GENERAL DISCUSSION
By means of the studies described in this thesis we aimed to gain more insight into the pathophysiological processes underlying cognitive decline and dementia in Parkinson’s disease (PD) by longitudinally studying local synchrony, functional connectivity and topology of the functional brain network in patients and healthy 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 conversion to Parkinson’s disease dementia (PDD).

LOCAL SYNCHRONY WITHIN BRAIN REGIONS
Our first specific research aim was to study local synchrony of brain activity in relation to clinical deterioration by means of spectral power analysis. Previous studies had demonstrated marked slowing of brain activity in demented, advanced-stage non-demented, and even in early-stage, drug-naïve PD patients when compared to healthy controls. However, the exact development of these changes over the disease course and the relation with clinical measures of disease progression remained unclear due to the cross-sectional nature of these studies. Results from our longitudinal magnetoencephalography (MEG) study have now made it clear that slowing of oscillatory brain activity is a continuous process in PD that gradually progresses over the course of the disease, developing in strong relation to a decline in cognitive function and, to a lesser extent, increasing motor impairments (chapter 2). The absence of longitudinal changes in oscillatory brain activity in control subjects strengthened our conclusion that this process is a disease-related phenomenon and does not reflect a general ageing effect.

Jointly, the decrease of the dominant peak frequency and the increases in low frequency (theta band) and decreases in high frequency (alpha and beta band) relative spectral power in our PD sample indicate that local information processing is disturbed over a wide frequency range. Alpha oscillations are known to play a role in a broad spectrum of cognitive functions and attentional behavioural processes. A disturbance of alpha oscillations could result in a loss of relative alpha power and a decrease of the peak frequency, as found in our study, with a shift of relative power to the adjacent theta band. Beta rhythmic activity may regulate both motor and cognitive performance, probably by a common (cortical) source. In PD, excessive beta activity has previously been reported in relation to motor impairment, and only within the basal ganglia-cortical loop. Specific involvement of beta oscillations in cognitive decline is less easily explained. Interestingly, however, widespread decreases in beta power have also been observed in Alzheimer’s disease (AD), which might imply involvement of the cholinergic system.

FUNCTIONAL CONNECTIVITY BETWEEN BRAIN REGIONS
Brain regions are not functionally isolated compartments, but form a highly integrated system in which different regions can influence each other. This aspect of brain function, i.e. the functional connectivity between brain regions, was assessed by means of resting-state MEG and functional magnetic resonance imaging (fMRI) in this thesis.
We first presented an analysis of MEG-derived functional connectivity in PD. In most previous neurophysiological studies functional connectivity was calculated at the sensor-level, and therefore the spatial distribution of the observed changes in functional connectivity had to be interpreted with care. Furthermore, in previous studies functional connectivity estimators sensitive to volume conduction (e.g. synchronization likelihood; SL) were used, which may have influenced the attribution to anatomical substrates as well. In this study, we applied a superior methodology that entails a source- instead of a sensor-level based analysis and a functional connectivity estimator insensitive to volume conduction (i.e. the phase lag index; PLI) (chapter 3). This method enabled us to longitudinally investigate the overall resting-state functional connectivity of individual cortical brain regions. Based on the neuropathological involvement of temporal, prefrontal and high order sensory association areas in the early clinical stages of PD, we hypothesized that these brain regions would display progressive changes in overall functional connectivity with the rest of the brain.\textsuperscript{8,124,187} Considering the pattern of development of neuropathological changes over the brain, we expected that among the selected brain regions those involved earliest in the pathological process (i.e. temporal areas) would display connectivity changes at an earlier disease stage than brain regions affected at a later pathological stage, which would show a change in functional connectivity with further progression of disease.

Two interesting observations were made in this study. First, in early-stage PD we found increased resting-state functional connectivity in the lower alpha band that changed into functional connectivity decreases over time. This reversal suggests a remapping of cortical functional connectivity with disease progression. Apparently, increased synchronization between brain areas takes place only in the early stages of the disease, possibly reflecting a compensatory mechanism to maintain adequate information processing.\textsuperscript{130} A similar phenomenon has been observed in other neurodegenerative diseases such as AD and multiple sclerosis.\textsuperscript{188-190} Alternatively, the early-stage excessive synchronization may be explained by the mechanism of pathological disinhibition. This mechanism has been demonstrated in anatomically and functionally realistic models and entails that damage due to excessive local neuronal activity leads to an initial increase and subsequent breakdown of functional connectivity.\textsuperscript{131}

