: mean difference -4.9, 95% CI -5.4 to -4.4; DIS: mean difference -3.1, 95% CI -3.6 to -2.6). Lower scores for patients in comparison with controls were also found when analyzing scores of women and men separately (women ID: mean difference -4.0, 95% CI -4.8 to -3.2; women DIS: mean difference -2.4, 95% CI -3.2 to -1.5; men ID: mean difference -5.4, 95% CI -6.0 to -4.8; men DIS: mean difference -3.5, 95% CI -4.1 to -2.9).

Overall, 293 patients had valid scores on both olfactory tests, of which 27% had no OI (n = 78, 42% men), 43% had impaired ID or impaired DIS (n = 126, 70% men), and 30% had both impaired ID and DIS (n = 89, 78% men).

Subgroup evaluations

Of 293 patients with valid scores on the ID test, 178 patients (61%) had an impaired ID. These patients were significantly more often men, older, had a significantly older age at onset, more severe PD as measured by H&Y, more motor, cognitive, and psychiatric problems, and experienced significantly more daytime sleepiness, compared to patients with normal ID (Table IX).

Of 295 patients with valid scores on the DIS test, 128 patients (43%) had an impaired DIS. Patients with an impaired DIS were more often men (X^2:8.3, p = 0.004), were younger (mean difference -6.9, 95% CI -9.2 to -4.7), and had a younger age at onset (mean difference -7.7, 95% CI -10.1 to -5.3) compared to patients with a normal DIS.

Patients with a tremor-dominant phenotype (n = 110) were younger than patients with a PIGD phenotype (n = 148) (mean difference -5.2, 95% CI -7.7 to -2.6), but had comparable olfactory scores after correcting for age influences (ID: F = 0.21, p = 0.646; DIS: F = 0.12, p

= 0.726), or when analyzing women and men separately (women ID: F = 2.29, p = 0.134; women DIS: F = 0.13, p = 0.722; men ID: F = 0.85, p = 0.357; men DIS: F = 0.13, p = 0.908).

Table IX. Characteristics of patients with Parkinson's disease with impaired and normal ID.

Characteristics	Impaired ID	Normal ID	95% CI	p-value
No. of patients	178	115	-	-
Sex (M/F)	133/45	57/58	-	< 0.001[2]
Age, in years; mean (SD)	62.6 (10.1)	56.3 (10.1)	4.0 to 8.7[1]	-
Disease duration, in years; mean (SD)	12.0 (6.1)	11.2 (6.1)	-0.7 to 2.2[1]	-
Age at onset, in years; mean (SD)	50.7 (11.1)	45.0 (10.5)	3.1 to 8.2[1]	-
Hoehn and Yahr stage (%; 1/2/3/4/5/missing) *	2/38/41/16/2/2	7/50/30/11/1/1	-	0.03[2]
Total LDE, mg/day; mean (SD)	713.4 (474.3)	637.9 (570.9)	-46.8 to 197.7[1]	-
SPES/SCOPA-motor score; mean (SD)	15.2 (5.5)	13.1 (5.3)	0.7 to 3.3[1]	-
SCOPA-COG score; mean (SD)	27.0 (5.9)	29.6 (5.3)	-4.0 to -1.3[1]	-
SCOPA-AUT score; mean (SD)	18.5 (8.2)	16.9 (8.2)	-0.3 to 3.6[1]	-
SCOPA-SLEEP NS score; mean (SD)	4.7 (3.6)	4.6 (3.3)	-0.7 to 2.2[1]	-
SCOPA-SLEEP DS; mean (SD)	5.4 (4.0)	4.3 (3.7)	0.2 to 2.0[1]	-
SCOPA-PC score; mean (SD)	2.4 (1.9)	1.8 (1.6)	0.2 to 1.0[1]	-
BDI score; mean (SD)	9.5 (6.2)	10.0 (6.6)	-2.0 to 1.0[1]	-

ID = identification; LDE = levodopa dosage equivalent; NS = nighttime sleep; DS = daytime sleepiness; SCOPA-PC = SCOPA-Psychiatric Complications; BDI = Beck Depression Inventory

* sum of percentages does not equal 100 due to rounding off

[1] student's T-tests for independent samples; X^2: Chi-square test

Determinants of ID and DIS scores

There were no significant moderate or strong correlations (r > 0.4) found between ID and DIS scores and other demographic or clinical variables. The multiple regression analysis revealed that age (15%) and sex (7%) accounted for the 22% explained variance of the ID score (total regression model; p < 0.001) where lower age and female sex were associated with higher ID scores. Age (6%), sex (5%), and disease duration (4%) together explained 15% of the variance of the DIS score (total regression model; p < 0.001) where lower age, female sex and shorter disease duration were associated with higher DIS scores (Table X).

Olfaction in relation to genotypic characteristics

DNA from 268 patients was screened for LRRK2 mutations and genotyped for APOE polymorphisms, whereas the SNCA-REP1 polymorphism was genotyped in 247 patients. DNA of 159 patients with an age at onset ≤ 50 years was screened for the SNCA A30P mutation and mutations in Parkin, PINK1, and DJ-1.

Table X. Determinants of ID and DIS scores in patients with Parkinson's disease.

	Variable[1,2]	R square	Standardized β
ID score	Age	0.15	-0.378
	Sex	0.07	-0.263
	Total	0.22	-
DIS score	Age	0.06	-0.203
	Sex	0.05	-0.243
	Disease duration	0.04	-0.180
	Total	0.15	-

ID = identification; DIS = discrimination

[1] Multiple forward linear regression analysis was used with the variables:
Age, sex, disease duration, total LDE, H&Y stage, cognitive functioning, autonomic functioning, depressive symptoms, nighttime sleep, daytime sleepiness, psychiatric complications

[2] Variables are ordered in the table as they appeared in the model

Mutation carriers

One patient had a mutation in heterozygous state in the LRKK2 gene. In total, six patients had homozygous or compound heterozygous mutations in Parkin (n = 5) or DJ-1 (n = 1). No patients had an A30P mutation in the SNCA gene or compound heterozygous or homozygous mutations in the PINK1 gene. The LRRK2 mutation carrier had impaired ID but normal DIS. The Parkin mutation carriers had either normal olfactory scores (n = 2) or normal ID but impaired DIS (n = 3). The DJ-1 mutation carrier had normal ID but impaired DIS (Table XI).

Table XI. Olfaction scores of mutation carriers.

Patient no.	Genotype	ID	DIS
1	Parkin homozygous	Normal	Normal
2	Parkin homozygous	Normal	Normal
3	Parkin homozygous	Normal	Impaired
4	Parkin homozygous	Normal	Impaired
5	Parkin compound heterozygous	Normal	Impaired
6	DJ-1 homozygous	Normal	Impaired
7	LRRK2 heterozygous	Impaired	Normal

ID = identification; DIS = discrimination

Influence of APOE genotype on olfaction

Of 268 patients in which APOE genotype was determined, 76 patients carried one (n = 71) or two (n = 5) APOEε4 allele(s). No age (mean difference -2.6, 95% CI -5.3 to 0.1) or sex (X^2:1.4, p = 0.243) differences existed between APOEε4 allele-positive or APOEε4 allele-negative patients. Olfactory scores were comparable between APOEε4 allele-positive and APOEε4 allele-negative PD patients (ID: mean difference 0.7, 95% CI -0.1 to 1.5; DIS: mean difference 0.3, 95% CI -0.4 to 1.0). Furthermore, 42 patients carried an APOEε2 allele. No

differences in age (mean difference 0.5, 95% CI -2.9 to 3.9) or sex (X^2:1.6, p = 0.205) were found between APOEε2 allele-positive or APOEε2 allele-negative patients. Olfactory scores were comparable between APOEε2 allele-positive and APOEε2 allele-negative PD patients (ID: mean difference 0.0, 95% CI -1.0 to 1.0; DIS: mean difference -0.5, 95% CI -1.4 to 0.3).

Influence of SNCA-REP1 polymorphism on olfaction

The SNCA-REP1 genotype was determined in the DNA of 247 patients. Four alleles (266, 268, 270 and 272) of this polymorphism were observed in our population. Only one copy of the 272-allele was present in our population and was therefore excluded from the analyses. The allele distribution in the groups with an impaired or normal ID (X^2:1.0, p = 0.617) or in the groups with an impaired or normal DIS (X^2:3.6, p = 0.167) were comparable.

DISCUSSION

The aims of this study were to assess the relations between OI and other impairment domains in PD and to evaluate characteristics of OI in patients with certain genotypic characteristics. In this study, olfactory scores were lower in men and older patients, and not influenced by smoking status, which is in line with other studies.[138;143;159;163] Contrary to the results of a previous study, olfactory scores did not differ between patients with a tremor-dominant or PIGD phenotype.[143] In our sample of non-demented PD patients, OI occurred in a large proportion of patients with PD, with ID being more frequently impaired (61%) than DIS (43%), which was also found in a previous study with a largely overlapping population.[182] Most other studies, however, reported higher percentages of impaired patients.[80;163] Differences between our results and results of others could be due to the use of different olfactory tests or differences in sample characteristics.

In our study a relatively high percentage of patients has normal olfaction. Concerning neuropathology in PD, evidence has been presented for a sequential involvement of different regions of the central nervous system.[183] Braak stage 1 reflects involvement of the olfactory bulb, the anterior olfactory nucleus, and the dorsal motor nucleus of the vagal nerve.[183] A longitudinal study in patients with PD showed that in some patients olfactory function improved over time.[184] Furthermore, a significant improvement of DIS was noted in patients with PD treated with subthalamic deep brain stimulation.[154] These findings suggest that OI cannot be accounted for by cell loss only and may indicate a role of other mechanisms like complex adjustments in neuronal activities and network interactions.[185] In view of the large percentage of patients with normal olfaction, our findings apparently indicate a differential vulnerability of the olfactory circuitry to the different disease mechanisms that may operate in PD.

An important finding of this study is the absence of relations between olfaction and other specific PD features. The lack of relation between OI and disease severity has also been described by others,[93] whereas relations with other non-motor symptoms, except for cognition,[84;167] have not been evaluated before. Here we show that OI has no relation with any of the other impairment domains of PD. Apparently, OI, like tremor [186] behaves as an independent feature of the disease. The lack of relations found between OI and other early non-motor symptoms of PD could be due to the long mean disease duration of our cohort (12 years). To reliably evaluate the relation between OI and other early non-motor symptoms, an incident patient cohort would be more appropriate.

Our study shows that homozygous or compound heterozygous Parkin and DJ-1 mutation carriers had normal ID, whereas the heterozygous LRRK2 mutation carrier had impaired ID. Three out of five mutation carriers had an impaired DIS. There were no homozygous or compound heterozygous PINK1 mutation carriers or SNCA mutation carriers in our cohort. Hitherto, the only studies evaluating olfaction in mutation carriers have been evaluating ID in homozygous and heterozygous (single and compound) Parkin mutation carriers and in LRRK2 mutation carriers.[168;169] These studies also reported normal ID in these patients,[168;169] in accordance with our findings in Parkin mutation carriers. Although our findings on ID in Parkin mutation carriers corroborate those of others, our results make it impossible to draw conclusions because of the few mutation carriers.

The APOEε2 allele and APOEε4 allele are both described as risk factors for PD.[187;188] The APOEε4 allele also is a well-known risk factor for Alzheimer's disease,[189] a disease that is also associated with OI.[190] Non-demented persons with at least one APOEε4 allele have been shown to have a significantly poorer ID than those without an ε4 allele,[190] which could indicate that the presence of an APOEε4 allele by itself is associated with OI. The results of our study show that in PD neither the ε4 nor the ε2 allele seems to contribute to OI. Finally, there was no significant effect from the different alleles of the SNCA-REP1 polymorphism on olfaction.

Conclusion

This study shows that OI in PD is unrelated to other impairment domains of the disease. Considering genotypic characteristics, Parkin and DJ-1 mutation carriers had normal ID scores whereas the APOE genotype (APOEε2 or APOEε4 alleles) and SNCA-REP1 polymorphism do not seem to influence olfaction in PD.

Chapter 5

Odour recognition memory is not independently impaired in Parkinson's disease

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Submitted

ABSTRACT

Aim The results of previous studies in small groups of Parkinson's disease (PD) patients are inconclusive with regard to the presence of an odour recognition memory impairment in PD. The aim of the present study was to investigate odour recognition memory in PD in a larger group of patients.

Methods Odour recognition memory and detection thresholds were assessed using components of the "Sniffin' Sticks" test battery in 55 non-demented PD patients (Hoehn and Yahr stages I-III) and 50 control subjects of comparable age and sex.

Results PD patients performed slightly but significantly worse than control subjects on the odour recognition memory task. After correction for odour detection scores, however, the difference in odour recognition memory performance between PD patients and controls was no longer statistically significant.

Conclusion These data indicate that odour recognition memory is not independently impaired in PD patients.

INTRODUCTION

Olfactory deficits in Parkinson's disease (PD) were first empirically documented in 1975 by Ansari and Johnson.[78] Over the ensuing years it has become clear that most PD patients have olfactory disturbances that are not restricted to a single functional measure but include impairments of odour detection, discrimination and identification (for review see [191]). Clinical deficits in the sense of smell may even precede the development of overt motor symptoms.[89;90]

Few studies have addressed odour recognition memory performance in PD patients. The results of these studies are inconclusive. A review paper based upon three small studies concluded that odour recognition memory is impaired in PD patients,[98] whereas a separate study suggested that odour recognition memory may be intact in PD.[192]

Reduced olfactory acuity may, at least theoretically, affect performance on other olfactory tasks and thus lead to an underestimation of the actual performance on the olfactory task in question. It has been argued that olfactory detection thresholds should therefore always be assessed in addition to the specific olfactory measure under consideration and used in appropriate statistical analyses to correct for impairments in odour detection.[37]

The aim of the present study was to investigate odour recognition memory in a larger group of PD patients.

METHODS

Subjects

This study was performed in 55 control subjects and 63 PD patients. Eight subjects (4 control subjects and 4 PD patients) had a score below 25 on the MMSE or a score below 27 on the CAMCOG on the day of olfactory testing, and were therefore excluded. In addition, five subjects (1 control subject and 4 PD patients) did not complete both olfactory tasks and were excluded. 55 non-demented PD patients (31 males and 24 females; mean age 62.0 years, range 50-73 years, Hoehn and Yahr (H&Y) stages I-III, disease duration 0-19 years), and 50 control subjects (27 males and 23 females; mean age 59.5 years, range 49-78 years) remained in the study. All PD patients were recruited from the outpatient clinic for movement disorders of the department of Neurology of the VU University Medical Center (VUMC) or via advertisements on Parkinson's disease-related websites on the internet. Parkinson's disease was diagnosed according to the United Kingdom Parkinson's Disease Society Brain Bank criteria. Four patients were drug-naive. Of the remaining PD patients, three patients were treated with levodopa monotherapy, five patients were on dopamine-agonist monotherapy, 18 patients were treated with a combination of both levodopa and a dopamine agonist, and 25 patients used levodopa, a

dopamine agonist, as well as other medication, including monoamine oxidase B (MAO-B) inhibitors, catechol-O-methyltransferase (COMT) inhibitors, parasympathicolytica and beta-blockers. Medicated patients were tested in the 'ON' state, and all patients were rated for disease stage by means of the modified H&Y scale,[193] and for motor symptom severity by the Unified Parkinson's Disease Rating Scale III (UPDRS-III). All control subjects were volunteers recruited among hospital employees and partners of patients and reported normal subjective olfactory function and had no history of major olfactory or (other) neurological disorders. All subjects provided written informed consent. The study was approved by the Medical Ethics Committee of the VUMC.

Olfactory function testing

Odour recognition memory and odour detection threshold were assessed using components of the "Sniffin' Sticks" test battery (Burghart, Wedel, Germany).[33]

For the *odour recognition memory* task, we used the odorants of the extended identification part of the "Sniffin' Sticks".[194] Subjects were presented with 8 target odorants, with an interval of approximately 10 sec between odorants, and were asked to memorize them. No verbal descriptions were provided. After a short break (1-5 min), in which instructions on the task were given, 16 odours (8 target odours, 8 distracters; Table XII) were presented and the subject was asked whether the odour had been smelled before. A fixed presentation order of the target and distracter odours was randomly selected at the onset of the study and used in for all participants. Odour recognition memory scores were calculated as "proportion correct" measure: correctly recognized target odours (0-8) plus correctly recognized distracter odours (0-8), divided by the total number of targets and distracters (16).

Table XII. Target and distracter odours used in the odour recognition memory task.

Target odours	Distracter odours
Lilac	Pear
Grass	Coke
Peach	Grapefruit
Raspberry	Ginger
Mushroom	Coconut
Onion	Melon
Honey	Smoked meat
Lavender	Chocolate

Odour detection threshold was assessed using a single-staircase, three-alternative forced-choice procedure, with a 1:2 dilution series of sixteen stages. Subjects had to identify the odour-containing pen when presented with three pens, two containing the solvent and

one the odorant (score 0-16). Subjects who were unable to correctly identify even the highest concentration of the odour-containing pens received a score of 0.
Subjects were blindfolded during the two tests to prevent visual identification of the sticks. Olfactory tests were administered birhinally in a quiet, well-ventilated room to avoid any background smell interfering with the test odours.

