1

GENERAL INTRODUCTION
PARKINSON’S DISEASE

Parkinson’s disease (PD) is a neurodegenerative movement disorder, named after James Parkinson who described some of its characteristic symptoms in ‘An essay on the shaking palsy’ in 1817. The four cardinal motor symptoms of the disease are brady-hypokinesia, tremor, rigidity and postural instability. An important neuropathological feature of PD is the degeneration of dopaminergic neurons in the substantia nigra and ventral tegmental area, leading to a progressive loss of dopaminergic input to the striatum and mesocorticolimbic areas. Surviving neurons are characterized by the presence of cytoplasmic inclusion bodies, referred to as Lewy-bodies after the neurologist F.H. Lewy. The discovery that PD is associated with a reduction of dopamine levels in certain brain areas led to the introduction of treatment with levodopa, a precursor of dopamine, in the mid-1960’s. This so-called dopamine replacement therapy evolved over the ensuing years, with optimization of administration regimes and the introduction of decarboxylase inhibitors to prevent the peripheral conversion of levodopa to dopamine. Although levodopa treatment leads to a marked improvement in motor symptoms, this treatment remains purely symptomatic; no disease-modifying therapy is available for PD up to this date.

Thanks to major advances in our understanding of the pathogenesis of PD over the past fifteen years, we now know that the neurodegenerative process in PD is not limited to the midbrain dopamine system. Neuropathological changes extend beyond the nigrostriatal system and involve other neurotransmitter systems as well, including the cholinergic (basal nucleus of Meynert), serotonergic (dorsal raphe nuclei) and noradrenergic (locus coeruleus) systems. Furthermore, Lewy-body pathology has been demonstrated in many other areas, such as the dorsal motor nucleus of the vagal nerve, the amygdala, the thalamus and neocortical brain regions. The neuropathological process seems to follow a predictable topographical course, first involving the lower brain stem and olfactory system, and then gradually spreading to ultimately affect widespread cortical areas. From a clinical point of view, the presence of a variety of non-motor symptoms, in addition to the mostly dopamine-related motor symptoms, is increasingly acknowledged. These symptoms include among others olfactory deficits, fatigue, autonomic dysfunction, sleep disturbances, depression, anxiety, psychotic symptoms and cognitive impairment. PD is therefore no longer seen as a pure movement disorder, but nowadays regarded as a multisystem disorder.

COGNITIVE IMPAIRMENT IN PARKINSON’S DISEASE

Mild cognitive impairment is common in PD, with an estimated cross-sectional prevalence ranging between 19 and 51%, and often already present at the time of the clinical diagnosis of PD. Substantial heterogeneity in the range of early-stage cognitive deficits exists, the most prevalent impairments involving memory, attention, visuospatial and/or executive functions. So far it remains unclear whether specific cognitive profiles are associated with a higher risk
of conversion to dementia. In some studies an association between impaired posteriorly located cognitive functions and increased dementia risk was found,\textsuperscript{19-21} while in other studies fronto-striatal executive deficits had predictive value for the development of dementia too.\textsuperscript{22-24} The development of PDD greatly affects the quality of life of PD patients and increases caregiver burden, health-related costs and the chances of being placed in a nursing-home.\textsuperscript{26-28}

Despite the improved awareness and growing attention for cognitive impairment and dementia in PD, the underlying pathophysiological mechanisms are still poorly understood. A number of neuropathological and neurochemical changes are thought to be associated with the development of cognitive impairment in PD, not surprisingly including the central dopaminergic denervation. In fact, dopaminergic deficits were the focus of attention in the first investigations trying to understand the neurochemical basis of cognitive dysfunction in PD.\textsuperscript{29,30} Early-stage cognitive impairment in PD, particularly executive dysfunction, is associated with fronto-striatal dopaminergic deficits as a result of nigrostriatal and mesocortical dopamine denervation.\textsuperscript{20} Dopaminergic medication may either improve or impair these early-stage cognitive impairments depending on the nature of the task and the baseline level of dopaminergic activity.\textsuperscript{31} The so-called overdose theory for cognitive function in PD entails that medication doses sufficient to restore dopamine function in the most-affected striatal region (i.e. dorsal striatum) may be excessive for less-affected parts of the striatum (i.e. the caudate nucleus and ventral striatum).