Second, in early-stage, drug-naive patients overall functional connectivity was changed for the temporal, but not the prefrontal and high order association areas. It is tempting to assume that these early-stage changes in functional connectivity of temporal brain regions are associated with the development of local cortical Lewy body pathology, which is known to affect limbic cortical brain regions already in the earliest clinical disease stages (Braak stage 4). The alleged link between the pattern of development of local cortical pathology and changes in functional connectivity is strengthened by the fact that our longitudinal analysis revealed decreases in functional connectivity for a wider array of cortical brain regions with further disease progression.\textsuperscript{8,123,124} Although we did not perform a whole-brain analysis, our data suggest that local cortical pathology within a brain region affects the functional interactions of that region with the rest of the brain.
The data obtained in our subsequent study using a different functional imaging technique, i.e. fMRI (chapter 4), confirmed and extended the results of our MEG study that disease progression in PD is associated with a loss of functional connectivity. In this fMRI study, we analyzed functional connectivity at a higher spatial resolution and using a whole-brain analysis that included both cortical and subcortical regions. In PD patients, functional connectivity with the rest of the brain was decreased for multiple brain regions, in particular occipital and temporal areas. The decreases in functional connectivity were progressive over time, and were closely associated with clinical deterioration, especially cognitive decline.

Interestingly, the reductions in functional connectivity that were most strongly related to decreasing cognitive function were those of the occipital brain regions. Furthermore, post-hoc analyses showed that the loss of functional connectivity of the occipital brain regions particularly involved connections with temporal brain areas, including the parahippocampal region. This would seem to indicate a disturbance in the so-called ventral stream of information processing, which is strongly related to (visuospatial) cognition. To a lesser extent, the dorsal stream appeared to be affected as well, since the connectivity between occipital and parietal regions was decreased in PD patients.

TOPOLOGICAL ORGANIZATION OF THE BRAIN NETWORK

As a third aspect we investigated the topological organization of the functional brain network in PD. Only a single study addressed this issue in PD so far. In this study, using fMRI, a loss of both local integration and global efficiency of the brain network was uncovered in advanced-stage patients compared to healthy controls. We aimed to expand on these data by studying early-stage and subsequent ongoing (longitudinal) changes in fundamental network properties, in relation to clinical features. To this end, we applied both a standard graph analysis method as well as the construction of a minimum spanning tree (MST) to our MEG data, the latter being a novel approach that allows a unique and unbiased characterization of brain networks (chapter 5).

In early-stage, drug-naïve PD patients, we found decreased local integration (with preserved global efficiency) and network decentralization compared to controls. Longitudinal analysis revealed a progressive impairment in local integration and an additional loss of global efficiency with disease progression. The MST results additionally showed a loss of relative importance of individual brain regions within the network with disease progression, predominantly involving orbitofrontal and temporal brain regions. Taken together, these changes indicate that the brain network organization in PD moves towards a more random and decentralized structure. Apparently, information processing in the parkinsonian brain is not only quantitatively impaired, but also less efficiently organized. This important observation may have clinical relevance, since the progressive changes in functional brain network organization were associated with increasing cognitive and motor impairments.
EVOLVEMENT OF BRAIN NETWORK ALTERATIONS OVER THE COURSE OF PD

Taken together, the results obtained using MEG and fMRI in the studies described in chapters 2, 3, 4 and 5 of this thesis demonstrate that in PD multiple aspects of the functional brain network, i.e. local synchrony, functional connectivity and the overall topological organization, are disturbed from the earliest clinical stages onward and evolve in close association with clinical impairments.

The earliest clinical stages of PD are first of all characterized by a widespread slowing of oscillatory brain activity, including a decrease in the dominant peak frequency and a shift from high frequency (alpha and beta band) to low frequency (theta band) relative spectral power. In addition, there is increased functional connectivity in the lower alpha band coupled with decreased functional connectivity in the delta band, predominantly in temporal brain regions. Lastly, reduced local integration and network decentralization are present within the brain network in early-stage PD.

With increasing disease duration, the process of oscillatory slowing is gradually progressive in strong correlation with cognitive decline, and to a lesser extent with increasing motor impairments. Furthermore, the initial increase in lower alpha band functional connectivity of temporal brain regions with the rest of the brain reverses, combined with concurrent decreases in higher alpha band functional connectivity that involve more widespread cortical areas in association with increasing impairments in both cognitive and motor function. The whole-brain fMRI analyses, although covering a somewhat later disease phase, confirm the loss of functional connectivity with disease progression, including the association with clinical measures of declining cognitive and motor function. Furthermore, the fMRI data expand the MEG findings by demonstrating that the decreases in functional connectivity are widespread and predominantly involve the posterior parts of the brain.

In addition to the changes in local synchrony and functional connectivity, disease progression in PD is characterized by a progressive disruption of overall brain network topology, including a further loss of local integration, a reduction in global efficiency, and a loss of relative importance of predominantly (orbito)frontal and temporal brain regions. These changes in brain network topology are also correlated with declining cognitive and motor function.