Data analysis

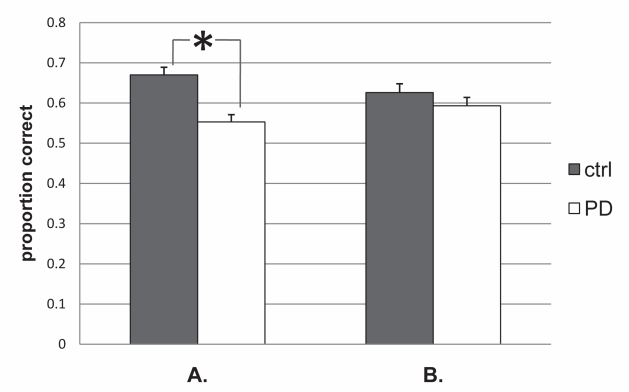
Since olfactory function is generally considered to be age- and sex-dependent [121;157] we used these terms as covariates in our analyses.
Odour recognition memory scores ('proportion correct') were analyzed by means of an analysis of variance (ANOVA), with 'group' as factor, and 'age', 'sex', and 'detection score' as covariates, using SPSS 15.0 for Windows (Chicago, IL, USA).
To explore sex differences in odour recognition memory performance, data were analyzed by means of an ANOVA, with 'sex' as factor, and 'group', 'age', and 'detection score' as covariates.
To investigate a possible relation between odour recognition memory performance and disease duration or severity, data of the PD patients were analyzed by means of an ANOVA with 'disease duration' (in years, based on first symptoms perceived by the patient), 'disease stage' (modified H&Y scale), 'motor symptom severity' (as measured by the UPDRS-III), 'sex', and 'age', as determinants.

RESULTS

The mean number of correctly recognized target odours (± SEM) was 6.3 ± 0.19 for control subjects, and 5.2 ± 0.23 for PD patients. The mean number of correct rejections (± SEM) was 4.4 ± 0.25 for control subjects, and 3.6 ± 0.22 for PD patients. Total odour recognition memory score ('proportion correct'; ± SEM) was 0.67 ± 0.019 for control subjects, and 0.55 ± 0.018 for PD patients (Figure 11A). Mean odour detection threshold score (± SEM) was 7.9 ± 0.41 for control subjects, and 2.4 ± 0.36 for PD patients.
Odour recognition memory scores were significantly lower in PD patients than in control subjects ($F [1,101] = 15.59$, $p < 0.001$), when corrected only for age and sex. When odour recognition memory scores were also adjusted for odour detection threshold scores, there was no significant difference between PD patients and control subjects ($F [1,100] = 0.87$, $p = 0.352$; Figure 11B).
There was no main effect of sex with respect to odour recognition memory scores ($F [1,100] = 0.65$, $p = 0.423$), when corrected for age and odour detection threshold scores. No relationship was found between disease duration ($F [1,49] = 2.63$, $p = 0.111$), disease

stage (F [1,49] = 1.08, p = 0.304) or motor symptom severity (F [1,49] = 0.89, p = 0.350) and odour recognition performance, when corrected for age and sex.

Figure 11. Odour recognition memory scores.



A. Mean odour recognition memory scores ('proportion correct') and standard errors of the mean, for control subjects and Parkinson's disease patients.
* indicates p-value < .05, based upon an ANOVA with 'group' as factor.
B. Mean odour recognition memory scores ('proportion correct') and standard errors of the mean, for control subjects and Parkinson's disease patients, after statistical correction for age, sex and odour detection threshold scores.

DISCUSSION

The results of the present study demonstrate that PD patients had slightly but significantly lower odour recognition memory scores than control subjects. However, when odour recognition memory scores were corrected for odour detection threshold scores, the difference between PD patients and control subjects lost statistical significance.

Few studies have previously addressed odour recognition memory performance in PD patients, and the results of these studies are inconclusive. In a meta-analysis Mesholam et al. concluded that odour recognition memory was impaired in PD.[98] However, the analysis was based on only three small studies, including a study by Kesslak et al. that lacked statistical significance due to the low number of subjects (n = 4) [195] and a study by Zucco et al. in which no difference had been found between PD patients and control subjects in odour recognition memory performance.[196] In the third study, an odour recognition paradigm was used that actually did not involve a memory component.[197] In contrast, the results of a separate study in PD patients suggested that odour recognition memory is intact.[192] Although our results without correction for odour detection threshold would seem to support the conclusion by Mesholam et al. that there is a slight impairment of odour recognition memory in PD, the present data indicate that this slight impairment is not independent of the deficit in odour detection.

To correct for the influence of olfactory acuity on odour recognition memory performance - a methodological issue that was previously raised [37] - we used a statistical correction for odour detection thresholds. Using this approach, we found no significant difference between PD patients and control subjects on the odour recognition memory task. Presumably, the PD patients were not able to perceive the target odours sufficiently well to memorize and recognize them afterwards. This finding suggests that an impairment in olfactory acuity may also underlie reduced performance on other olfactory tasks in PD, which would be in line with the suggestion by Doty et al. that most olfactory tests measure a common source of variance.[34] However, this does not seem to apply to all olfactory measures, since in at least one recent study odour identification and discrimination deficits were independent from the increase in odour detection threshold.[81] The current findings suggest that the olfactory impairments in PD, which appear to involve several specific olfactory functions, do not include odour recognition memory.

In Alzheimer's disease (AD), previous studies have reported odour recognition memory to be impaired,[98;192] even when corrected for odour detection thresholds.[198-200] Therefore, testing of odour recognition memory may prove useful in the differential diagnosis between PD patients and AD patients, in particular in the context of early diagnostic procedures. Future studies directly comparing groups of PD and AD patients are necessary to confirm this.

Conclusion

In conclusion, the present data indicate that odour recognition memory is not independently impaired in PD patients.

Chapter 6

Extended testing across, not within, tasks raises diagnostic accuracy of olfactory testing in Parkinson's disease

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Submitted

ABSTRACT

Aim To determine whether extended olfactory testing within a single olfactory task and/or across olfactory tasks increases diagnostic accuracy of olfactory testing in Parkinson's disease (PD).

Methods Olfactory function was assessed using an extended version of the "Sniffin' Sticks", comprising 32-item odour identification and discrimination tasks, and a detection threshold task in 52 PD patients and 50 controls, all aged between 49 and 78 years. ROC curves based on sensitivity and specificity estimates were used to compare the diagnostic accuracy of extended and combined olfactory testing.

Results There was no significant difference in diagnostic accuracy between the 16-item and the 32-item versions of the odour identification or discrimination test. The single olfactory test that was best in discriminating between PD patients and controls was a 16-item odour identification test. A combination of the 16-item identification test and the detection threshold task had a significantly higher area under the curve than the 16-item odour identification test alone.

Conclusion Extended testing across, and not within, olfactory tasks increases diagnostic accuracy of olfactory testing in PD. A combination of an odour detection threshold task and a 16-item odour identification test had the highest sensitivity and specificity in distinguishing between PD patients and controls.

INTRODUCTION

Olfactory dysfunction is a frequent symptom in Parkinson's disease (PD). Even in early stage, untreated PD patients, deficits in olfactory function have been demonstrated,[79;81;160] which is supported by recent neuropathological studies demonstrating that the olfactory bulb and anterior olfactory nucleus may be among the induction sites of PD pathology.[19] In later pathological stages, the olfactory bulb and tract are among the brain regions where Lewy bodies and Lewy neurites are particularly abundant.[16] Impairments in the sense of smell may even precede the development of overt motor symptoms,[23;89;90] and prospective studies in first degree relatives of PD patients,[89] subjects with idiopathic hyposmia,[90] and in a large cohort of Asian men [91] have shown that olfactory loss is associated with an increased risk of developing PD. Olfactory testing could therefore be valuable in establishing an early diagnosis of PD when other clinical (motor) symptoms are not apparent yet, presumably before significant loss of dopaminergic neurons has already occurred.

Since the first study on olfactory deficits in PD, reporting an increase in odour detection thresholds,[78] it has become clear that most PD patients have olfactory disturbances that are not restricted to a single functional measure but also include impairments of odour discrimination and identification.[80-85] So far the only olfactory measure that does not appear to be independently impaired is odour recognition memory *(unpublished observations)*.

In order to reliably assess olfactory function in clinical practice, many psychophysical tests have been developed that provide a quantitative measure of olfactory function, such as the University of Pennsylvania Smell Identification Test (UPSIT) and the "Sniffin' Sticks". The UPSIT is a 40-item, forced-choice odour identification test, developed for the US population.[32] The "Sniffin' Sticks" is an olfactory test battery that can be used to assess three different aspects of olfactory function: Odour identification, discrimination and detection,[33] each consisting of 16 items. Odour detection threshold testing measures the lowest concentration of an odorant that can be perceived by a subject. Odour identification testing involves the perception and naming of an odour presented. An odour discrimination task measures the ability to differentiate between a set of odorants. The main differences between these the UPSIT and the "Sniffin'Sticks" are the number of items (within a single task) and the functions tested. We have previously observed in a large population of PD patients that an odour identification test is better at distinguishing PD patients from control subjects than an odour discrimination test and that adding a discrimination test to an identification test does not improve diagnostic accuracy.[182] Although this would suggest that combining multiple olfactory tests is not useful, this type of analysis was not performed for other combinations, in particular those including odour detection. Nor do we know whether the diagnostic accuracy of olfactory testing in PD

might be increased by extending the number of items within a test of a single olfactory measure.

The present study was set out to determine whether extended olfactory testing within a single test and/or a combination of tests involving different olfactory functions can increase diagnostic accuracy of olfactory testing in PD patients.

METHODS

Subjects

This study was performed in 52 non-demented PD patients (29 males and 23 females; mean age 61.8 years, range 50-73 years, Hoehn and Yahr (H&Y) stages I-III, disease duration 0-19 years), and 50 control subjects (27 males and 23 females; mean age 59.5 years, range 49-78 years). All PD patients were recruited from the outpatient clinic of the department of Neurology of the VU University Medical Center (VUMC) or via advertisements on PD-related websites on the internet. PD was diagnosed according to the United Kingdom Parkinson's Disease Society Brain Bank criteria.[155] Four patients were drug-naive. Of the remaining PD patients, two patients were treated with levodopa monotherapy, five patients were on dopamine-agonist monotherapy, 18 patients were treated with a combination of both levodopa and a dopamine agonist, and 23 patients used levodopa, a dopamine agonist, as well as other medication, including monoamine oxidase B (MAO-B) inhibitors, catechol-O-methyltransferase (COMT) inhibitors, anticholinergics and/or beta-blockers. For an overview of subjects characteristics, see table XIII. Medicated patients were tested in the 'ON' state, and all patients were rated for disease stage by means of the modified H&Y scale.[193] All control subjects were volunteers recruited among hospital employees and partners of patients and reported normal subjective olfactory function. All subjects reported no history of major chronic olfactory or (other) neurological disorders. All subjects provided written informed consent. The study was approved by the Medical Ethics Committee of the VUMC.

Table XIII. Subject characteristics.

	PD patients	control subjects
Age, in years; mean (SD)	61.8 (7.0)	59.5 (7.6)
Sex (M/F)	29/23	27/23
Disease duration, in years; mean (SD)	6.7 (4.3)	-
H&Y stage (1/1.5/2/2.5/3)	2/2/19/25/4	-
PD medication (levodopa/dopamine agonist/other PD medication) *	30/39/23	-

PD = Parkinson's disease; H&Y = Hoehn and Yahr

* Sum does not equal total number of PD patients due to subjects using a combination of medication

Olfactory function testing

An extended version of the Sniffin' Sticks test battery (Burghart, Wedel, Germany), which employs reusable felt-tip pens ("sticks") containing odorants dissolved in propylene glycol, was used.[33]

First, *odour detection thresholds* were assessed using a single-staircase, three-alternative forced-choice procedure, with a 1:2 dilution series of sixteen stages. Subjects were blindfolded and had to identify the odour-containing pen when presented with three pens, two containing the solvent and one the odorant (score 0-16). Subsequently, odorants 17-32 of the *odour identification* test were presented in suprathreshold intensity in a 4-alternative forced-choice format with verbal descriptors. Each stick was held approximately 2 cm in front of the nostrils for 2-3 sec, with an interval of 20-30 sec between each stick. Next, in the *odour discrimination* task, subjects were blindfolded again and presented with 32 odour-triplets, with an interval of 30 sec between each triplet. Each triplet consisted of two identical and one aberrant odorant. Subjects were asked to select the odd odour out of the three odorants presented, without the need to recognize or name the odours. Lastly, subjects were presented with odorants 1-16 of the odour identification test.

The order of olfactory testing was the same for all participants, with short breaks in between. Olfactory tests were administered birhinally in a quiet, well-ventilated room to avoid any background smell interfering with the test odours.

Data analysis

Olfactory scores were defined as the total number of correct answers. For the odour identification and discrimination tasks, test scores were calculated for the first 16-items in the task (the standard versions of the tasks) and for the total of 32-items (the extended versions of the tasks).

To determine whether the olfactory measures were independently impaired in PD patients when compared to control subjects, we used an analysis of variance (ANOVA) with 'group' as factor, and 'age', 'sex', and 'detection score' as covariates.

To establish whether extended olfactory testing within a single test is useful for discriminating between PD patients and control subjects, we plotted receiver operating characteristic (ROC) curves based on sensitivity and specificity estimates, and calculated the area under the curve (AUC) for the 16- and 32-item versions of the odour identification and discrimination tasks separately. To compare the diagnostic accuracy of the olfactory tests, we used a nonparametric analysis of the areas under the correlated ROC curves.[201]

To determine whether combining tests across different olfactory measures would further improve the diagnostic value compared to the best single olfactory test, we first converted olfactory test scores to standardized z-scores. Subsequently, we used the best single

olfactory test, added the other olfactory tests, and performed similar analyses as mentioned above for the single extended tasks.
ANOVAs were analyzed using SPSS 15.0 for Windows (Chicago, IL, USA); ROC curves were analyzed using Stata 10.0 (StataCorp LP, College Station, TX, USA).

RESULTS

Olfactory test scores

PD patients scored significantly worse than control subjects on each of the olfactory tests, also when corrected for age, sex and odour detection thresholds (see Table XIV).

Table XIV. Mean olfactory test scores for PD patients and control subjects, and areas under the curve (AUC) for the individual tests.

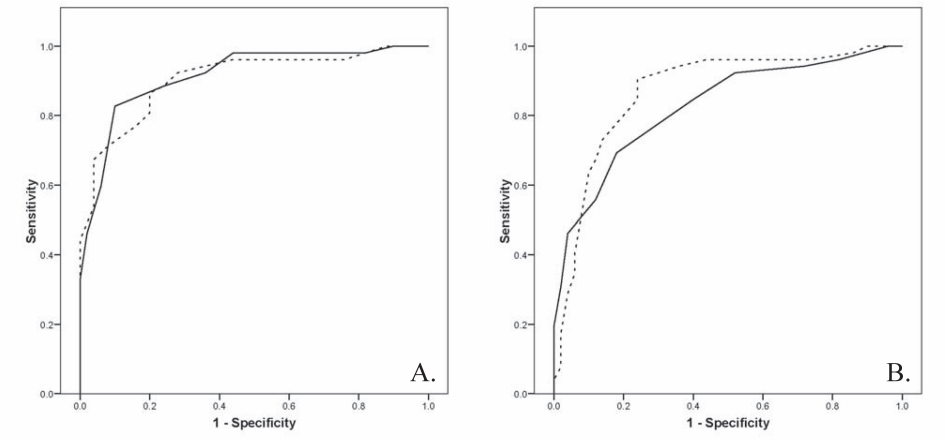
	PD patients	Control subjects	*p*-value	AUC
ID-16	7.0	12.3	< 0.001	0.91
ID-32	13.9	22.4	< 0.001	0.91
DIS-16	8.0	11.2	0.013	0.83
DIS-32	15.5	22.4	0.001	0.87
THR	2.5	7.9	< 0.001	0.90

PD = Parkinson's disease; ID-16 = 16-item identification test; ID-32 = 32-item identification test; DIS-16 = 16-item discrimination test; DIS-32 = 32-item discrimination test; THR = detection threshold test.
p-values correspond to an ANOVA with 'group' as factor, and 'age', 'sex', and 'detection score' as covariates.

Extended testing within a single olfactory test

ROC curves were plotted and corresponding AUCs were calculated for each olfactory test separately (see Table XIV). The 32-item odour identification test (AUC = 0.91, sensitivity 0.87, specificity 0.80) was not better at discriminating between PD patients and control subjects than the 16-item identification test (AUC = 0.91, sensitivity 0.83, specificity 0.90; p = 0.63; Figure 12A). The extended 32-item odour discrimination test (AUC = 0.87, sensitivity 0.90, specificity 0.76) was not better at discriminating between PD patients and control subjects than the 16-item discrimination test (AUC = 0.83, sensitivity 0.69, specificity 0.82; p = 0.09; Figure 12B).

Figure 12. Receiver Operating Characteristic (ROC) curves relating sensitivity and specificity estimates for the 16-item and 32-item versions of the odour identification and discrimination tests.



A. Odour identification test: Solid line represents the 16-item identification test; dotted line represents the 32-item identification test.

B. Odour discrimination test: Solid line represents the 16-item discrimination test; dotted line represents the 32-item discrimination test.

Extended testing across different olfactory measures

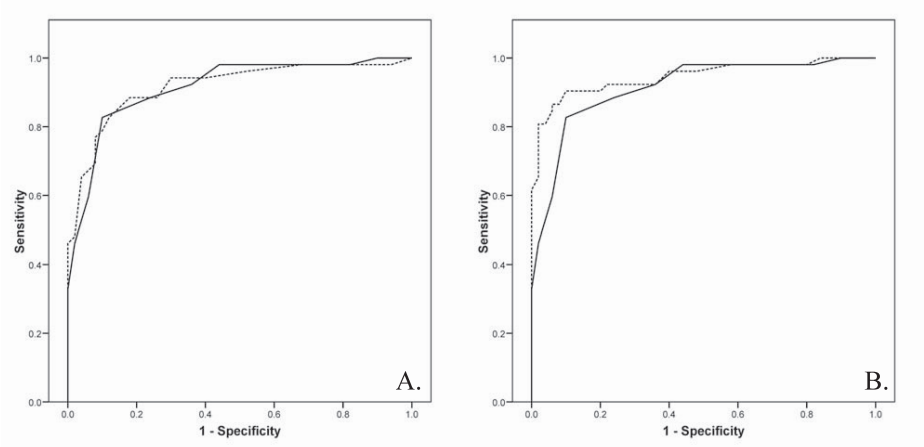
ROC curves were plotted and corresponding AUCs were calculated for combinations of olfactory tasks (using converted standardized z-scores) comprising the best single olfactory test (16-item odour identification test; see above) with the addition of one or more of the other olfactory tests (see Table XV). A combination of the 16-item odour identification test and the 16-item discrimination test (AUC = 0.91, sensitivity 0.81, specificity 0.90) did not significantly increase the AUC when compared to the identification test by itself (p = 0.80; Figure 13A). A combination of the 16-item odour identification test and the detection threshold test significantly improved the AUC compared to the single odour identification test (AUC = 0.95, sensitivity 0.90, specificity 0.92; p = 0.04; Figure 13B). Adding both the 16-item odour discrimination task and the odour detection threshold task to the odour identification test did not improve the AUC further (AUC = 0.94, sensitivity 0.81, specificity 0.96; p = 0.16 for comparison with identification testing only; Figure 13C).