In addition to the loss of dopamine, cholinergic deficits have been reported at the level of the frontal and temporal cortex early in the course of PD.\textsuperscript{32,33} These cholinergic deficits are due to degeneration of the basal forebrain cholinergic nuclei and ascending cholinergic pathways.\textsuperscript{20,32,34} Neuropsychological correlates of these cholinergic deficits include impairments in memory and visuospatial abilities.\textsuperscript{35-37} Lastly, a parallel noradrenergic deficit, stemming from degeneration of the locus coeruleus, may also contribute to cognitive impairment in the early stages of PD.\textsuperscript{20} In spite of the prominent involvement of dopaminergic, other monoaminergic and cholinergic systems, this by itself is not considered sufficient to explain the progressive cognitive decline, ultimately leading to dementia, which occurs in part of the PD population. Clinicopathological studies have demonstrated an association between the extent of cortical Lewy body pathology and the severity of dementia in PD.\textsuperscript{38,39} From a neuropathological point of view it would therefore seem plausible that progression to PDD is caused by the spreading of Lewy body pathology to limbic and neocortical brain areas. In line with this assumption, neuroimaging studies have shown substantial atrophy throughout the brain in PDD. Specifically, PDD was associated with a loss of gray matter volume in the (para)limbic system, including the amygdala, hippocampus and parahippocampal cortices, and the anterior cingulate cortex.\textsuperscript{40-42} In a substantial proportion of patients, however, the correlation between Lewy body pathology and the severity of cognitive symptoms is poor.\textsuperscript{43} This implies that the relationship between
neuropathological changes and cognitive decline is not straightforward. It has been suggested that synaptic dysfunction rather than neuronal loss per se might be of importance. 44 From the above it will be clear that, in spite of a rapid increase in knowledge, the exact relationship between cognitive decline in PD and the underlying neuropathological and neurochemical substrates is far from being fully elucidated. Not surprisingly, therefore, effective treatment options for cognitive decline and dementia in PD are lacking. To date, symptomatic treatment with cholinesterase inhibitors is the only approved treatment for dementia in PD, with modest benefit in at least the early stages of dementia. 45 A better understanding of the pathophysiological processes involved in cognitive dysfunction and the development of dementia in PD is urgently needed, in particular for the development of future targeted treatment strategies. As will be discussed in the following paragraphs, functional brain imaging techniques provide us with an opportunity to study the pathophysiological processes involved in PD-related cognitive decline in great detail in a completely non-invasive way.

NEURAL OSCILLATIONS AND RESTING-STATE BRAIN ACTIVITY

Information is continuously being processed and transported within and between the many different regions of our brains. A crucial part of this processing is accomplished by neurons; brain cells specialized in signal reception and transmission. In individual neurons, activity can appear either as fluctuations in membrane potential or as action potentials, which subsequently lead to activation of other (post-synaptic) neurons. At the level of neuronal ensembles, the (synchronized) activity of large numbers of neurons is usually observed as repetitive, rhythmic neural activity, i.e. macroscopic oscillations.

Oscillatory brain activity is traditionally classified into different frequency bands, which include the delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz) and gamma (30–70 Hz) bands. 46 Oscillatory brain activity can be detected outside the human head by means of functional brain imaging techniques such as electroencephalography (EEG) and magnetoencephalography (MEG; see box 1). In functional magnetic resonance imaging (fMRI; see box 1), brain activity is measured in an indirect way by detecting changes in blood oxygen level (the blood-oxygen-level-dependent (BOLD) contrast) induced by fluctuations in neuronal activity. 47, 48 Low frequency fluctuations in the BOLD signal are used as input for the fMRI analysis; i.e. this signal is not decomposed into different frequency bands, but analyzed in its entirety, with an emphasis on low frequency (0.01-0.1 Hz) components.