LINKING FUNCTIONAL TO STRUCTURAL CHANGES

The changes in local synchrony, functional connectivity and network topology were spatially distributed in our studies, i.e. not all brain regions were affected to the same degree or at the same disease stage. This interesting observation implies that certain brain regions are selectively vulnerable to progressive brain network injury and raises the question whether structural changes underlie these functional disturbances. Previous imaging studies in early-stage PD patients have failed to directly relate functional to structural changes so far. Although neuropathological changes in the form of Lewy body pathology are widespread
in the brain of PD patients, apparently their load is, at least in early disease stages, not heavy enough to cause structural abnormalities (i.e. atrophy) detectable with the present structural imaging techniques. At the macroscopic level of in vivo brain imaging it thus appears that functional changes may antedate structural changes.

Based upon the results described in this thesis, we hypothesize that there may be a direct relationship between neuropathological lesions and changes in functional brain dynamics. Considering for example the loss of relative importance of individual brain regions within the functional brain network, such regions might be selectively vulnerable to the pathological accumulation of alpha-synuclein. Although this issue still awaits investigation in PD, promising results have emerged from studies in AD, in which a substantial spatial overlap between functional network hubs and brain regions showing large amounts of AD-related pathology (amyloid-β deposition) has been demonstrated. Interestingly, in a computational network model excessive neuronal activity reproduced several neurophysiological hallmarks of AD, such as oscillatory slowing, loss of synchronization, hub vulnerability and disruptions in functional network topology, via the simulation of activity dependent degeneration. Activity-dependent degeneration thus provides a potential explanation for hub vulnerability, at least in AD. We suggest that a similar approach could be used to explore the relationship between the distribution of PD-specific pathology and changes in brain network structure and function, and hence with clinical disease progression, thus bridging the gap between neuropathological changes and clinical symptomatology that still exists in PD. Another promising approach is the combination of post-mortem imaging with high-resolution MRI-scanners and histopathological examination of the same brain tissue. This may provide a link between the results of in vivo brain imaging and neuropathological changes in PD, an approach that has yielded good results in other neurodegenerative diseases.

**FUNCTIONAL IMAGING MEASURES AS MARKERS OF COGNITIVE DECLINE IN PD**

The associations of the longitudinal changes in local synchrony, functional connectivity and brain network topology with declining cognitive and motor function in our studies suggest that these imaging measures definitely hold promise as surrogate markers of disease progression in PD. Reflecting underlying disease mechanisms, surrogate markers may lead to new pathophysiological insights that may serve as targets for the development of future treatment strategies aimed at the improvement of cognitive function in PD. In addition, surrogate markers could be used to monitor disease progression and treatment effects. As an example, MEG has been used to demonstrate the ameliorating effect of the cholinesterase inhibitor rivastigmine on oscillatory slowing in PDD.

Great potential lies in the use of functional imaging markers to predict clinical deterioration. This holds in particular for PDD, which develops in a substantial proportion of PD patients after prolonged disease duration and has a profound socio-economic impact. In this thesis we demonstrated that slowing of oscillatory brain activity in PD carries a strong
predictive value for the future conversion to PDD. Moreover, we demonstrated that combining neurophysiological markers with measures of cognitive function substantially improves dementia risk profiling over the predictive value of cognitive impairments or neurophysiological markers alone (chapter 6).

Our observations imply that neurophysiological markers could effectively complement cognitive assessment in the clinical workup of PD patients to establish the risk of conversion to PDD. This could benefit routine clinical care, since establishing a high risk of conversion to PDD at an early disease stage would enable more careful patient monitoring. Furthermore, patients and caregivers could be provided with better prognostic information that would help them to anticipate PDD-related problems such as psychosis, caregiver distress and nursing home placement. A high risk of PDD may even warrant earlier treatment with cholinesterase inhibitors, although the efficacy of this approach would first have to be assessed in an intervention study. Lastly, the identification of high- and low-risk subgroups of PD patients is essential for the future development and evaluation of therapies to effectively treat cognitive decline and dementia in PD.

METHODOLOGICAL CONSIDERATIONS

In the computation of functional connectivity between brain regions, different coupling measures can be used. When choosing a functional connectivity estimator for MEG data, the most important issue to take into account is volume conduction, which refers to the fact that adjacent MEG sensors are likely to pick up signals from the same underlying sources, thereby overestimating the presence of real functional interactions between brain areas. In the MEG studies in this thesis, we chose the PLI as a functional connectivity estimator. Since the PLI discards any (near) zero lag synchronization, it is relatively insensitive to volume conduction. The drawback of this method, however, is that it also discards true connectivity at very small phase differences (i.e. at short-distance), since at zero phase lag there is no way to distinguish between true and spurious synchronization. Thus, although we conformed to the safest approach by excluding zero lag coupling from all our analyses, this means that we might have underestimated true short-distance connectivity changes in our sample.