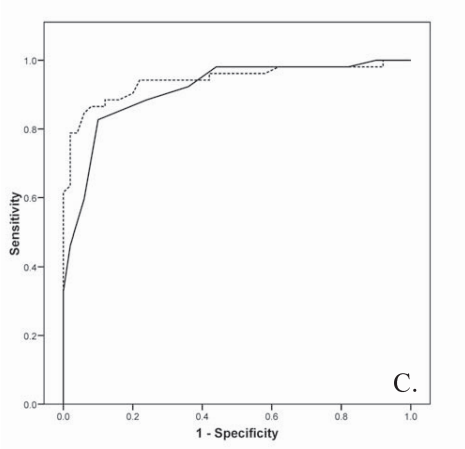
Table XV. Optimal cut-off z-scores, associated sensitivity and specificity estimates, and area under the curve (AUC) for combined tests, determined from ROC curves (Figure 13).

	Cut-off z-score	Sensitivity	Specificity	AUC
ID-16 + DIS-16	-0.148	0.81	0.90	0.91
ID-16 + THR *	-0.215	0.90	0.92	0.95
ID-16 + DIS-16 + THR	-0.736	0.81	0.96	0.94

ID-16 = 16-item identification test; DIS-16 = 16-item discrimination test; THR = detection threshold test.
* indicates that the AUC is significantly different from the AUC of the 16-item identification test alone (p-value < .05).

Figure 13. Receiver Operating Characteristic (ROC) curves relating sensitivity and specificity estimates for the combinations of olfactory tests.





A. Solid line represents the 16-item identification test; dotted line represents the 16-item identification test combined with the 16-item discrimination test.
B. Solid line represents the 16-item identification test; dotted line represents the 16-item identification test combined with the detection threshold test
C. Solid line represents the 16-item identification test; dotted line represents the 16-item identification test combined with the 16-item discrimination test and the detection threshold test.

DISCUSSION

The present study shows that extended testing within a single olfactory test (odour identification or discrimination) does not improve the diagnostic accuracy of olfactory testing in PD. A combination of an odour detection task and a 16-item odour identification task best discriminated between PD patients and control subjects.

Extended, 32-item, odour identification testing was not better at distinguishing between PD patients and control subjects than a 16-item identification task. Similarly, there was no significant improvement in diagnostic accuracy when comparing the 32-item with the 16-item version of the odour discrimination task, even though there was a trend towards an increase of the AUC. Apparently, 16-items are sufficient to detect olfactory deficits within

a single test, and increasing the number of items in the olfactory tests does not increase test accuracy of the Sniffin'Sticks. These findings do not necessarily imply that the diagnostic potential of the identification part of the "Sniffin' Sticks" is superior to the 40-item UPSIT. In order to reliably assess this, a direct comparison between the two tests is necessary, preferably in both healthy controls and PD patients. Additionally, since the UPSIT and "Sniffin' Sticks" do not consist of identical odorants, item analyses for the odour identification tests could result in a set of odours that increases the diagnostic accuracy of this test further.

In line with our previous observations in a larger group of PD patients, the present data confirm that adding an odour discrimination task to a combination of olfactory tests that includes an odour identification task does not further improve the diagnostic value of the olfactory test (battery).[182] By contrast, combining tests of odour identification and odour detection does improve the diagnostic accuracy of olfactory testing. Thus, the present findings support the notion that the olfactory impairment in PD is not based on a single common underlying deficit, such as an increased odour detection threshold,[34;37] but reflects a disturbance of multiple olfactory functions. This is further confirmed by our observation that odour identification and discrimination are impaired independent of increased odour detection thresholds in PD patients.

In this study, the best combination of olfactory tests to distinguish between PD patients and control subjects was a combination of the odour detection task and the 16-item odour identification task of the "Sniffin' Sticks" test battery. Not surprisingly, these two tasks had the highest AUC based on the individual ROC curves and, in addition, displayed the largest relative difference in test scores between PD patients and control subjects. These results partly correspond to recent findings by Lötsch et al., asserting that combined testing of several components of olfaction provides the most significant approach to the diagnosis of smell loss.[35] In their study, involving primarily healthy subjects with or without olfactory loss, these authors found odour detection thresholds to be the most important function to assess when screening for olfactory loss. The present findings, however, indicate that this is different when trying to distinguish between patients with Parkinson's disease and controls: The 16-item odour identification task is the best individual task to discriminate between PD patients and control subjects, which tallies with previous findings using the "Sniffin' Sticks".[159]

In contrast with the severe olfactory impairments in PD, olfactory function in most other degenerative (movement) disorders is either spared or only mildly affected.[86;98;202;203] Future studies will have to determine which combination of olfactory tests is most useful in the differential diagnosis between PD and other parkinsonian syndromes, such as multiple system atrophy and progressive supranuclear palsy, or Alzheimer's disease.

Conclusion

A combination of tests assessing different olfactory functions improves the diagnostic value of olfactory testing in PD to a greater extent than increasing the number of trials within a test of a single olfactory function. The best combination of olfactory tests to distinguish PD patients from control subjects is a combination of an odour detection task and a 16-item identification test.

Section III

Neurophysiological studies of olfactory function

Chapter 7

Signal-to-noise ratio of chemosensory event-related potentials

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ABSTRACT

Aim We investigated the influence of the number of stimuli on signal-to-noise (S/N) ratio of CSERP.

Methods CSERP from 20 normosmic subjects were obtained in response to stimulation with two olfactory (H_2S and PEA) and a trigeminal (CO_2) stimulant. For each of these odours, 160 stimuli were delivered into the right nostril (duration 200 ms, mean ISI 30 sec) using a constant-flow, air-dilution olfactometer. For each EEG recording site (Fz, Cz, Pz, C3, C4), peak-to-peak amplitude N1P2 and noise amplitude levels were determined. Subsequently, S/N ratios were calculated.

Results The S/N ratios for olfactory ERP generally improved for H_2S and PEA. For responses to PEA, S/N ratios increased significantly up to 80 averages (S/N ratio = 5.6). The number of stimuli for an optimal S/N ratio for trigeminal ERP was slightly lower, i.e. 60 averages (S/N ratio = 7.9).

Conclusion S/N N1P2 ratios in olfactory and trigeminal ERP significantly improve with an increasing number of responses averaged under these experimental conditions. This is mainly due to a reduction of noise level. Applying more stimuli has little additional effect on S/N ratio due to a concomitant decrease in signal amplitude.

Significance An optimal S/N ratio is essential when recording CSERP in neurodegenerative disorders, where responses may be of low amplitude, and for medico-legal purposes.

INTRODUCTION

In 1966, Finkenzeller, and in 1967, Allison and Goff first described cerebral potentials, which they assumed to be of olfactory origin. Measurement of chemosensory event-related potentials (CSERP) has since become a useful method to quantify olfactory function in a manner relatively independent of subjective biases (for review see [63]). Despite this long-standing use, methods for appropriate stimulation are still under debate, as olfactory ERP (OERP) components are affected by the same factors that influence ERP in other modalities, such as variations in interstimulus interval (ISI), stimulus duration, stimulus concentration, and type of stimulus.[67;204-207]

Since ERP reflect cognitive processing, attention has a major influence on their appearance.[208] Most subjects have difficulty maintaining vigilance and attention during long test sessions. Experiments should therefore not be excessively lengthy. Other than by choosing short ISIs, this can be achieved by minimizing the number of stimuli. However, little is known about the influence of the number of stimuli on CSERP latency, amplitude, and signal-to-noise ratio. According to previous research,[204;209] the absolute minimum number of averages per ERP is 8 records. Although this number of stimuli may produce meaningful results, there tends to be a high noise level. So far, this issue has not been investigated systematically.

The aim of the present study was to determine the number of stimuli that is required to obtain an optimal signal-to-noise ratio in recording of the general amplitude of the CSERP.

METHODS

Subjects

Twenty subjects (11 male, 9 female, aged between 15-35 years, mean age 23.9 years), recruited from the University of Dresden Medical School, were included in this study. Only subjects with normal olfactory function, as determined by administration of the odour identification part of the "Sniffin' Sticks" test battery,[33;40] were included. Subjects provided written informed consent. The study was approved by the Ethics Committee of the University of Dresden Medical School.

Test procedures

In a training session before the actual experiment, subjects were instructed to perform a special breathing technique (velopharyngeal closure) that avoids respiratory airflow in the nasal cavity during ERP recording [64] and were acquainted with the experimental condition. Subjects were installed comfortably in an air-conditioned room. They received white noise (approximately 50 Hz) through headphones to mask switching clicks of the stimulation

device. During the actual EEG experiment, subjects performed a tracking task on a computer screen in order to maintain vigilance and to reduce unwanted eye movements.[63] They were instructed to hold a white dot inside a larger, moving square using a joystick. Following each stimulus presentation, a visual analogue scale was presented on screen which subjects used to rate the intensity of the presented stimulus by moving a marker on the scale. The left hand end of the scale was defined as "no sensation" (0 estimation units [EU]), the right hand end as "maximum intense sensation" (100 EU).

Chemosensory event-related potentials (CSERP)

Chemosensory ERP were obtained in response to stimulation by two pure olfactory (H_2S, 6.8 ppm, and phenylethyl alcohol [PEA], 20% v/v) and one trigeminal (CO_2, 44% v/v) stimulant in suprathreshold concentrations. As only one odorant was used per session, the order of sessions was randomized across subjects to minimize possible sequence effects. During a single session, 160 stimuli of a stimulant were delivered into the right nostril (stimulus duration 200 ms, mean interstimulus interval 30 sec, range 25-35 sec) using an air-dilution olfactometer (OM6b, Burghart, Wedel, Germany). This olfactometer allows for application of rectangular-shaped chemical stimuli. Mechanical stimulation is avoided by embedding these stimuli in a constant flow of odourless, humidified air of controlled temperature (8 l/min, 36°C, 80% relative humidity). In addition to the training session, three sessions (one for each stimulant) were completed, each lasting approximately 90 min, with a short break after each 30 min of recording.

EEG was recorded from 5 positions of the international 10/20 system (Fz, Cz, Pz, C3, C4; see insert in Figure 14), referenced to linked earlobes A1 and A2 (bandpass filter 0.2-30 Hz; 8-channel EEG amplifier, SIR, Röttenbach, Germany). Possible eye blinks were registered from the Fp2 site. A 1500 ms post-stimulus period was recorded, as well as a 500 ms pre-stimulus period.

Data analysis

The raw data were divided into blocks of 20 consecutive stimuli. After removing trials containing artefacts (such as eyeblinks or motor artefacts), responses were averaged separately for each odorant. Peaks N1 and P2 were then marked for each recording site as defined by Kobal (see insert in Figure 14).[64] As peak latencies exhibit relatively large interindividual variability, the temporal search windows for components were set at 200-700 ms for N1, and 300-800 ms for P2.[209] Subsequently, peak-to-peak amplitudes N1P2 were determined. Noise levels were calculated as the average of two heuristically selected maxima and minima of spontaneous EEG during the 500 ms pre-stimulus interval. Dividing the N1P2 amplitude by the average noise level yielded the signal-to-noise (S/N) ratio.

ERP results were submitted to analyses of variance for repeated measures (rm-ANOVA) performed separately for amplitude N1P2 (A-N1P2), noise in the pre-trigger period, the signal-to-noise ratio for amplitude N1P2 (S/N N1P2), and for intensity ratings. Within-subject factors 'sequence' (averages for records 1-20, 1-40, 1-60, …, 1-160) and, in case of the ERP 'position' (recording sites Fz, Cz, Pz, C3, and C4) were used. 'Sex' was used as a between-subject factor. In order to have a more conservative measure of effects, degrees of freedom were corrected according to Greenhouse-Geisser. Only significant main effects will be reported plus significant interactions. Reporting significant effects of the factor 'recording site' was deemed not informative as, for example, the amplitude of ERP at the different sites is typically different;[63] however, interactions between this and other factors will be discussed, as this indicates different behaviour of the recordings over the various recording sites. Eta^2 (η^2)-values are presented for significant results of the ANOVAs as a measure of statistical power. Bonferroni tests were applied for post-hoc testing. The level of significance was set at 0.05. All analyses were performed using SPSS software (version 12.0; Chicago, IL, USA).

RESULTS

Chemosensory event-related potentials

Descriptive statistics for results obtained at position Cz are shown in Table XVI (see also Figure 14).

Trigeminal stimulation with CO_2

For A-N1P2 a significant effect of the factor 'sequence' was found ($F [7,98] = 16.3$, $p < 0.001$; $\eta^2 = 0.54$) suggesting that the amplitude decreased with the number of averages. Similar observations were made for the general noise level ($F [7,98] = 33.6$, $p < 0.001$; $\eta^2 = 0.71$). Specifically, noise levels for averages over trials 1-20 and 1-40 were significantly higher compared to all other averages, except for the comparison between noise levels for averages over trials 1-40 and 1-60. In addition, noise level for the average over trials 1-60 was significantly higher than noise levels for averages over trials 1-100, 1-120, and 1-160. Furthermore, a higher noise level was found for the average over trials 1-80 compared to the noise level for the average over trials 1-120.

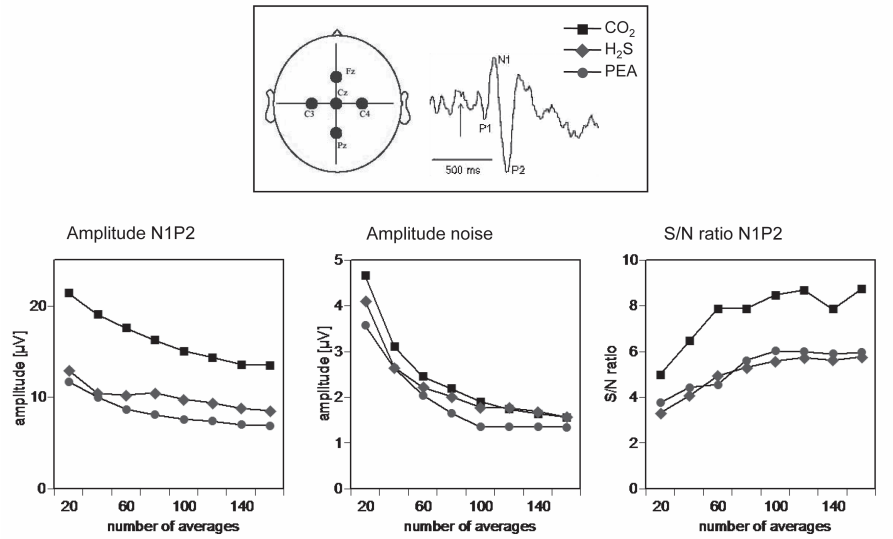
S/N N1P2 increased with the number of averages ($F [7,98] = 8.47$, $p < 0.001$; $\eta^2 = 0.38$). Post-hoc Bonferroni testing indicated that S/N N1P2 for the average over trials 1-20 was significantly smaller than that for all other averages, and S/N N1P2 for the average over trials 1-40 was still significantly smaller than that for the average over trials 1-120. All other pairs of S/N N1P2 were not significantly different from each other indicating that there was no further improvement of the S/N ratio from 60 trials onward (S/N ratio = 7.9). With

regard to the factor 'sex' generally higher S/N N1P2 was found in women compared to men (women: mean = 9.55, SEM = 0.78; men: mean = 7.11, SEM = 0.53; F [1,14] = 6.68, p = 0.022; η^2 = 0.32).

Table XVI. Descriptive statistics (means, standard errors of means [SEM]) of investigated parameters (A-N1P2 [in µV]; general noise level [in µV]; signal-to-noise ratio S/N N1P2; intensity ratings [in estimation units]), separately for responses to CO_2, H_2S and PEA obtained at recording site Cz.