Oscillatory brain activity has been linked to many cognitive functions such as information transfer, perception, executive control and memory. 49 When studying cognition, it would seem rational to measure brain activity during a mental task, and task-based experiments have indeed produced quite important results. However, widespread intrinsic processing is continuously taking place in the brain, even in the absence of external stimuli. In recent years, the study of resting-state brain activity has received increasing attention. 50 We now
Box 1 Functional brain imaging techniques explained.

**Magnetoencephalography (MEG)** records the electromagnetic fields that are generated outside the human head as a result of the simultaneous input to large numbers of neurons. MEG can measure variations in brain activity at the scale of milliseconds, i.e. it has a high temporal resolution. It is a completely non-invasive and silent technique that does not require radiation or the application of strong magnetic fields. Since the weak magnetic fields generated by the brain are measured from the outside of the head, there is uncertainty about the exact sources of the underlying neuronal activity. Mathematical techniques are used to estimate the activity of the underlying sources and thus provide a solution for this so-called inverse problem. \(^54-56\) Source activity is reconstructed with variable spatial resolution, \(^57\) the highest resolution being achieved at the cortical level. \(^58\)

**Functional magnetic resonance imaging (fMRI)** measures brain activity in an indirect way by detecting changes in blood oxygen level (the blood-oxygen-level-dependent (BOLD) contrast) induced by fluctuations in neuronal activity. \(^47,48\) As neurons do not have an internal energy reserve, they are completely dependent on the blood stream to provide them with oxygen whenever it is needed. When a brain area becomes more active, the metabolic need for oxygen in this area will increase. The vasculature of the brain responds to this need by releasing more oxygen into the tissues than is really necessary. The change in the ratio between oxygenated and de-oxygenated hemoglobin induces a fluctuation of the magnetic field of the brain tissue over time, which is measurable with fMRI. fMRI can localize brain activity at a high spatial resolution (i.e. in the order of 1 millimeter) both at the cortical and subcortical level, but, in contrast to MEG, only at a relatively low temporal resolution.

In this thesis, both MEG and fMRI are used complementary to each other to study resting-state brain activity patterns in PD, thereby combining the advantages of both techniques.

know that the resting-state of the brain represents a condition in which the brain is not idle, but rather displays robust, characteristic patterns of activity that are associated with cognitive functions and can be disrupted in disease conditions. \(^48,51-53\) The studies in this thesis therefore focus on resting-state brain activity, also because task performance and understanding task instructions can be difficult in patients with cognitive impairments.

## A NETWORK PERSPECTIVE ON HUMAN BRAIN FUNCTION

Although individual brain regions are often associated with specific functions, each brain region needs to continuously exchange information with other regions. Hence, together they form one integrative network of functionally (and structurally) linked regions. By exploring our brain as a complex communication network, we can learn essential things about the way our brain functions. \(^59,60\) In the analysis of functional brain activity, MEG and fMRI signal properties can be extracted using different analysis approaches that each capture a distinct aspect of the dynamics within the brain network. In this thesis, three different approaches are used, i.e. the analysis of local synchrony, functional connectivity and the topological organization of the brain network:

**Local synchrony**

A certain degree of synchronous neural activity is a prerequisite for recording EEG or MEG signals at the level of the skull. The strength of *local synchrony* can be measured by applying...
power spectral analysis to the registered signals by way of a Fast Fourier Transformation (FFT). This results in the decomposition of the EEG or MEG signal into absolute or relative power of local oscillatory activity in the different frequency bands and provides information about the local activity within a brain region.\textsuperscript{61}

**Functional connectivity**
In addition to measures of local synchrony within brain regions, measures of synchronization of activity between distributed brain regions can be computed in order to characterize the strength of communication or integration of information processing between brain regions. The functional coupling between two brain regions can be estimated by computing the statistical correlations or interdependencies between the time-activity series generated by the two brain regions. When the focus is on undirected statistical interdependence, this is generally referred to as ‘functional connectivity’.\textsuperscript{62} When the directionality of the interactions is considered this is called ‘effective connectivity’.\textsuperscript{63} In this thesis, where we focus on functional connectivity, the phase lag index (PLI) and the synchronization likelihood (SL) were computed as measures of functional coupling between brain regions in the MEG and fMRI signal, respectively.\textsuperscript{64,65}