Averages	A-N1P2		Noise level		S/N N1P2		Intensity	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Trigeminal stimulation (CO_2) - n > 18								
1-20	21.39	2.75	4.66	0.52	4.98	0.58	34.3	3.5
1-40	19.05	1.93	3.11	0.24	6.47	0.63	32.9	3.6
1-60	17.58	1.86	2.45	0.19	7.88	0.99	32.3	3.5
1-80	16.24	1.90	2.19	0.18	7.87	0.88	31.9	3.4
1-100	15.05	1.74	1.90	0.14	8.47	0.98	31.3	3.3
1-120	14.34	1.72	1.74	0.13	8.68	0.89	31.2	3.2
1-140	13.58	1.65	1.64	0.10	7.86	0.74	31.1	3.2
1-160	13.49	1.66	1.56	0.11	8.73	0.89	30.8	3.1
Olfactory stimulation (H_2S) - n > 17								
1-20	12.95	1.00	4.11	0.31	3.30	0.26	18.6	2.9
1-40	10.42	1.01	2.65	0.17	4.06	0.38	16.8	3.0
1-60	10.22	1.08	2.22	0.17	4.94	0.52	16.5	3.0
1-80	10.47	1.22	2.01	0.13	5.29	0.55	15.9	2.9
1-100	9.75	1.21	1.78	0.11	5.57	0.67	15.1	2.9
1-120	9.37	1.16	1.77	0.12	5.73	0.86	14.9	2.9
1-140	8.77	1.16	1.68	0.12	5.62	0.88	14.6	2.9
1-160	8.52	1.07	1.57	0.12	5.77	0.77	14.3	2.8
Olfactory stimulation (PEA) - n > 18								
1-20	11.72	1.02	3.57	0.30	3.76	0.45	18.0	3.3
1-40	10.00	0.72	2.64	0.22	4.42	0.49	16.2	3.0
1-60	8.67	0.71	2.04	0.13	4.55	0.48	15.8	2.9
1-80	8.09	0.71	1.65	0.14	5.60	0.67	15.4	2.9
1-100	7.57	0.74	1.35	0.08	6.02	0.70	15.1	2.9
1-120	7.37	0.72	1.35	0.13	5.99	0.67	14.9	2.9
1-140	7.00	0.67	1.35	0.11	5.90	0.83	13.2	2.5
1-160	6.87	0.68	1.34	0.12	5.97	0.88	13.0	2.5

Figure 14. Mean amplitudes N1P2 (left), general noise level (middle), and S/N ratio for amplitude N1P2 (right) with increasing number of stimuli at midline recording position Cz, in response to PEA (n > 18), H_2S (n > 17), and CO_2 (n > 18).



Please note the different scaling of the Y-axes. For standard errors of means see Table XVI. The olfactory event-related potential in the insert is an average over 160 trials in a single subject in response to H_2S.

Olfactory stimulation with PEA

A-N1P2 decreased in relation to the number of trials (F [7,105] = 23.2, $p < 0.001$; $\eta^2 = 0.61$), as did the noise level (F [7,105] = 56.4, $p < 0.001$; $\eta^2 = 0.79$). Interestingly, for A-N1P2 the change varied as a function of the recording position (F [28,420] = 3.43, $p = 0.005$; $\eta^2 = 0.19$). It was most pronounced for recording sites Pz and Cz, and least pronounced at recording site Fz.

Noise levels were significantly different between averages over trials 1-20, 1-40, 1-60, and 1-80 and all other averages.

S/N N1P2 increased with the number of trials (F [7,105] = 10.7, $p < 0.001$; $\eta^2 = 0.42$). Averages over trials 1-20 differed significantly from averages over trials 1-100, 1-120, 1-140, and 1-160, and averages over trials 1-60 were significantly different from those over trials 1-120. All other pairs of S/N N1P2 were not significantly different from each other. Thus, averaging more than 80 trials did not show further improvement of S/N N1P2 (S/N ratio = 5.6).

In addition, there was an interaction between factors 'sequence' and 'sex' (F [7,105] = 3.49, $p = 0.026$; $\eta^2 = 0.19$) indicating that the increase in S/N N1P2 was less pronounced in women compared to men, when up to 80 stimuli were used for averaging. The opposite occurred when more stimuli were utilized for averaging.

Olfactory stimulation with H_2S

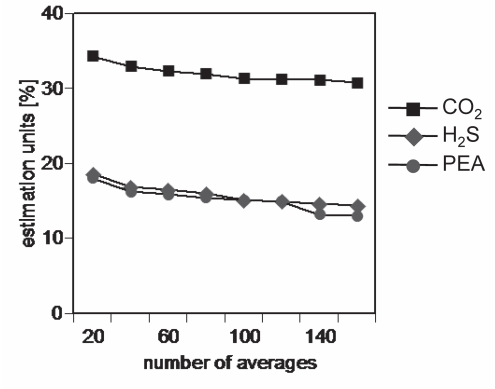
A-N1P2 in response to olfactory stimulation with H_2S decreased with increasing number of trials ($F [7,77] = 14.4$, $p < 0.001$; $\eta^2 = 0.57$). In addition, the general noise level decreased with averaging more stimuli ($F [7,77] = 36.8$, $p < 0.001$; $\eta^2 = 0.77$). Post-hoc Bonferroni tests indicated that noise levels for averages over trials 1-20 and 1-40 were significantly different from noise levels for all other averages, except for the comparison between noise levels for the averages over trials 1-40 and 1-60. In addition, noise level for the average over trials 1-60 stimuli was significantly higher than that for the average over trials 1-100.

A significant effect of averaging was found for S/N N1P2 ($F [7,77] = 4.28$, $p < 0.016$; $\eta^2 = 0.28$). However, post-hoc Bonferroni tests did not yield significant differences between S/N N1P2 when averaging 1-20, 1-40 or up to 160 trials.

Psychophysical data

The overall perceived intensity for CO_2 was rated higher than that of H_2S and PEA. When CO_2 was used, there was a trend towards a decrease in intensity ratings with an increase in the number of trials ($F [7,133] = 3.42$, $p = 0.061$; $\eta^2 = 0.15$) (Figure 15). This effect was significant for the olfactory stimuli PEA ($F [7,126] = 5.38$, $p = 0.023$; $\eta^2 = 0.23$) and H_2S ($F [7,126] = 4.12$, $p = 0.046$; $\eta^2 = 0.19$). When performing Bonferroni post-hoc testing, a significant difference between the various trials was present only for H_2S in the comparison of averages over trials 1-20 with that over trials 1-100.

Figure 15. Mean intensity ratings with increasing number of stimuli, for PEA (n > 18), H_2S (n > 18), and CO_2 (n = 20).



For standard errors of means see Table XVI.

DISCUSSION

The present study revealed that the S/N ratio of olfactory ERP significantly improves with an increasing number of stimuli. For PEA the optimal number of stimuli was found to be approximately 80. For H_2S, a significant effect of averaging was found, indicating an increase of S/N ratio for repeated averaging. However, no significant differences were found after post-hoc testing, probably because Bonferroni correction is too conservative an adjustment for this analysis. The optimal number of stimuli for the trigeminal ERP is slightly lower, i.e. 60 stimuli. These results are mainly due to a reduction of the noise level with increasing numbers of responses averaged and a concomitant decrease of signal amplitudes that is initially less pronounced. Further increases in the number of stimuli have little additional effect due to a subsequent parallel decline in both signal amplitudes and noise level, resulting in a plateau for S/N ratios. Since the different peaks of the ERP may represent different psychological processes, and thus show different physical features under different conditions, these findings hold true only for S/N N1P2 ratios in experiments under similar circumstances. For instance, the amplitude of the late positivity in CSERP studies increases with higher concentration,[210] longer duration,[206] prolonged ISI,[207] or when the stimulus is attended.[208]

In the present study, all identical stimuli were given within a single session, in order to maximize the number of consecutive stimuli of one odorant without exhausting the subject by a lengthy experiment. In future studies, multiple odorants can be applied in a randomized design within a single session – using the optimal number of stimuli determined in the present study – without the experiment becoming excessively lengthy. This may further reduce habituation and produce even better signal-to-noise ratios.

S/N ratio was generally larger in women compared to men. This relates to previous research indicating that women have larger ERP amplitudes in response to chemosensory stimuli than men.[211;212] In addition, men had a larger benefit from averaging over an increasing number of trials compared to women in terms of the S/N ratio which may be an expression of the idea that the S/N ratio reached an optimum in women while there was still room for improvement in the S/N ratio of men. Although not investigated in the present study, other factors like smoking [213] or hormonal status [214] can also be expected to affect the S/N ratio.

The present results compare only partly to previous work with regard to the improvement of the S/N ratio of ERP. The S/N ratio of ERP has been assumed to increase according to the formula $N/\sqrt{(N)}$ – N being the number of trials averaged – which implies a steady increase of the S/N ratio.[215] The present results, however, clearly indicated that a plateau is reached after averaging 60-80 stimuli. It has been argued that this discrepancy between theoretical and measured behaviour of the S/N ratio may be due to the increased

occurrence of artefacts or decrease in vigilance with accompanying slowing of the EEG that occur when the experiment prolongs.[216]

The observed decrease in signal amplitude with increased number of stimuli might be caused by adaptation or habituation to the stimulant – which is indicated by the decrease of the averaged intensity ratings, at least for olfactory stimuli. Considering the observation by Kobal,[64] using electro-olfactograms (EOG) from the nasal mucosa, that peripheral olfactory chemical receptors show hardly any adaptation at all, the reduction in responses must hence originate in central neuronal structures (habituation) (see also [217]). Alternatively, the decrease in signal amplitude may be brought about by jitter of individual ERP – meaning the temporal variation of peak amplitudes when recording repeated responses to an identical stimulus. This question was addressed through ancillary analyses where responses were analyzed for consecutive blocks of 20 stimuli each, i.e., the response to stimuli 1-20, 21-40, 41-60, and 61-80. Results from rm-ANOVAs conducted separately for the three stimulants and peaks N1 and P2 (and factor 'recording site) suggested that latencies did not change significantly in response to repeated stimulation ($p > 0.10$), which confirms and extends previous experiments on trigeminal ERP by Hummel et al.[218] From this result, it can be concluded that ERP peak latencies are relatively stable when up to 80 stimuli are presented, indicating that it is unlikely that jitter of ERP responses is responsible for amplitude decreases with increasing numbers of stimuli. Rather, the result supports the hypothesis that the signal rises more clearly from the noise with the process of repeated stimulation.

The pronounced decrease of response amplitudes has no behavioural equivalent, i.e., it is not evident from the psychophysical ratings that are reduced only mildly. This discrepancy between ERP data and psychophysical ratings might reflect differential mechanisms of habituation. CSERP amplitudes, as a measure of the objective physiological response of the olfactory or trigeminal system to a stimulus, may decrease rapidly as a result of habituation to the repetitive character of stimulation. Yet, intensity ratings, as a subjective measure of the effect of a stimulus on the organism as a whole, may be more resistant to habituation as a result of the influence of higher order cognitive and affective processes. In other words, this would suggest that, following repeated exposure to the same stimulus, a smaller number of neurons producing a smaller ERP amplitude are necessary to produce the same percept at the level of intensity; the observed discrepancy could be explained by a learning effect, such that cortical circuits are used more efficiently.

Conclusion

The results of the present study highlight the importance of using a sufficient number of stimuli when recording CSERP, which can be of particular value in patients with (neurological) disorders associated with olfactory impairments, such as Parkinson's disease, where responses might be reduced in amplitude. Moreover, these results will also

have an effect on the practical conduct of medico-legal investigations in patients with olfactory loss where measures of utmost reliability are required. Application of the present results is also highly valuable in experimental investigations aimed at localization of sources of olfactory ERP components, e.g. in magnetoencephalographic studies, where a high S/N ratio is of crucial significance.

Chapter 8

Advanced time-series analysis of MEG data as a method to explore olfactory function in healthy controls and Parkinson's disease patients

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Submitted

ABSTRACT

Aim To determine whether time-series analysis of magnetoencephalography (MEG) data is a suitable method to study brain activity related to olfactory information processing, and to detect differences in odour-induced brain activity between Parkinson's disease (PD) patients and controls.

Methods Whole head 151-channel MEG recordings were obtained in 21 controls and 20 PD patients during a 10-min olfactory stimulus paradigm, consisting of 10 alternating rest-stimulus cycles (30 sec each), using phenylethyl alcohol administered by means of a Burghart olfactometer. Relative spectral power and synchronization likelihood (SL; an unbiased measure of functional connectivity) were calculated for delta, theta, alpha1, alpha2, beta and gamma frequency bands.

Results In controls, olfactory stimulation produced an increase in theta power and a decrease in beta power. In PD patients there was a decrease in alpha1 power. No significant interaction between group and condition was found for spectral power. SL analysis revealed a significantly different response to olfactory stimulation in PD patients then in controls. In controls, the odour stimulus induced a decrease in local beta band SL. The response in PD patients involved a decrease in intrahemispheric alpha2 band SL.

Conclusion This is the first study to show that time-series analysis of MEG data, including spectral power and SL, can be used to detect odour-induced changes in brain activity. In addition, differences in odour-induced brain activity were found between PD patients and controls using analysis of SL, but not of spectral power. These differences may reflect olfactory dysfunction and abnormal olfactory information processing in PD patients.

INTRODUCTION

Olfactory dysfunction is a frequent symptom in Parkinson's disease (PD),[78;85] that may even precede the development of overt motor symptoms.[23;89-91] Pathological studies support these observations by demonstrating that the olfactory bulb and tract may be among the induction sites of PD pathology and show an abundance of Lewy bodies and Lewy neurites in later pathological stages.[16;19] The pathophysiology underlying the olfactory deficits in PD is far from being elucidated. In pathological studies, neuronal loss has been observed in the olfactory bulb and tracts of PD patients,[100] whereas others have reported a doubling of the number of dopaminergic neurons in the olfactory bulb.[102] Structural imaging studies have revealed disruption of the olfactory tract,[104] but no abnormalities of olfactory bulb volume.[105] A recent functional MRI study pointed to yet other brain areas that may be involved in PD-related olfactory dysfunction: After olfactory stimulation, neuronal activity in the amygdala and hippocampus was lower in PD patients when compared to control subjects.[106]

Another way to study olfactory information processing is by using electrophysiological techniques. When the brain processes a stimulus, two types of changes may occur in the electroencephalogram (EEG) or magnetoencephalogram (MEG): Evoked activities, which are exactly time-locked to the stimulus, and induced activities, which are changes in the EEG that are not phase-locked to the stimulus. The most basic approach to study the effects of olfactory stimulation was taken by Moncrieff.[50] He presented healthy subjects with different odours while recording their EEG, and found that several odours reduced alpha activity. Subsequent studies using EEG have found both increases and decreases of spectral power in almost all frequency bands upon olfactory stimulation.[51;53-59] Much of the variation in these studies can probably be attributed to differences in EEG recording techniques and conditions, and in the type and quality of odours presented. Olfactory evoked magnetic fields have been found bilaterally in the anterior-central parts of the insula, the parainsular cortex, the superior temporal sulcus,[72;75] and near the orbitofrontal sulcus.[76] A recent MEG study using frequency analysis combined with a beamforming technique, reported olfactory event-related desynchronization in the beta and gamma band, in the right precentral gyrus, frontal gyri, and the superior parietal lobe gyrus.[71]

In PD patients, electrophysiological studies have shown that olfactory event-related potentials have prolonged latencies when compared to controls, whereas amplitudes are similar.[80;108] MEG studies of olfactory information processing have so far not been performed in PD patients. However, advanced time-series analysis techniques of resting-state MEG data have been used in a number of recent studies in PD patients to show changes in both frequency distribution and functional connectivity between brain areas.[109-111] Furthermore, these same analysis techniques have proven their use in studying

resting-state data in a number of other neurological conditions,[112-115] but can also be applied to task-related MEG data.[115;116]

The aim of the present study was to determine whether time-series analysis of MEG data, including spectral power (as a measure of local synchronization) and synchronization likelihood (as a measure of functional connectivity), can be used to study olfactory information processing in healthy subjects, and also to detect differences in task-related brain activity during olfactory stimulation between PD patients and healthy controls.

METHODS

Subjects

23 healthy control subjects and 21 PD patients participated in this study. Due to considerable dental artefacts in the MEG recordings of two subjects, and technical problems during the MEG recording of one subject, the final study population consisted of 21 control subjects (9 male; mean age 56.3 years, range 49-73 years) and 20 PD patients (12 male; mean age 61.5 years, range 50-73 years; Hoehn and Yahr stage I-III). All PD patients were recruited from the outpatient clinic of the department of Neurology of the VU University Medical Center (VUMC) or via advertisements on PD-related websites on the internet. Parkinson's disease was diagnosed according to the United Kingdom Parkinson's Disease Society Brain Bank criteria.[155] Three patients were drug-naive. Of the remaining PD patients, two patients were treated with levodopa monotherapy, three patients were on dopamine-agonist monotherapy, five patients were treated with a combination of both levodopa and a dopamine agonist, and seven patients used levodopa, a dopamine agonist as well as other medication, including monoamine oxidase B (MAO-B) inhibitors, catechol-O-methyltransferase (COMT) inhibitors, anticholinergics, and/or beta-blockers. Medicated patients were tested 'ON' medication, and all patients were rated for disease stage by means of the modified Hoehn and Yahr scale.[193] Control subjects were volunteers recruited among hospital employees and partners of patients, and reported normal subjective olfactory function and no history of major olfactory or neurological disorders. All subjects underwent olfactory testing by means of the "Sniffin' Sticks" test battery.[33] Both an odour detection threshold score, and a composite TDI (threshold, discrimination, identification) score were used to assess olfactory function; higher test scores indicate better olfactory function.

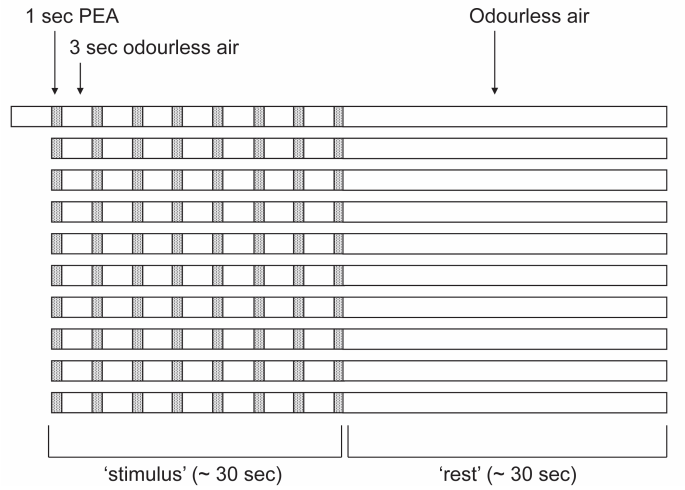
The study was approved by the Medical Ethics Committee of the VUMC, and all subjects gave written informed consent.

MEG data acquisition

MEG data were acquired using a 151-channel whole-head axial gradiometer MEG system (CTF Systems Inc., Port Coquitlam, BC, Canada). Average distance between sensors in this system is 3.1 cm. Patients were seated in a magnetically shielded room (Vacuum-schmelze GmbH, Hanau, Germany). The recording pass-band was 0–200 Hz with a sample rate of 625 Hz. A third-order software gradient was applied. At the beginning and at the end of the measurement, head position relative to the coordinate system of the helmet was recorded by leading small alternating currents through three position coils situated at the left and right pre-auricular points and the nasion on the subject's head.

MEG recordings were made during a 10-min olfactory stimulus paradigm, consisting of 10 alternating rest-stimulus cycles (30 sec each). Phenylethyl alcohol (PEA) was presented in a suprathreshold concentration (40% v/v) unilaterally into the right nostril using an air-dilution olfactometer (OM6b, Burghart, Wedel, Germany) asynchronous to breathing, for 1 sec every 4 sec during the 30 sec 'stimulus' period; during the 30 sec 'rest' period, subjects received odourless air (Figure 16). Mechanical stimulation was avoided by embedding the olfactory stimuli in a constant flow of odourless, humidified air of controlled temperature (8 l/min, 36°C, 80% relative humidity). All subjects were asked to breathe through their mouth to avoid respiratory airflow in the nasal cavity, to keep their eyes open and to avoid eye blinking or other ocular movement as much as possible. In addition, they received white noise (approximately 50 dBA) through headphones to mask switching clicks of the olfactory stimulation device.

Figure 16. A schematic representation of the olfactory stimulus protocol.



Phenylethyl alcohol (PEA, 40% v/v) was delivered for 1 sec every 4 sec during a 30 sec period in the 'stimulus' condition. During the 30 sec 'rest' period, subjects received odourless air. A total of 10 alternating rest-stimulus cycles were presented. Four sec of odourless air preceded the first stimulus condition; MEG recordings acquired during these four sec were not used in the analyses.

MEG data analysis

For each cycle, a rest and a stimulus epoch of approximately 6.56 sec (sample rate 625 Hz; 4096 samples per epoch) free of significant artefacts as detected by visual inspection, were selected. For further off-line processing and data analysis, epochs were converted to ASCII-files and imported into the DIGEEGXP 2.0 software package (CJ Stam, Amsterdam, the Netherlands). Subsequently, the MEG data were digitally filtered off-line with a band-pass of 1–48 Hz.

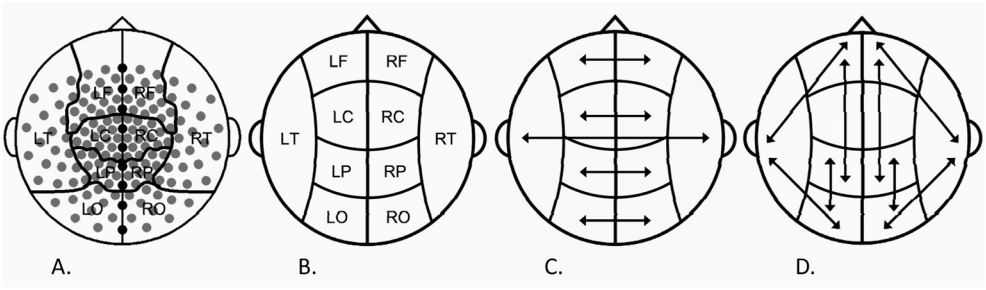
Relative spectral power was calculated in the following frequency bands: 1–4 Hz (delta), 4–8 Hz (theta), 8–10 Hz (alpha1), 10–13 Hz (alpha2), 13–30 Hz (beta) and 30–48 Hz (gamma). The MEG channels were grouped into regions of interest (ROIs), roughly corresponding to the major cortical areas (frontal, central, temporal, parietal and occipital) on the left and right side of the brain. The nine midline channels were left out of this clustering, leaving a total of 141 channels divided over 10 ROIs (Figure 17A and B). Furthermore, seven channels (one above the left frontal, the left occipital, the right central, the right frontal and the right parietal region, and two above the right temporal region) were excluded in all patients because of technical problems during the recordings in some of the patients. Power values for these channels were left out of the averaging, ensuring that the mean relative power in a ROI containing bad channels was not distorted. Fast Fourier Transformation was separately applied for every subject on all epochs in the previously mentioned frequency bands. Mean relative power averaged over all included channels was used in the primary statistical analysis ('overall spectral power').

Functional connectivity between all pair-wise combinations of MEG channels was computed with synchronization likelihood (SL).[219] SL is a general measure of the correlation or synchronization between two time series that is sensitive to linear as well as non-linear interdependencies. In case of total synchrony the value of synchronization likelihood is 1, while for completely independent systems it equals 0. Parameter settings used for SL computation were explicitly based on the frequency content of the data (for lags and embedding dimensions used, see [220]).

SL was computed for the same epochs as aforementioned, in the same frequency bands. Digital, zero-phase lag filtering was done off-line. MEG channels were grouped into (left and right) central, frontal, occipital, parietal and temporal regions, ROIs (Figure 17A). Midline sensors and the aforementioned channels containing artefacts were excluded from averaging. Ten local SL measures were computed per epoch by averaging the SL values of all possible sensor pairs within each ROI (Figure 17B). Five interhemispheric SL measures were computed per epoch by averaging the SL values of all possible sensor combinations between two homologous ROIs involved in the specific measure (Figure 17C). Eight intrahemispheric SL measures were computed per epoch by averaging the SL values of all possible sensor combinations between the two ROIs involved in the specific measure (Figure 17D). Within ROI (local) SL, between ROI intrahemispheric SL and between

ROI interhemispheric SL represent overall weighted averages (based on the number of possible sensor combinations) of the aforementioned specific SL measures.

Figure 17. Sensor clustering and selection of relative spectral power and synchronization likelihood (SL) measures.



A. Clustering of MEG sensors above major cortical areas; midline sensors were excluded from spectral power and SL analysis
B. Schematic representation of regions of interest (ROIs) used to calculate spectral power and short-distance local SL
C. Long-distance interhemispheric connections used to calculate SL
D. Long-distance intrahemispheric connections used to calculate SL
Arrows indicate SL connections used.
L = left, R = right, F = frontal, C = central, P = parietal, O = occipital, T = temporal.

Statistical analysis

For each frequency band separately, we used a multilevel general linear model with a compound symmetric covariance structure, with 'epoch' as level-1 units, and subjects as level-2 units to determine changes in overall relative power and overall SL measures (local, inter- and intrahemispheric SL) in response to the olfactory stimulus in both groups (PD patients and control subjects). 'Condition' (rest versus stimulus) was used as fixed factor. Parameters were estimated by the method of restricted maximum likelihood.

To determine whether PD patients responded differently to an olfactory stimulus compared to control subjects, we performed similar analyses, with 'condition', 'group' (PD patients and control subjects) and the interaction 'condition*group' as fixed factors.

When overall relative power, local, interhemispheric or intrahemispheric SL showed statistically significant effects within a frequency band for 'condition' or the interaction 'condition*group', we performed an exploratory post-hoc analysis for each ROI or short- or long-distance connection (Figure 17B-D) within the frequency band of interest to obtain an indication of the regional distribution of the effect.

To determine if there were differences in olfactory test scores between PD patients and control subjects, we used the univariate general linear model UNIANOVA, with 'group' (PD patients and control subjects) as factor, and corrected for 'age' (covariate) and 'sex' (factor).

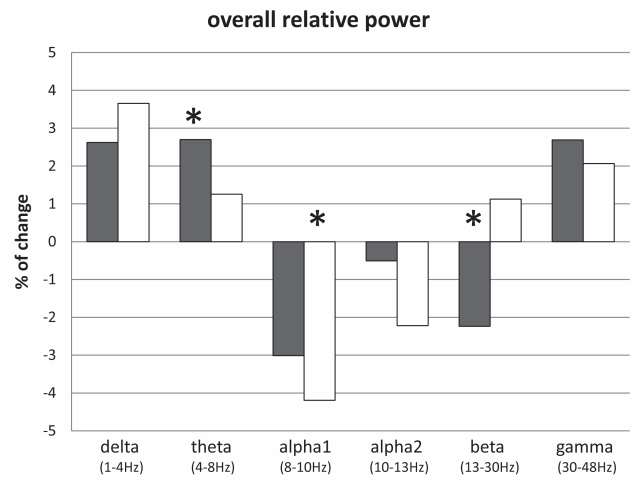
We studied the relationship between spectral power, SL and olfactory function by first subtracting overall relative power, local, interhemispheric and intrahemispheric SL in the rest condition from the same measures in the stimulus condition for all epochs and, subsequently, averaging these values for each subject. Pearson correlation coefficients were then computed to determine the correlation between the changes in relative spectral power or SL measures and odour detection threshold scores or composite TDI scores, measured with the "Sniffin' Sticks" test battery, for each group separately.
Data were analyzed using SPSS 15.0 for Windows (Chicago, IL, USA).

RESULTS

Relative spectral power

Control subjects and PD patients showed a similar pattern of changes in overall spectral power for the stimulus compared to the rest condition (Figure 18): A power increase in the lower frequency bands (delta and theta), a power decrease in alpha1 and alpha2 bands and a power increase in the gamma band. In the beta band, control subjects showed a power decrease, whereas PD patients showed an increase. The overall similarity in the patterns of changes in spectral power is reflected in the absence of a significant interaction effect for overall spectral power between group (PD and control subjects) and condition (rest and stimulus) in any of the frequency bands (Figure 18).

Figure 18. Percentage of change in relative spectral power (stimulus compared to rest condition), for each frequency band.



Grey bars represent control subjects, white bars represent Parkinson's disease patients.
* indicates *p*-value < 0.05, when comparing all rest and stimulus epochs in a multi-level statistical model

Statistical analysis revealed a significant increase in overall spectral power for the stimulus compared to the rest condition for control subjects in the theta frequency band (F [1,383] = 5.93, p = 0.015). Post-hoc analyses indicated that this increase in power was mainly over bilateral central and temporal regions (Table XVII a). Also in control subjects, a significant decrease in overall spectral power for the stimulus compared to the rest condition was found in the beta frequency band (F [1,383] = 5.98, p = 0.015). Post-hoc analyses indicated that this decrease in power mainly involved bilateral central regions, and the right temporal region (Table XVII b).

In PD patients, a significant decrease in overall spectral power for the stimulus compared to the rest condition was found in the alpha1 frequency band (F [1,366] = 5.59, p = 0.019). Post-hoc analyses indicated that this decrease in power mainly involved bilateral central and parietal regions and the left temporal region (Table XVII c).

Table XVII a. Relative spectral power in the theta band for control subjects.

Relative power theta (4-8 Hz)	rest	odour	*p*-value
overall	0.1689	0.1735	**0.015**
LC	0.1505	0.1556	**0.045**
LF	0.1982	0.2017	0.233
LO	0.1535	0.1557	0.390
LP	0.1394	0.1437	0.160
LT	0.1781	0.1836	**0.045**
RC	0.1461	0.1534	**0.006**
RF	0.2010	0.2060	0.104
RO	0.1559	0.1571	0.684
RP	0.1371	0.1418	0.120
RT	0.1809	0.1860	**0.045**

Table XVII b. Relative spectral power in the beta band for control subjects.

Relative power beta (13-30 Hz)	rest	odour	*p*-value
overall	0.3193	0.3119	**0.015**
LC	0.3817	0.3701	**0.006**
LF	0.2983	0.2911	0.092
LO	0.2999	0.2978	0.560
LP	0.3717	0.3664	0.287
LT	0.2664	0.2609	0.148
RC	0.3967	0.3859	**0.015**
RF	0.2978	0.2902	0.058
RO	0.3009	0.2948	0.075
RP	0.3832	0.3754	0.104
RT	0.2733	0.2662	**0.043**

Table XVII c. Relative spectral power in the alpha1 band for PD patients.

Relative power alpha1 (8-10 Hz)	rest	odour	*p*-value
overall	0.1174	0.1107	**0.019**
LC	0.1012	0.0941	**0.019**
LF	0.0903	0.0874	0.399
LO	0.1698	0.1600	0.051
LP	0.1353	0.1229	**0.002**
LT	0.1404	0.1312	**0.022**
RC	0.0851	0.0799	**0.034**
RF	0.0751	0.0732	0.403
RO	0.1610	0.1532	0.134
RP	0.1257	0.1143	**0.011**
RT	0.1262	0.1217	0.241

All *p*-values are determined by a multi-level model, comparing all rest and stimulus epochs of control subjects. Analysis of regional changes was performed to explore the distribution of the odour-induced changes within a frequency band.
A and B. After Holm-Bonferroni correction for multiple comparisons, regional *p*-values lost significance.
C. After Holm-Bonferroni correction for multiple comparisons, only the LP regional *p*-value remained significant.
L = left, R = right, C = central, F = frontal, O = occipital, P = parietal, T = temporal.

Synchronization likelihood

Local SL

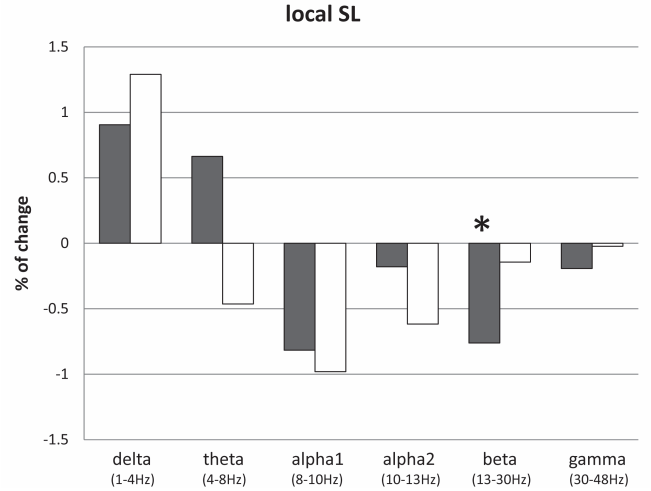
Control subjects and PD patients showed a similar pattern in local SL for the stimulus compared to the rest condition (Figure 19A): An increase in the delta band, and a decrease in the alpha1, alpha2, beta and gamma bands in functional connectivity were found for both control subjects and PD patients. In the theta band, control subjects showed an increase in local SL, whereas PD patients showed a decrease.

Statistical analysis revealed only a significant decrease in local SL for the stimulus compared to the rest condition for control subjects in the beta frequency band ($F [1,411] = 4.59$, $p = 0.033$). Post-hoc analyses indicated that this decrease in functional connectivity mainly involved connections within the left central and frontal regions (Table XVIII a).

In PD patients there were no significant differences in local SL for the stimulus compared to the rest condition in any of the frequency bands (Figure 19A).

There was no significant interaction effect for local SL between group (PD and control subjects) and condition (rest and stimulus) in any of the frequency bands (Figure 19A).

Figure 19A. Percentage of change in local synchronization likelihood (stimulus compared to rest condition), for each frequency band.

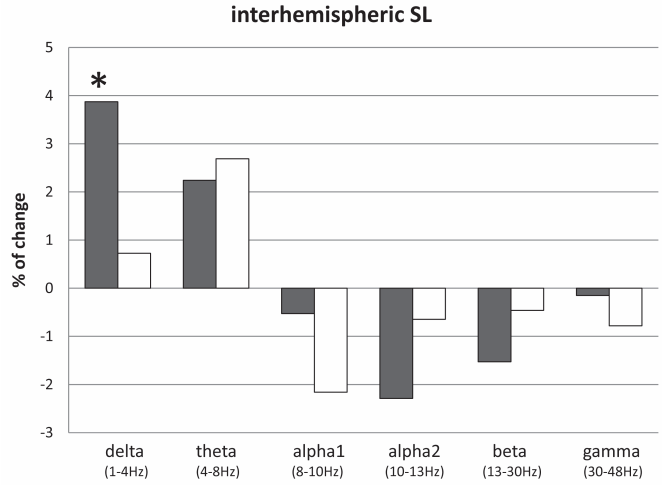


Grey bars represent control subjects, white bars represent Parkinson's disease patients.
* indicates *p*-value < 0.05, when comparing all rest and stimulus epochs in a multi-level statistical model

Interhemispheric SL

Control subjects and PD patients showed a similar pattern in interhemispheric SL for the stimulus compared to the rest condition (Figure 19B): An increase in the lower frequency bands (delta and theta), and decreases in the alpha1, alpha2, beta and gamma bands were found in both control subjects and PD patients.

Figure 19B. Percentage of change in interhemispheric synchronization likelihood (stimulus compared to rest condition), for each frequency band.



Grey bars represent control subjects, white bars represent Parkinson's disease patients.
* indicates *p*-value < 0.05, when comparing all rest and stimulus epochs in a multi-level statistical model.

Statistical analysis revealed only a significant increase in interhemispheric SL for the stimulus compared to the rest condition for control subjects in the delta frequency band (F [1,383] = 4.84, p = 0.028). Post-hoc analyses indicated that this decrease in functional connectivity mainly involved connections between the temporal regions of both hemispheres (Table XVIII a).

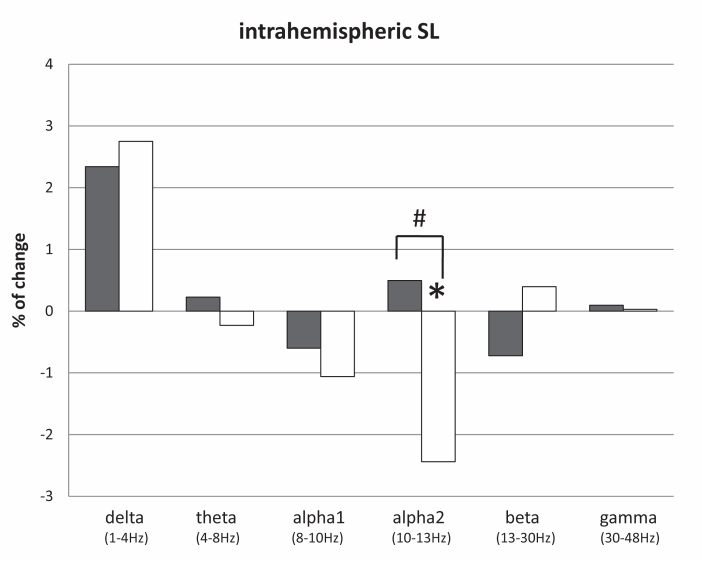
In PD patients, here were no significant differences in interhemispheric SL for the stimulus compared to the rest condition in any of the frequency bands (Figure 19B).