**Topological organization**
In addition to the strength of functional connectivity, the complexity and overall organization of the functional connections within the brain network provide important information. By applying graph theoretical methods (a graph is a mathematical representation of a network) new insight can be obtained in how communication between brain regions is organized.\textsuperscript{66-68} A graph consists of nodes (=vertices, representing brain regions), and the connections between them (=edges, representing functional connectivity values). The topological organization of the brain network can be captured by a number of specific graph measures. Over the past decade, we have come to appreciate that brain networks in humans are organized according to a highly efficient topology that combines a high level of local integration (i.e. dense local clustering of connections) with a high level of global efficiency (i.e. the existence of critical long-distance connections); i.e. a so-called small-world organization.\textsuperscript{69,70} In addition, brain networks in healthy subjects contain a subset of relatively important, highly connected regions (‘hubs’),\textsuperscript{71} that are also mutually and densely interconnected, forming a connectivity backbone or ‘rich club’ crucial for efficient information processing.\textsuperscript{72} In this thesis, we assessed local integration, global efficiency and relative nodal importance as fundamental network properties by means of graph-derived measures.

**BRAIN NETWORK DYNAMICS IN PARKINSON’S DISEASE**
Focal as well as diffuse brain diseases can profoundly disrupt the functional brain network. The disruption can be detected in the resting-state of the brain when assessing the three different aspects of the dynamics of the brain network described in the previous section.\textsuperscript{73-78}
In the case of PD, power spectral analysis of MEG and EEG recordings has revealed a slowing of resting-state brain activity in early stage drug-naïve, as well as advanced stage non-demented PD patients. Dementia in PD is associated with a further slowing in comparison to both healthy subjects and non-demented PD patients. Functional connectivity in PD has only been studied cross-sectionally using EEG, MEG and fMRI. Excessive resting-state cortico-cortical coupling in the EEG / MEG has been reported in both early-stage and advanced-stage non-demented patients. In contrast to the observations in non-demented patients, dementia in PD appears to be associated with decreases in functional connectivity in fronto-temporal and parietal regions. In both non-demented and demented PD patients some of the changes in functional connectivity were correlated cross-sectionally with cognitive performance. In fMRI studies in PD patients, concurrent decreases and increases in functional connectivity between different combinations of brain regions have been reported, suggesting differential involvement of distinct brain regions in PD-related connectivity changes. Most studies to date focused on the assessment of cortico-striatal pathways in relation to motor symptomatology, with results indicative of a remapping of cerebral connectivity in response to dopamine depletion. In a single study investigating resting-state fMRI connectivity changes in early-stage non-demented PD patients, reduced connectivity in the posterior parts of the default mode network was associated with neuropsychological test performance. In the same study, no structural atrophy was found in PD patients, suggesting that functional changes may precede structural abnormalities in PD. The overall topological organization of the brain network in PD has only been investigated in a single fMRI study to date. In this study, the brain network in PD patients was characterized by a loss of both local integration and global efficiency compared to healthy controls. The results of the aforementioned (cross-sectional) studies in PD suggest that changes in the different aspects of the functional brain network (i.e. local synchrony, functional connectivity and the topological organization) may reflect important pathophysiological mechanisms underlying cognitive decline and dementia in PD. Many questions still remain though. First, the development of slowing of oscillatory brain activity over the course of the disease has never been confirmed in a longitudinal setting, nor have the changes in local synchrony been related to clinical measures of cognitive decline or motor impairment in individual patients. Second, the analyses of functional connectivity using MEG (and fMRI) have provided diverging observations, possibly due to methodological differences and/or heterogeneity in patient characteristics. In most previous neurophysiological studies functional connectivity was calculated at the sensor-level. Consequently, the spatial distribution
of the changes in functional connectivity had to be interpreted with care, since volume conduction and field spread might have influenced the attribution to anatomical substrates. Furthermore, the use of functional connectivity estimators that are sensitive to volume conduction (e.g. SL) may have confounded previous results as well. Moreover, a longitudinal assessment of functional connectivity in PD patients and its relationship with clinical features has never been performed.