There was no significant interaction effect for local SL between group (PD and control subjects) and condition (rest and stimulus) in any of the frequency bands (Figure 19B).

Intrahemispheric SL

Control subjects showed an increase in alpha2 intrahemispheric SL and a decrease in the beta band, whereas PD patients showed the opposite pattern. Both control subjects and PD patients showed an increase in intrahemispheric SL in the delta band, and a decrease in the alpha1 band. There were no changes in theta or gamma band intrahemispheric SL (Figure 19C).

Figure 19C. Percentage of change in intrahemispheric synchronization likelihood (stimulus compared to rest condition), for each frequency band.



Grey bars represent control subjects, white bars represent Parkinson's disease patients.
* indicates p-value < 0.05, when comparing all rest and stimulus epochs in a multi-level statistical model;
# indicates p-value < 0.05 for the group*condition interaction in a multi-level statistical model

Statistical analysis revealed no significant differences in intrahemispheric SL for control subjects in any of the frequency bands. In PD patients, only the decrease in intrahemispheric alpha2 SL for the stimulus compared to the rest condition was statistically significant ($F [1,375] = 9.64$, $p = 0.002$). Post-hoc analyses indicated that this decrease in functional connectivity mainly involved fronto-parietal and fronto-temporal connections in the right hemisphere (Table XVIII c).

Furthermore, in the alpha2 band, a significant interaction effect for intrahemispheric SL between group (PD and control subjects) and condition (rest and stimulus) was found ($F [1,749] = 4.25$, $p = 0.039$; Figure 19C). Post-hoc analyses indicated that this interaction effect mainly involved fronto-parietal connections in the right hemisphere (Table XVIII c).

Table XVIII a. Local synchronization likelihood in the beta band for control subjects.

SL local beta (13-30 Hz)	rest	odour	*p*-value
local	0.1149	0.1142	**0.033**
LC	0.1188	0.1172	**0.009**
LF	0.1125	0.1110	**0.032**
LO	0.1252	0.1253	0.879
LP	0.2017	0.1997	0.103
LT	0.0887	0.0886	0.777
RC	0.1073	0.1071	0.753
RF	0.1076	0.1071	0.397
RO	0.1403	0.1394	0.091
RP	0.1583	0.1575	0.376
RT	0.0765	0.0764	0.669

Table XVIII b. Interhemispheric synchronization likelihood in the delta band for control subjects.

SL inter delta (1-4 Hz)	rest	odour	*p*-value
inter	0.0785	0.0822	**0.028**
inter C	0.0662	0.0655	0.710
inter F	0.0773	0.0804	0.266
inter O	0.0715	0.0737	0.336
inter P	0.0618	0.0633	0.583
inter T	0.0987	0.1078	**0.012**

Table XVIII c. Intrahemispheric synchronization likelihood in the alpha2 band for PD patients and control subjects.

SL intra alpha2 (10-13 Hz)	PD patients			Control subjects		Group * condition interaction
	rest	odour	*p*-value	rest	odour	*p*-value
intra	0.0252	0.0245	**0.002**	0.0247	0.0249	**0.009**
left FT	0.0269	0.0261	0.091	0.0270	0.0268	0.397
left FP	0.0208	0.0203	0.272	0.0204	0.0213	0.054
left PO	0.0318	0.0305	0.059	0.0296	0.0292	0.297
left TO	0.0254	0.0253	0.831	0.0253	0.0261	0.126
right FT	0.0261	0.0251	**0.012**	0.0255	0.0255	0.107
right FP	0.0188	0.0179	**0.016**	0.0184	0.0186	**0.038**
right PO	0.0312	0.0308	0.617	0.0276	0.0274	0.833
right TO	0.0227	0.0222	0.176	0.0229	0.0232	0.138

All *p*-values are determined by a multi-level model, comparing all rest and stimulus epochs of control subjects or Parkinson's disease (PD) patients. All *p*-values for group*condition interactions are determined by a multi-level statistical model, comparing the difference between all rest and stimulus epochs of control subjects with the difference between all rest and stimulus epochs of PD patients. Analysis of regional changes was performed to explore the distribution of the odour-induced changes within a frequency band. After Holm-Bonferroni correction for multiple comparisons, regional *p*-values lost significance.
L = left, R = right, C = central, F = frontal, O = occipital, P = parietal, T = temporal.
FT = fronto-temporal, FP = fronto-parietal, PO = parietal-occipital, TO = temporal-occipital.

Correlations with olfactory function

PD patients had lower mean olfactory test scores (based on odour detection threshold scores and composite TDI scores on the "Sniffin' Sticks" test battery) compared to control subjects, when corrected for age and sex (mean detection score control subjects = 8.0, PD patients = 2.3, F [1,37] = 44.7, $p < 0.001$; mean TDI score control subjects = 31.0, PD patients = 17.9, F [1,37] = 70.5, $p < 0.001$).
In control subjects, correlations were found between overall relative power and local SL in the alpha1 band and TDI scores ($r = 0.50$, $p = 0.020$, and $r = 0.44$, $p = 0.049$ respectively), and between intrahemispheric SL in the alpha1 band and odour detection threshold scores ($r = 0.47$, $p = 0.031$). In PD patients, no correlations were found between olfactory test scores and overall relative power or local, interhemispheric or intrahemispheric SL.

DISCUSSION

This is the first report using time-series analysis of MEG data to study odour-induced changes in spectral power and functional connectivity in controls and PD patients. The pattern of these changes in controls and PD patients was similar for spectral power, but differed for functional connectivity in the alpha2 band.

The present study showed a significant increase in overall theta band power and a decrease in overall beta band power for control subjects. Although different conclusions have been generated as to which frequency bands are involved in olfactory information processing due to variations in data analysis methods and stimulation paradigms for odour responses, our present data confirm previous findings in the theta and beta band in healthy subjects.[55;71;221] A study by Klemm et al. showed widespread increases in the theta band in response to a variety of odours, especially over the left anterior group of EEG electrodes and the right hemisphere.[55] Increases in evoked theta have been reported in response to various other sensory stimuli, such as visual and auditory stimulation.[222;223] The odour-induced changes in theta rhythm we observed are therefore probably associated with non-specific sensory processing.

Our findings for spectral power in the beta band are concurrent with a recent MEG study using intravenous application of odorous stimuli, reporting event-related desynchronization in the beta (and gamma) band, mainly in the right precentral gyrus, and superior and middle frontal gyri.[71] In addition, a study by Kemp et al. partly supports our findings, stating that elderly subjects with intact olfactory function displayed a decrease in beta band power in the odour condition, which was not specific to particular brain regions but rather an overall effect.[221]

A decrease in relative spectral power in the alpha1 band was seen in both controls and PD patients, although this reached significance only in the PD patients. All subjects were told beforehand that odorous stimuli would be delivered; however, PD patients may not have been able to perceive any odour during the whole of the experiment, due to their olfactory dysfunction. This might have resulted in an increased state of vigilance or attention ("searching the odour"), compared to control subjects, and therefore in a significant decrease in (centro-parietal) spectral power in the alpha1 band.[224;225] Another explanation for the findings in the alpha1 band might be derived from a series of studies by Lorig et al. who demonstrated a decrease in central alpha band power after low-concentration odorous stimulation in healthy subjects.[226;227] Possibly, suprathreshold odour exposure in PD patients who suffer from olfactory deficits is similar to low-level odour exposure in healthy subjects and thus induces a decrease in alpha power.

The absence of a significant difference in odorant-induced changes in spectral power between PD patients and control subjects in this study corresponds to the results of a previous study by Gori et al. that failed to find any alterations in the EEG of PD patients after olfactory stimuli compared to control subjects.[228] Spectral analysis apparently is not a suitable method to study differences in olfactory information processing between PD patients and controls.

Synchronization likelihood is a measure of the integration of neuronal activity between brain regions ('functional connectivity'), which is essential to normal brain function in a

resting-state condition, but also plays a key role in cognitive functioning. A number of recent studies have shown changes in functional connectivity between brain areas in the resting-state condition in various neurological disorders compared to healthy controls.[112-115] Moreover, analysis of functional connectivity has also proven a useful tool to study task-related brain activity, for instance in a working memory paradigm.[115;229] The present study showed that the analysis of functional connectivity is also a suitable method to study olfactory information processing, and moreover, to detect odour-induced differences in ongoing brain activity between healthy control subjects and PD patients. Olfactory stimulation induced a decrease in local SL in the beta frequency band in the control subjects and an increase in interhemispheric SL in the delta frequency band compared to the rest condition. Considering the decrease in the beta band in both overall relative power and local SL, this frequency band might be particularly sensitive to olfactory stimuli. Furthermore, an increase in functional connectivity between both hemispheres in the delta band was found in control subjects. A previous EEG study on odour-induced changes in functional connectivity revealed a decrease in coherence in the delta band in the frontal region.[52] However, findings on functional connectivity in the delta frequency band should be interpreted with caution, as slow-wave artefacts as a result of breathing or ocular movement that might have passed the visual inspection unnoticed.
The most interesting observation was an odour-induced decrease in intrahemispheric SL in the alpha2 band in PD patients, which was significantly different from the response to olfactory stimulation in control subjects. Apparently, in addition to the changes in functional connectivity in the resting state,[111] PD patients also have a defective functional coupling within hemispheres in a stimulus condition.

Correlations between olfactory function and changes in spectral power or functional connectivity were found in the alpha1 band, only for control subjects. The lack of a similar relationship between the scores on a psychophysical test and measures of local synchrony or functional connectivity in PD patients may be due to the differences in odour delivery between the psychophysical tests and the olfactory stimulation paradigm during the MEG registrations. The olfactory stimulus-paradigm used during MEG recordings is a 'passive' method of odour delivery, whereas psychophysical testing demands a more 'active' approach in order to perceive the odour. Since PD patients are reported to have difficulty sniffing,[107] the reduced sniff vigour in PD patients might (partly) explain the lack of a correlation in the present study between the neurophysiological parameters, as measured by MEG, and the psychophysical test scores.

Conclusion

In conclusion, the present study showed that time-series analysis of MEG data is a suitable method to detect odour-induced changes in brain activity. Furthermore, we demonstrated that there are differences in odour-induced functional connectivity, but not spectral power, between PD patients and control subjects.

General discussion

At present it is quite firmly established that olfactory dysfunction is one of the first clinical manifestations of Parkinson's disease (PD).[79;81] Bearing in mind the recently introduced Braak staging system (see Figure 1) in which the olfactory system is one of the induction sites of the neuropathological process in PD,[16] and considering that the onset of dopaminergic neuronal loss probably antedates the clinical diagnosis by about 3-7 years,[230] olfactory testing could be highly valuable in establishing an early diagnosis of PD when other clinical (motor) symptoms are not apparent yet. Also in the early clinical motor stages of PD, olfactory testing may contribute to the accuracy of the clinical diagnosis. In specific clinical situations, olfactory testing may help to differentiate between PD and other neurodegenerative disorders. Yet, still little is known about the involvement of the various specific olfactory modalities and their relationship to motor and other non-motor disease characteristics.

Furthermore, the pathophysiology of olfactory deficits in PD is far from being fully elucidated. Ultimately, we need to know how the known pathological changes contribute to the clinical olfactory deficits observed in PD. Therefore, olfactory imaging studies, structural as well as functional, are necessary to provide additional information.

From this perspective, the following research questions were addressed in this thesis:

- What is the prevalence and nature of impairments in the different specific olfactory modalities in PD and how do they relate to other (motor and non-motor) disease characteristics?
- Which (combination of) olfactory test(s) is best at discriminating PD patients from control subjects?
- Is it possible to explore the neurophysiological basis of olfactory (dys)function by means of MEG in healthy controls and PD patients?

PREVALENCE AND NATURE OF OLFACTORY DEFICITS IN PD

In order to assess the prevalence of olfactory deficits in PD in three different modalities (odour identification, discrimination and detection threshold), we formed a large sample of PD patients from three populations in Australia, Germany, and the Netherlands. In this study (**chapter 2**), age-independent criteria for hyposmia were first applied. These criteria had been derived previously from a group of 18-35 year old healthy subjects, considered to be the standard population in terms of normal olfactory sensitivity.[117] Using these age-independent criteria, only 3.3% out of a total of 400 PD patients were normosmic.

However, since olfactory function is age-related,[121] to reliably determine the prevalence of olfactory dysfunction in PD patients, normative values for a matched control population

are necessary. In addition to age, cultural influences also affect a subjects' performance on olfactory tests. While odour threshold values are not culture dependent,[129] performance on odour identification (and discrimination) tests relies on prior exposure to, and familiarity with the odours used.[130] Normative values for these tests may therefore be influenced by the cultural background of the reference population. In **chapter 1**, we provided age-specific normative values for the Dutch population (over 45 years of age) for the two culture-dependent components of the "Sniffin' Sticks" test battery: Odour identification and odour discrimination, and found them to be comparable to the German normative data for subjects over 55 years.[117] However, the values for the Ducth population were lower than the values recently reported for the Greek population,[128] which might be explained by cultural differences, such as a more important role of odours in the Greek cuisine.

Subsequently, in **chapter 3** we compared these normative values to a large population of Dutch PD patients from two university medical centres. The prevalence of an olfactory deficit in PD patients on either the odour identification or discrimination task in this study was 73%. Clearly, these figures are lower than those in **chapter 2,** obtained using age-independent normative values derived from healthy young subjects. This is not very surprising, since olfactory function declines with age, even in healthy subjects.[121] Also in **chapter 2**, when comparing the combined TDI scores of the (partly overlapping) multinational PD patients to (German) normative data in relation to the subjects' age,[117] the percentage of PD patients with an olfactory deficit was only 74.5%. Using a different olfactory test, the 40-item UPSIT (University of Pennsylvania Smell Identification Test), Doty et al. reported a much higher prevalence (90%) of olfactory dysfunction in PD.[85] This difference may be related to the method of individually matching PD patients with control subjects used by Doty et al. rather than to the olfactory test used, since Hawkes et al. reported olfactory dysfunction in 74% of PD patients, using the same 40-item UPSIT.[80] Apparently, taking all data in consideration, a significant minority of PD patients does not suffer from olfactory dysfunction.

It has been suggested that PD patients with intact olfactory function may have been misdiagnosed and in reality suffer from a different neurodegenerative disorder that is not accompanied by olfactory dysfunction.[231] Therefore, in this thesis, great care was taken to establish a precise diagnosis according to the United Kingdom Parkinson's Disease Society Brain Bank criteria.[155] Furthermore, although the Braak staging system suggests that olfactory bulb pathology is essential for a pathological diagnosis of PD,[16] this does not necessarily imply that olfactory dysfunction should be present in all PD patients. Intact olfactory function in many PD patients, as demonstrated in the studies presented in this thesis, could indicate that olfactory deficits in PD may require additional pathology in other brain areas. Moreover, the validity of the Braak staging system and the proposed topographical spreading of Lewy pathology has been questioned recently.[232-235] The results

of a post-mortem study demonstrated that the dorsal motor nucleus of the vagal nerve is not the induction site in all PD brains.[234] The same could also hold true for the olfactory bulb. Along this line of reasoning, there may be a subgroup of PD patients without Lewy pathology in the olfactory bulb and, hence, without clinical olfactory deficits. Studies relating pathological data to clinical olfactory data of PD patients are necessary to address this issue.

When focussing on the (age-independent) results from the individual olfactory tests in **chapter 2**, 85% and 87% of PD patients had deviant odour detection threshold or odour discrimination scores, respectively, whereas 96% scored outside the normosmic range for odour identification. Age-dependent results from **chapter 3** showed an impairment in odour identification for 65% of PD patients relative to the performance of controls, whereas 42% of patients were impaired on the odour discrimination task. These different percentages of impairment for the specific modalities indicate that the sensitivity of olfactory testing for a diagnosis of PD depends on the test used to establish this diagnosis. Reduced olfactory acuity may affect performance on other olfactory tasks and thus lead to an overestimation of the actual deficit on the olfactory task in question. It has been argued that olfactory detection thresholds should therefore always be assessed in addition to the specific olfactory modality under consideration and used in appropriate statistical analyses to correct for impairments in odour detection.[37] In **chapter 6** we showed that odour identification and odour discrimination are impaired independent of increased odour detection thresholds in PD patients. By contrast, the results described in **chapter 5** indicate that PD patients have a slight impairment of odour recognition memory that appears to be fully accounted for by an increased odour detection threshold. These findings argue against the notion that all olfactory impairments in PD would be based on a single common underlying deficit, such as an increased odour detection threshold.[34;37] The pyshophysical data presented in this thesis suggest that olfactory dysfunction in PD entails a disturbance of multiple, but not all, olfactory modalities.

RELATIONSHIP BETWEEN OLFACTORY (DYS)FUNCTION AND OTHER DISEASE CHARACTERISTICS

The results presented in this thesis reveal that there is a relatively large percentage of normosmic PD patients when using age-specific normative values. Therefore, olfactory function might contribute to the phenotypic characterization of PD patients. Consequently, we wanted to determine the relationship between the different olfactory modalities and other domains in PD, including general disease characteristics, and specific motor and non-motor features.

Odour identification performance in PD was found to be related to age and sex, but independent of disease duration or stage (**chapters 3 and 4**), which is in accordance with previous reports [85] and supported by the pathological staging system by Braak et al. that shows that the olfactory bulb is involved in the earliest pathological stages.[16]

The novel finding that odour discrimination performance decreases with disease duration in PD (**chapters 3 and 4**), partly relates to previous observations in a smaller sample of patients, in which disease stage and severity accounted for part of the variance in discrimination scores of PD patients.[81] In addition, others have shown that odour discrimination performance in PD patients improves after stereotactic neurosurgical treatment using deep brain stimulation,[154] concurrent with clinical motor improvement.

The differential characteristics of the odour identification and discrimination deficits in PD suggest that these olfactory modalities involve at least partly distinct components of the olfactory information processing system. Several imaging studies (in healthy controls) support this notion by demonstrating that olfactory functions are mediated by common, as well as task-specific regions in the brain.[42;44;45] Additionally, different cognitive components are involved in these two olfactory modalities: Working memory is critical when assessing odour discrimination, whereas language capacity or semantic memory is involved in identification (for review see [36]).

Our findings suggest that part of the PD patients may suffer from an odour identification deficit in the early phases of the disease, and develop an impairment in odour discrimination later on. This would also provide a further argument in favour of the notion that the olfactory deficits in PD may not solely depend on pathology in the olfactory bulb. Additional support for the latter notion comes from a recent fMRI study, pointing to other brain areas that may also be involved in PD-related olfactory dysfunction: the amygdala and the hippocampus.[106]

In the olfactory bulb, specific ensembles of activated glomeruli are activated by each odorant.[166] If Lewy pathology in the bulb somehow leads to a 'shift' in these representations, this could result in an alteration in recognition and thus in an incorrect identification of odorants. In odour discrimination testing, there is no need to recognize a specific odorant as such. Therefore, in PD, odour discrimination performance may be more resistant to olfactory bulb pathology than odour identification performance. Odour discrimination deficits may arise either with progressive degenerative changes in the olfactory bulb or, alternatively, when other brain structures become affected by the disease process. An independent progression over time of the different olfactory deficits in PD is compatible with a differential vulnerability to disease pathology. This could be investigated further, preferably by means of functional neuroimaging techniques and by studies relating pathological data to clinical olfactory data.

The presence of differential patterns of olfactory impairment might be related to other aspects of phenotypical heterogeneity among PD patients. This issue was addressed in **chapter 4**. Although odour discrimination deficits turned out to be related to disease duration, there were no other significant correlations between olfactory function and motor or (other) non-motor symptoms in PD, such as cognitive status, psychiatric complications, sleep or autonomic function. Moreover, as described in both **chapter 2 and 4** there were no significant differences with regard to olfactory test scores (as measured by TDI or separate olfactory modalities) between patients with different motor phenotypes (tremor-dominant, akinetic-rigid, postural instability gait difficulty or mixed). Consistent with previous reports,[79] we did not find a relationship between the use of dopaminergic medication and olfactory performance on the identification or discrimination task (**chapter 4**). In combination with the pathological observation that the number of dopaminergic neurons in the olfactory bulb is doubled in PD,[102] this strengthens the notion that the olfactory deficit in PD is independent of the dopaminergic deficit that is associated with the characteristic motor symptoms.

In the study described in **chapter 4** we did not test odour detection thresholds and therefore could not analyze the relationship between odour detection thresholds and other PD characteristics. Since a detection threshold test is often considered as a more peripheral measure of olfactory function, it may be hypothesized that such deficits are present in the early stages of the disease already and do not progress. However, since the experimental design of an odour detection threshold test resembles that of an odour discrimination test [33] and may therefore involve working memory, this should be explored carefully.

DIAGNOSTIC VALUE OF OLFACTORY TESTING IN PD

In considering the use of olfactory testing as a diagnostic procedure in patients suspected of suffering from PD, or as a component of screening strategies for the detection of PD in the premotor phase, the question arises which individual test or combination of tests would be best to use. As part of this thesis we aimed to determine which (combination of) olfactory test(s) is best at discriminating PD patients from control subjects. From the results described in **chapters 2 and 3** we learned that odour identification is more frequently impaired in PD than odour discrimination and odour detection, and that an odour identification test allows a better discrimination between patients and controls. As described in **chapter 5**, odour recognition memory did not appear to be independently impaired in PD. Consequently, odour recognition memory testing is not useful as a diagnostic tool to differentiate between PD patients and control subjects.

The most widely used odour identification test, the UPSIT, consists of 40 items, whereas we used a 16-item odour identification subtest of the "Sniffin' Sticks" test battery. Therefore, in **chapter 6**, we used extended versions of the odour identification and discrimination parts of the "Sniffin' Sticks" to determine whether this would increase diagnostic accuracy of olfactory testing in PD. We found that adding more items within a single olfactory modality did not results in a better discrimination between PD patients and control subjects. These findings do not necessarily imply that the diagnostic potential of the identification part of the "Sniffin' Sticks" is superior to the 40-item UPSIT. In order to reliably assess this, a direct comparison between the two tests is necessary, preferably in both healthy controls and PD patients. Additionally, since the UPSIT and "Sniffin' Sticks" do not consist of identical odorants, item analyses for the odour identification tests could result in a set of odours that increases the diagnostic accuracy of this test further. However, this type of analysis would most likely be influenced by cross-cultural differences [130] and therefore yield different sets of odours depending on the population tested, limiting its practical use.

In **chapter 6** we also demonstrated that, in contrast to the lack of effect of adding more items to a test of a single olfactory modality, combining different olfactory modalities did increase the diagnostic accuracy of olfactory testing in PD. The combination of an odour identification and a detection threshold task turned out to be the best in differentiating between PD patients and control subjects. It would be quite interesting to apply this combination of olfactory tests in a prospective study, to assess whether this combination is also the most useful to distinguish healthy controls from subjects in the presymptomatic phase of PD. Observations in an ongoing prospective study in a cohort of asymptomatic PD relatives [236] surprisingly suggest that performance on an odour discrimination task was the best predictor for developing PD. This finding, however, may be related to the small number of subjects developing PD in this study. In fact, analyzed separately, worse performance on each olfactory test (including also odour detection and odour identification) was associated with an increased risk of future PD.

The accuracy of the clinical diagnosis of PD is 90% at most.[237] Among the cases of clinically misdiagnosed PD, the most frequent causes are multiple system atrophy,[86;237-239] progressive supranuclear palsy,[86;92;237;239] and Alzheimer's disease (AD).[239] Although we did not provide direct data on the potential of olfactory testing as a tool for differential diagnosis in this thesis, testing of odour recognition memory may prove useful in distinguishing between PD patients and AD patients, since odour recognition memory appears to be impaired in AD,[98;240] even when corrected for odour detection thresholds,[198-200] and not in PD (**chapter 5**). This might be of particular value in screening for presymptomatic cases, when a definite diagnosis of AD or PD is not yet offered. Future studies directly comparing groups of PD and AD patients are necessary to confirm this.

Furthermore, in **chapter 4** we confirmed the previous observation that PD patients with a Parkin mutation have normal odour identification scores [168] and extended this observation by showing that the same may hold for PD patients with a DJ-1 mutation. These observations suggest that odour identification performance may be useful for differentiating between idiopathic PD and certain genetic forms of PD.
Although odour discrimination performance does not appear to play a significant role in distinguishing between PD patients and control subjects (**chapter 6**), this does not imply that this specific olfactory test could not be of significant value in distinguishing between different neurodegenerative disorders. Future studies will have to determine which combination of olfactory tests is most useful in the differential diagnosis between PD and other parkinsonian syndromes, such as multiple system atrophy and progressive supranuclear palsy.

NEUROPHYSIOLOGICAL STUDIES OF OLFACTORY FUNCTION

One of the aims of this thesis work was to explore the potential of recording olfactory event-related brain activity by means of MEG to serve both as a biological marker of impaired olfactory function in PD and as a means to study the pathophysiology of these olfactory deficits. As described in **chapter 7**, we first determined the number of chemosensory stimuli needed to obtain an optimal signal-to-noise (S/N) ratio for studying olfactory event-related responses by means of an olfactometer and EEG in healthy controls. The S/N ratio of olfactory and trigeminal ERP significantly improved up to 60-80 stimuli, mainly due to a reduction of the noise level with increasing numbers of responses averaged and a concomitant decrease of signal amplitudes. We then performed a pilot study applying our EEG results to MEG. In previous studies in healthy controls, olfactory event-related magnetic fields had been reported, albeit with a source distribution that varied from one research group to another.[72;74-76;241] In our pilot study in 23 PD patients and 22 controls, we were unable to obtain consistent olfactory event-related magnetic fields in each individual, not even in healthy subjects (*unpublished observations*). Possibly the orientation of olfactory sources is more radial than tangential to the skull surface. Since MEG is relatively less sensitive to radial sources, this would make EEG more suitable to detect olfactory event-related magnetic fields than MEG.[70] At the time of our pilot study, parallel studies in PD patients using time-series analysis MEG data had revealed important changes in functional interactions between brain areas in the resting state.[111;242] Therefore, we chose to shift focus of our studies of olfactory event-related brain activity towards time-series analyses of MEG data as a means to gain more insight in the neurophysiological basis of olfactory information processing deficits in PD.

In **chapter 8** we showed for the first time that time-series analysis of MEG data, including spectral power and synchronization likelihood, can be used to detect odour-induced changes in brain activity in healthy subjects. At present it is unclear whether these changes are specifically associated with olfaction, represent non-specific task-related effects of sensory processing, or are merely arousal-induced. With respect to spectral power, we found changes in the theta, beta and alpha1 band. Event-related desynchronization in the alpha and beta bands, similar to that in our findings, is generally interpreted as an electrophysiological correlate of activated cortical areas involved in processing of sensory or cognitive information.[243] Klimesch suggested that alpha and theta power respond in different and opposite ways, with alpha power decreasing in a task condition, and theta power increasing,[244] a pattern that corresponds to our findings during olfactory stimulation. An interesting focus for additional olfactory MEG research would be to localize the observed odour-induced changes in spectral power and functional connectivity by means of a beamforming technique (for a review and detailed description on beamforming techniques, see [245]), to determine whether they are confined to anatomical olfactory areas or have a distribution compatible with non-specific task-induced changes.

In other mammals, high-frequency gamma oscillations have been recorded from the olfactory bulb.[246] We were not able to detect changes in the gamma band, most likely because MEG is most suitable to detect activity from (tangential sources in) the cortex and provides limited information on activity from deep-lying structures such as the olfactory bulb.[70]

A slowing of resting-state oscillatory brain activity in PD patients has previously been described by means of spectral power analysis on MEG data.[109;110] Since the response in EEG or MEG to a stimulus depends on the level of ongoing activity, these resting-state changes might have an influence on task-related data obtained during olfactory stimulation in PD patients: The decrease in spectral power in control subjects during the olfactory stimulus might correspond to the decrease in power in a (s)lower frequency band in PD patients. However, those resting-state data were obtained in an eyes-closed condition, and are therefore not directly comparable to our task-related data from subjects with their eyes open, since eye-opening significantly influences the power spectrum.[247] The absence of a significant difference in odour-induced changes in spectral power between PD patients and control subjects in this thesis corresponds to the results of a previous study by Gori et al. that failed to find any alterations in the EEG of PD patients after olfactory stimulation compared to control subjects.[228] Spectral power analysis apparently is not a suitable method to study differences in olfactory information processing between PD patients and controls.

In addition, in **chapter 8**, differences in odour-induced brain activity were found between PD patients and controls using analysis of SL. These differences may reflect olfactory

dysfunction and abnormal olfactory information processing in PD patients. Apparently, in addition to the changes in functional connectivity in the resting state,[111] PD patients also have a defective functional coupling within hemispheres in a stimulus condition. Future olfactory imaging studies in otherwise healthy subjects with olfactory deficits unrelated to PD could reveal whether the observed changes in functional connectivity in PD patients are associated with olfactory dysfunction in general or, instead, related specifically to olfactory dysfunction in PD.

As SL measures statistical interdependencies *between* sensors within or across regions of interest (ROI), it is fundamentally different from spectral power within a ROI; spectral power within a ROI is an average of the local field potentials measured at each individual sensor within that ROI and reflects the synchronous activity of underlying populations of neurons. It is therefore not surprising that spectral power showed a different pattern of changes during odour stimulation compared to SL, in both PD patients and healthy controls.

In the study described in **chapter 8**, we used a 'passive' odour-delivery method, based on odour perception only. To further investigate the neurophysiological correlates of olfactory information processing, and in particular its dysfunction in PD, it would be interesting to perform similar experiments with the addition of several more complex olfactory tasks, such as odour identification or discrimination. This has been done previously in healthy controls by means of positron emission tomography (PET),[42;44;45] showing that olfactory functions are differentially mediated by task-specific regions in the brain. As argued above on the basis of the results of the studies presented in this thesis, odour identification deficits are presumably present in the early stages of PD, whereas odour discrimination performance may develop later in the course of the disease. Future MEG studies could reveal differential patterns of task-related connectivity changes during performance of these different olfactory tasks. Subsequently, a longitudinal approach could provide more insight in the (sequential) involvement of different brain regions and/or networks underlying olfactory dysfunction in PD.

CONCLUSIONS

In short, based on the studies presented in this thesis, the following conclusions can be drawn:

The impairment of olfactory function in PD involves multiple, but not all, olfactory modalities: Odour recognition memory is not independently impaired in PD.

Approximately 25% of PD patients do not have any impairment of olfactory function.

With the exception of odour discrimination, which is associated with disease duration, olfactory dysfunction in PD is not related to motor or (other) non-motor characteristics, indicating that olfaction is a largely independent feature of the disease process in PD.

Adding more items to a test of a single olfactory modality does not improve its diagnostic value in discriminating between PD patients and controls. By contrast, combining tests of different olfactory modalities improves the diagnostic accuracy of olfactory testing in PD. A combination of an odour detection threshold task and a 16-item odour identification test best distinguishes between PD patients and controls.

Time-series analysis of MEG data is a suitable method to study odour-induced changes in brain activity. In addition, this method can detect differences in odour-induced changes in brain activity between PD patients and controls using analysis of functional connectivity, but not of spectral power.

Summary

Olfactory deficits in Parkinson's disease (PD) were first empirically documented in 1975 by Ansari and Johnson. Over the ensuing years it has become clear that most PD patients have olfactory disturbances that are not restricted to a single functional modality. Even in early stage, untreated PD patients, deficits in olfactory function have been demonstrated, which is supported by recent neuropathological studies demonstrating that the olfactory bulb and anterior olfactory nucleus structures may be among the induction sites of PD pathology. In later pathological stages, the olfactory bulb and tract are among the brain regions where Lewy bodies and Lewy neurites, the characteristic neuropathological features of PD, are particularly abundant. Since impairments in the sense of smell may even precede the development of overt motor symptoms, olfactory testing could prove valuable in establishing an early diagnosis of PD when other clinical (motor) symptoms are not apparent yet. Also in the early clinical motor stages of PD, olfactory testing may be useful as a diagnostic tool, both for distinguishing between PD patients and controls, and in differentiating between PD and other neurodegenerative disorders. Furthermore, the pathophysiology underlying the olfactory deficits in PD is far from being elucidated.

The following research questions were addressed in this thesis:

- What is the prevalence and nature of impairments in the different specific olfactory modalities in PD and how do they relate to other (motor and non-motor) disease characteristics?
- Which (combination of) olfactory test(s) is best in discriminating PD patients from control subjects?
- Is it possible to explore the neurophysiological basis of olfactory (dys)function by means of magnetoencephalography (MEG) in healthy controls and PD patients?

Prevalence and nature of olfactory deficits in PD

The "Sniffin' Sticks" is a multimodal olfactory test battery that can be used to assess three different aspects of olfactory function: odour identification, discrimination and detection, each consisting of 16 items.

In **chapter 1**, we provided age-specific normative values for the Dutch population (over 45 years of age) for the two culture-dependent components of the "Sniffin' Sticks" test battery: odour identification and odour discrimination. In **chapter 3,** we used these age-dependent normative values to study the prevalence of deficits on the odour identification and discrimination task in a large population of Dutch PD patients from two university medical centres. The prevalence of an olfactory deficit in PD patients on any of the two tasks in this study was 73%. In **chapter 2**, we assessed the prevalence of olfactory

deficits (odour identification, discrimination and detection threshold) in PD in a large sample of PD patients from three populations in Australia, Germany, and the Netherlands. When we applied age-independent criteria for hyposmia, only 3.3% out of a total of 400 PD patients were normosmic. However, when applying age-specific criteria, as we did for the Dutch cohort in **chapter 3,** 25.5% of patients were normosmic. From these data we concluded that, apparently, a significant minority of PD patients does not suffer from olfactory dysfunction.

The results in **chapter 3** further demonstrate an impairment in odour identification in 65% of PD patients relative to the performance of controls, and an impairment in odour discrimination in 42% of patients. The results described in **chapter 5** indicate that PD patients have a slight impairment of odour recognition memory that appears to be fully accounted for by an increase in odour detection threshold. Taken together, these findings argue against the notion that the olfactory impairments in PD would be based on a single common underlying deficit, such as an increased odour detection threshold, but suggest that olfactory dysfunction in PD entails a disturbance of multiple, but not all, olfactory modalities.

Relationship between olfactory (dys)function and other disease characteristics

Since approximately 25% of PD patients do not appear to have olfactory deficits (see above), olfactory function might contribute to the phenotypic characterization of PD patients. Therefore, we wanted to determine the relationship between the different olfactory modalities and other PD characteristics.

The results described in **chapter 3** show that odour identification performance in PD is related to age and sex, but independent of disease duration or stage. By contrast, odour discrimination performance was found to decrease with disease duration in PD.