Third, the topological organization of the brain network in PD has only been studied once. The observations in this fMRI study have not been confirmed using other techniques, nor have relative regional importance ('hubness'), the longitudinal development of changes, or the relationship with clinical measures of disease severity ever been addressed.

Fourth, an important and clinically relevant question is whether changes in resting-state brain activity in non-demented PD patients precede the development of progressive cognitive decline and thus might serve as predictive markers of future conversion to dementia.

AIMS, RESEARCH QUESTIONS AND OUTLINE OF THE THESIS
The main objective of this thesis was to gain more insight into the pathophysiological processes underlying PD-related cognitive decline and dementia by longitudinally studying local synchrony, functional connectivity and the topological organization of the functional brain network in PD patients and controls. In addition, we aimed to identify potential surrogate markers of clinical disease progression in PD, in particular related to cognitive decline, and to find predictors for the development of dementia.

The studies described in this thesis were performed in a cohort of PD patients and healthy controls who were longitudinally followed for seven years. At baseline, a total of 70 non-demented patients (disease duration 0-13 years) with idiopathic PD and 21 healthy controls were prospectively included at the outpatient clinic for movement disorders of the VU University Medical Center. Participants underwent longitudinal motor and cognitive assessments, as well as repeated MEG and structural MRI recordings, at baseline and at two follow-up visits scheduled 4 and 7 years after the baseline visit. fMRI was acquired twice, at the first and second follow-up visits.

We addressed the following specific research questions:

1. Is PD associated with progressive slowing of resting-state brain activity when studied longitudinally within subjects using MEG; and is there a correlation with clinical deterioration, in particular cognitive decline?

2. Is PD associated with changes in MEG-derived resting-state functional connectivity when using a data analysis method that is insensitive to volume conduction and performed at the source- instead of the sensor-level? If so, are these changes in the strength of functional connectivity progressive when analyzed longitudinally; and do these changes correlate with motor and/or cognitive measures of disease progression?
3. Can changes in resting-state functional connectivity in PD be demonstrated using a different functional brain imaging technique, i.e. whole-brain fMRI; and are these changes progressive over time in relation to cognitive decline and/or worsening motor function?

4. Is PD associated with changes in the topological organization of the functional brain network; more specifically, is there a loss of local integration and/or global efficiency; are these changes progressive over the course of the disease when analyzed longitudinally; do these changes over time correlate with clinical measures of disease severity?

5. Can changes in functional brain activity in non-demented PD patients predict future conversion to dementia; if so, does the predictive value compare favorably with the predictive value of cognitive test performance; is there added value of combining neurophysiological and cognitive measures?

To answer the first research question, we performed a longitudinal analysis of changes in local synchrony of brain activity measured using MEG in both PD patients and controls, the results of which are described in chapter 2.

The second and third research question are addressed in chapters 3 and 4, respectively, in which we describe the results of longitudinal assessments of functional connectivity in relation to clinical measures of disease progression in PD patients and controls. Chapter 3 is a description of a longitudinal study in which we used MEG and a seed-based approach to study the functional connectivity of specific cortical brain regions known to be neuropathologically affected at an early disease stage. In chapter 4, we describe a longitudinal study of fMRI-derived whole-brain functional connectivity in relation to cognitive decline and worsening motor function.

Chapter 5 comprises a longitudinal study of the overall topological organization of the functional brain network in PD. In this study, we applied two different mathematical approaches to the construction of graphs representing the whole-brain MEG-derived brain network in relation to clinical measures of disease progression in PD patients compared to controls.

In chapter 6 we address the final research question by describing the results of a study assessing the predictive value of local synchrony of brain activity (in initially non-demented PD patients) for future conversion to dementia over a seven-year follow-up period. In this study, we also evaluated whether the prediction of dementia would be improved by combining neurophysiological and neuropsychological variables.

In chapter 7 the main findings of this thesis are discussed along with recommendations for future research.