Chapter 4 addresses the relationship between olfactory impairment and other aspects of phenotypical heterogeneity among PD patients. Apart from the above-mentioned association between odour discrimination deficits and disease duration, there were no other significant correlations between olfactory function and motor or (other) non-motor symptoms in PD, such as cognitive status, psychiatric complications, sleep or autonomic function. Moreover, there were no significant differences in olfactory test scores (either measured as a combined test score or each of three olfactory modalities separate) between patients with different motor phenotypes (tremor-dominant, akinetic-rigid, postural instability gait difficulty or mixed (**chapters 2 and 4**).

Diagnostic value of olfactory testing in PD

The results of the studies described in **chapters 2 and 3** show that odour identification is more frequently impaired in PD than odour discrimination and odour detection, and that an odour identification test allows a better differentiation between patients and controls.

Odour recognition memory, was not independently impaired in PD (**chapter 5**), and is therefore not useful as a diagnostic tool to differentiate between PD patients and control subjects.

In **chapter 6**, we used extended versions of the odour identification and discrimination parts of the "Sniffin' Sticks" and found that adding more items within a single olfactory modality does not improve the diagnostic accuracy of these tests. By contrast, combining different olfactory modalities did increase diagnostic accuracy. A combination of an odour identification and a detection threshold task turned out to be the best in differentiating between PD patients and control subjects.

Neurophysiological studies of olfactory function

In **chapter 7**, we determined the number of chemosensory stimuli needed to obtain an optimal signal-to-noise (S/N) ratio for studying olfactory event-related responses by means of an olfactometer and electroencephalography (EEG) in healthy controls. The S/N ratio of olfactory and trigeminal event-related potentials significantly improved up to 60-80 stimuli, mainly due to a reduction of the noise level. However, in a pilot study involving both healthy controls and PD patients, applying our EEG results to MEG, we were unable to obtain consistent olfactory event-related magnetic fields (*unpublished observations*).

Therefore, we changed focus towards time-series analyses of MEG data instead, as a means to gain more insight in the neurophysiological aspects of olfactory information processing in healthy controls and the pathophysiology of olfactory dysfunction in PD. **Chapter 8** describes the results of a study in which we were able to show for the first time that time-series analysis of MEG data, including spectral power and synchronization likelihood (a general measure of functional connectivity between brain areas), can be used to detect odour-induced changes in brain activity in healthy subjects. In addition, we found differences in odour-induced changes in brain activity between PD patients and controls using analysis of functional connectivity, but not of spectral power. These differences in functional connectivity may reflect abnormal olfactory information processing in PD patients that leads to the clinically observed olfactory impairments.

General discussion

In the general discussion, the data presented in the various chapters of this thesis were combined and a consideration of the potential implications as well as future research perspectives was provided. The most striking observations from the first two sections of this thesis are A) that apparently approximately 25% of PD patients do not suffer from olfactory dysfunction, B) that the impairment of olfactory function in PD entails a disturbance of multiple, but not all, olfactory modalities, and C) that a combination of an odour detection threshold test and an identification test is the best in distinguishing PD patients from controls. Furthermore, differential characteristics of the odour identification

and discrimination deficits in PD suggest that these olfactory modalities involve at least partly differential components of the olfactory information processing system.

From the last section, we can conclude that time-series analysis of MEG data is a suitable method to study odour-induced changes in brain activity. In addition, differences in odour-induced functional connectivity were found between PD patients and controls. The results obtained may be used in future olfactory neuroimaging studies to further investigate the pathophysiology of olfactory dysfunction in PD, in particular moving beyond the mere administration of odorants to the use of more complex tasks, such as odour identification or discrimination.

Samenvatting

De eerste beschrijving van een reukstoornis bij de ziekte van Parkinson (ZvP) dateert uit 1975. Sindsdien is het duidelijk geworden dat Parkinson-patiënten reukstoornissen hebben die niet beperkt blijven tot een enkele modaliteit. Zelfs in de vroegste en onbehandelde stadia van de ziekte zijn reukstoornissen aanwezig, wat in overeenstemming is met de resultaten van recente post-mortem studies. De voor de ZvP kenmerkende neuropathologische veranderingen (Lewy lichaampjes en neurieten) worden als eerste waargenomen in het olfactoire systeem en het verlengde merg en breiden zich in de loop van de ziekte volgens een vast patroon uit over de hersenen. Omdat een afname van het reukvermogen vooraf kan gaan aan de motorische symptomen van de ZvP, kunnen reuktests een belangrijk onderdeel vormen van een toekomstige screeningbatterij voor de vroege (presymptomatische) detectie van de ZvP. Ook in vroege klinische stadia van de ZvP zouden reuktests kunnen bijdragen aan het onderscheid tussen patienten met de ZvP en gezonde controles, en aan het onderscheid tussen de ZvP en andere neurodegeneratieve aandoeningen. De pathofysiologie van olfactoire disfunctie bij de ZvP is echter nog verre van opgehelderd.

De volgende onderzoeksvragen komen aan de orde in dit proefschrift:

- Wat zijn de prevalentie en kenmerken van stoornissen van de verschillende aspecten van het reukvermogen bij de ZvP, en hoe zijn deze gerelateerd aan andere (motorische en niet-motorische) ziektekarakteristieken?
- Welke (combinatie van) reuktest(s) is het meest geschikt om patiënten met de ZvP te onderscheiden van gezonde controles?
- Is het mogelijk de neurofysiologische achtergrond van olfactoire (dis)functie te onderzoeken bij gezonde controles en Parkinson-patiënten, met behulp van magnetoencefalografie (MEG)?

Prevalentie en kenmerken van reukstoornissen bij de ziekte van Parkinson

De “Sniffin’ Sticks” is een multimodale olfactoire testbatterij, die gebruikt kan worden om drie verschillende aspecten van het reukvermogen te meten: geuridentificatie, -discriminatie, en -detectie, allen bestaande uit 16 items.

In **hoofdstuk 1** hebben wij leeftijdsafhankelijke normaalwaarden vastgesteld voor de Nederlandse populatie (van 45 jaar en ouder), voor de twee cultuur-afhankelijke onderdelen van de “Sniffin’ Sticks” testbatterij: geuridentificatie en geurdiscriminatie. Vervolgens zijn deze leeftijdsafhankelijke normaalwaarden in **hoofdstuk 3** gebruikt om de aanwezigheid van een stoornis van het geuridentificatie of -discriminatievermogen te meten in een grote groep Nederlandse Parkinson-patiënten uit twee academische

ziekenhuizen. Een stoornis op een van beide reuktaken kwam voor bij 73% van de patiënten.

In **hoofdstuk 2** hebben wij bij een grote groep Parkinson-patiënten uit drie landen (Australie, Duitsland en Nederland) vastgesteld dat, op basis van leeftijdsonafhankelijke normaalwaarden, slechts 3.3% van de 400 Parkinson-patiënten een normaal reukvermogen heeft. Bij gebruik van leeftijdsafhankelijke normaalwaarden bleek 25.5% van de patiënten een intact reukvermogen te hebben. Uit deze resultaten concluderen wij dat blijkbaar bij een significante minderheid van de patiënten met de ZvP het reukvermogen intact is.

De resultaten in **hoofdstuk 3** laten zien dat 65% van de Parkinson-patiënten een stoornis heeft van het geuridentificatievermogen, terwijl 42% van de patiënten slecht scoorde op de geurdiscriminatietaak. De resultaten beschreven in **hoofdstuk 5** maken duidelijk dat Parkinson-patiënten een geringe afname hebben in hun vermogen om geuren te onthouden en herkennen ten opzichte van gezonde controles, maar dit blijkt volledig te verklaren te zijn door een verhoogde geurdetectie drempel.

Uit bovenstaande resultaten kunnen we concluderen dat de stoornis van het reukvermogen bij de ZvP blijkbaar niet op één gemeenschappelijk onderliggende factor berust, zoals een verhoogde geurdetectie drempel, maar verschillende, doch niet alle, aspecten van het reukvermogen behelst.

Relatie tussen reukstoornissen en andere ziekteverschijnselen

Aangezien ongeveer 25% van de patiënten met de ZvP geen reukstoornissen heeft (zie bovenstaande), zou het reukvermogen kunnen bijdragen aan de fenotypering van Parkinson-patiënten. Om deze reden hebben we gekeken naar de relatie tussen de stoornissen van verschillende aspecten van het reukvermogen en overige ziekteverschijnselen.

Het vermogen om geuren te identificeren bleek bij Parkinson-patiënten gerelateerd te zijn aan leeftijd en geslacht, maar onafhankelijk van ziekteduur of ernst. Het vermogen om geuren te onderscheiden bleek echter wel gerelateerd te zijn aan ziekteduur (**hoofdstuk 3**).

Hoofdstuk 4 beschrijft de relatie tussen reukstoornissen en overige aspecten van de fenotypische heterogeniteit bij Parkinson-patiënten. Behalve de hierboven beschreven relatie tussen stoornissen van het geurdiscriminatievermogen en ziekteduur, zijn er geen significante relaties tussen het reukvermogen van Parkinson-patiënten en andere ziekteverschijnselen gevonden, zoals cognitieve stoornissen, psychiatrische complicaties, slaap, autonome functie of motorische functie. Bovendien bleken er geen significante verschillen te zijn in reukscores tussen patiënten met verschillende motorische fenotypes (tremor-dominant, akinetisch-rigide, houdings- en balansstoornissen (**hoofdstuk 2 en 4**).

Diagnostische waarde van reuktests bij de ziekte van Parkinson

In **hoofdstuk 2 en 3** is aangetoond dat het geuridentificatie vermogen vaker is aangedaan bij de ZvP dan het geurdiscriminatie- of geurdetectievermogen, en dat een geuridentificatietest beter onderscheid maakt tussen Parkinson-patiënten en gezonde controles. Het vermogen om geuren te onthouden en herkennen blijkt niet onafhankelijk gestoord te zijn bij de ZvP (**hoofdstuk 5**), en heeft dan ook geen waarde voor het onderscheiden van Parkinson patiënten en controles.

In **hoofdstuk 6** hebben wij verlengde versies van de geuridentificatie- en geurdiscriminatietaken van de "Sniffin' Sticks" gebruikt. Uit de resultaten kwam naar voren dat het toevoegen van meer items binnen een test van een enkele reukmodaliteit geen significant effect heeft op de diagnostische waarde van de test. In tegenstelling hiermee heeft het combineren van taken die verschillende aspecten van het reukvermogen testen wel een positieve invloed op de diagnostische waarde. Een combinatie van een geurdetectietaak en een geuridentificatietaak bleek het best in staat Parkinson-patiënten van gezonde controles te onderscheiden.

Neurofysiologisch onderzoek van het reukvermogen

In **hoofdstuk 7** hebben wij door middel van electroencefalografie (EEG) bij gezonde controles het aantal chemosensorische stimuli bepaald dat nodig is voor een optimale signaal-ruis verhouding. Deze signaal-ruis verhouding van zowel olfactoire als trigeminale 'event-related potentials' verbetert significant tot 60-80 stimuli, voornamelijk dankzij een afname van het ruisniveau als resultaat van het middelen van meer waarnemingen. Vervolgens zijn deze resultaten gebruikt in een pilot MEG studie bij gezonde controles en Parkinson-patiënten. Het bleek echter niet mogelijk om consistente veranderingen in magnetische velden te meten onder invloed van de aangeboden geurstimuli.

Derhalve hebben wij ons vervolgens gericht op het analyseren van locale synchronisatie van hersenactiviteit (frequentie-analyse) en functionele connectiviteit binnen en tussen hersengebieden (*synchronization likelihood*), om op deze manier meer inzicht te krijgen in de neurofysiologische processen die betrokken zijn bij het verwerken van olfactoire informatie bij zowel gezonde controles als patiënten met de ZvP (**hoofdstuk 8**). De belangrijkste en nieuwe bevinding van deze studie was dat zowel frequentie-analyse als berekening van *synchronization likelihood* bruikbaar zijn om geur-geïnduceerde veranderingen in de hersenen te meten. Bovendien werden verschillen gevonden in functionele connectiviteit, maar niet in frequentie-inhoud, onder invloed van geurstimuli tussen Parkinson-patiënten en gezonde controles. Deze verschillen in functionele connectiviteit vormen wellicht een afspiegeling van de verstoorde verwerking van olfactoire informatie bij de ZvP die leidt tot de objectief en subjectief aanwezige reukstoornissen.

Discussie

In de discussie wordt een overzicht gegeven van de resultaten van de diverse studies beschreven in dit proefschrift en worden suggesties voor toekomstig onderzoek gedaan. De meest opvallende bevindingen uit de eerste twee secties van dit proefschrift zijn dat A) ongeveer 25% van de Parkinson-patiënten geen reukstoornis hebben, B) dat de stoornis van het reukvermogen bij de ZvP verschillende, doch niet alle, aspecten van het reukvermogen omvat, en C) dat een combinatie van een geurdetectietaak en een geuridentificatietaak het best in staat is Parkinson-patiënten van gezonde controles te onderscheiden. Bovendien suggereren de verschillen in reukstoornissen bij de ZvP dat deze aspecten van het reukvermogen verschillende componenten omvatten van hoe reukinformatie in de hersenen wordt verwerkt.

Uit de laatste sectie van dit proefschrift is gebleken dat bepaalde MEG analyse methodes geschikt zijn om geur-geïnduceerde veranderingen in de hersenen te meten. Bovendien bleken verschillen in geur-geïnduceerde functionele connectiviteit tussen Parkinson-patiënten en gezonde controles aantoonbaar. Aan de hand van deze eerste resultaten kunnen vervolgstudies met behulp van MEG of andere beeldvormende technieken worden opgezet om de pathofysiologie van reukstoornissen bij de ZvP verder te onderzoeken, in het bijzonder door hersenactiviteit te meten tijdens meer complex reuktaken, zoals geuridentificatie en geurdiscriminatie.

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List of abbreviations

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LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
AUC	Area Under the Curve
CAMCOG	CAMbridge COGnition examination
CO_2	carbondioxide
CSERP	ChemoSensory Event-Related Potential
DIS	Discrimination task
EEG	ElectroEncephaloGraphy
ERP	Event-Related Potential
fMRI	functional Magnetic Resonance Imaging
H&Y	Hoehn and Yahr
H_2S	hydrogensulphide
ID	Identification task
ISI	InterStimulus Interval
LUMC	Leiden University Medical Center
MEG	MagnetoEncephaloGraphy
MMSE	Mini Mental State Examination
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	Magnetic Resonance Imaging
OERP	Olfactory Event-Related Potential
OI	Olfactory Impairment
PD	Parkinson's Disease
PEA	PhenylEthyl Alcohol
PET	Positron Emission Tomography
ROC	Receiver Operating Characteristic
ROI	Region Of Interest
S/N	Signal to Noise
SL	Synchronization Likelihood
TDI	sum score of Threshold, Discrimination and Identification task
THR	Threshold task
UPDRS	Unified Parkinson's Disease Rating Scale
UPSIT	University of Pennsylvania Smell Identification Test
VUMC	VU University Medical Center

Curriculum Vitae
List of publications
Dankwoord

CURRICULUM VITAE

Sanne Boesveldt werd geboren op 23 december 1980 in Amsterdam. Van 1992 tot 1998 heeft zij met goed gevolg het VWO doorlopen aan het Alkwin Kollege in Uithoorn. Aansluitend is zij begonnen aan de studie Medische Biologie aan de Faculteit der Aard- en Levenswetenschappen van de Vrije Universiteit in Amsterdam. Wegens interesse in de neurobiologie volgde zij de richting neurowetenschappen. Na een afstudeerstage bij het Nederlands Instituut voor Hersenonderzoek, waarvoor zij onder andere 3 maanden aan het 'Laboratory of Neuromodulation and Cognitive Processes', University P. & M. Curie in Parijs verbleef, behaalde zij in 2003 de academische graad van doctorandus.
Op 1 april 2004 kwam zij in dienst bij de afdeling Neurologie van het VU medisch centrum in Amsterdam. Onder leiding van prof.dr. E.Ch. Wolters, prof.dr. C.J. Stam en dr. H.W. Berendse deed zij promotieonderzoek naar reukstoornissen bij de ziekte van Parkinson, zoals beschreven in dit proefschrift. Tijdens deze periode werd haar belangstelling voor reukonderzoek gewekt, en heeft zij tevens 3 maanden stage gelopen bij de 'Smell and Taste Clinic' van de University of Dresden Medical School (onder leiding van prof.dr. T. Hummel).
Na haar promotie vertrekt zij naar Philadelphia voor een postdoc positie van 2 jaar bij het 'Cognitive Neuroimaging Laboratory' van Monell Chemical Senses Center, onder begeleiding van dr. J.N. Lundström.

LIST OF (PEER-REVIEWED) PUBLICATIONS

Boesveldt S, Haehner A, Berendse HW, Hummel T. Signal-to-noise ratio of chemosensory event-related potentials. Clin Neurophysiol 2007;118:690-695.

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Haehner A, **Boesveldt S**, Berendse HW, MacKay-Sim A, Fleischmann J, Silburn PA, Johnston AN, Mellick GD, Reichmann H, Hummel T. Prevalence of smell loss in Parkinson's disease – A multicenter study. *Submitted.*

Boesveldt S, de Muinck Keizer RJO, Wolters ECh, Berendse HW. Odor recognition memory is not independently impaired in Parkinson's disease. *Submitted.*

Boesveldt S, de Muinck Keizer RJO, Wolters ECh, Berendse HW. Extended testing across, not within, tasks raises diagnostic accuracy of olfactory testing in Parkinson's disease. *Submitted*.

Boesveldt S, Stam CJ, Knol DL, Verbunt JPA, Berendse HW. Advanced time-series analysis of MEG data as a method to explore olfactory function in healthy controls and Parkinson's disease patients. *Submitted*.

Verbaan D, **Boesveldt S**, van Rooden SM, Visser M, Marinus J, Macedo MG, Fang Y, Heutink P, Berendse HW, van Hilten JJ. Is olfactory impairment in Parkinson's disease related to phenotypic or genotypic characteristics? *Submitted.*

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