In most fMRI connectivity studies Pearson correlations have been used to compute functional connectivity. This measure only takes into account linear synchronization properties and therefore gives rise to difficulties in the interpretation of negative correlations. In line with our MEG studies, we preferred a functional connectivity estimator sensitive to both linear and non-linear interdependencies and therefore chose SL for this purpose, considering that PLI is less suitable for brief signals with a very low sample frequency such as the blood-oxygen-level-dependent (BOLD) signal of fMRI. Since BOLD signals do not suffer from volume conduction effects, we were able to use SL in spite of the sensitivity of this measure to volume conduction.

In chapter 5 of this thesis we used the MST as a methodological solution to the normalization problems that are associated with conventional graph analysis. An important caveat of the
MST method is that not all, but only the ‘core’ connections are taken into account (to prevent the formation of loops). As such, the choice of MST might have led to an underestimation of the contribution of ignored connections and clustering to information processing in the brain network. However, in our analyses we have shown that the MST captured clinically relevant changes in network topology that were in agreement with the results derived from more commonly used techniques in the same patient and control groups. Furthermore, our observations are supported by the results of other brain imaging studies that have recently demonstrated the value of the MST in capturing functional network changes in child development and epilepsy.166,198

RECOMMENDATIONS FOR FUTURE RESEARCH

The replacement of the MEG equipment after the first follow-up measurement of our cohort study and the fact that fMRI acquisition was only available from the first follow-up measurement onward prevented us from performing a direct comparative longitudinal multimodal MEG-fMRI analysis on our data. To further increase our understanding of the pathophysiological processes underlying PD-related cognitive decline, combining multiple imaging modalities in future studies should be encouraged in order to merge results derived from different structural and functional imaging modalities.

In the field of functional brain network analysis there is a rapidly growing availability of graph theoretical measures and methods. In this thesis we chose to analyze a selection of fundamental network properties (i.e. local integration, global efficiency, and the relative importance (centrality) of individual brain regions) based on the existing literature. In order to stimulate a judicious application of the best-suited graph measures, rather than the random use of a wide array of measures, future methodological studies that systematically compare the available measures are indispensable.

Taking a translational approach to further elucidate the pathophysiology of PD-related cognitive decline is a promising avenue of research. In particular, the use of animal models to replicate empirical findings in recordings of human brain activity might be particularly fruitful. Neurophysiological recordings in animal models mimicking the alterations in functional brain network characteristics that occur in patients will allow experimental modulation of neurotransmitter systems, which might teach us more about the impact of both depletion and/or replacement of particular neurotransmitters on brain network features.

As demonstrated in this thesis, neurophysiological markers that reflect slowing of brain activity can improve the identification of PD patients at a high risk for conversion to dementia. The availability of MEG is at present mostly limited to research centers, but a more cost-effective variant may become available in the near future.199 In clinical practice, the widely-available electroencephalographic recordings might serve as a good alternative for the purpose of dementia prediction.24 The next important step is a validation study using larger independent patient samples that should also aim to establish absolute cut-off values for the neurophysiological predictors of dementia.
From the results in this thesis, it is clear that changes in local synchrony, functional connectivity and brain network topology already occur in early-stage PD. It is therefore tempting to hypothesize that changes in these functional imaging measures might even precede the onset of the classical motor symptoms of PD, and hence be of use as an early diagnostic tool. The early diagnostic potential of these measures could initially be assessed in asymptomatic relatives of patients with familial forms of PD. Obviously, the development of functional imaging measures derived from MEG or fMRI as an early diagnostic tool would also require studies assessing the specificity of these measures for PD compared to other parkinsonian syndromes.

Differentiating PDD from other dementia syndromes such as AD and Lewy body dementia can be challenging in clinical practice. Studies directly comparing functional brain network characteristics in these diseases might reveal distinctive features that can be used to differentiate between these dementia syndromes.

CONCLUSIONS

The studies in this thesis have made clear that alterations in local synchrony, functional connectivity and the topology of the functional brain network are present in early-stage, drug-naïve PD patients and, with increasing disease duration, evolve in close association with clinical deterioration, in particular cognitive decline.

Early-stage, drug-naïve PD is characterized by widespread slowing of brain activity, changes in overall functional connectivity of predominantly temporal brain regions, and an impairment of local integration within the brain network. With increasing disease duration, oscillatory slowing continues to progress, decreases in functional connectivity between brain areas become more widespread, and changes in overall brain network topology are indicative of a further loss of efficiency of information processing, now also including the global network level.

The changes that occur with disease progression in the different aspects of the functional brain network, i.e. local synchrony, functional connectivity and network topology, are each associated with increasing cognitive and motor impairments and therefore hold promise as surrogate markers of disease progression in PD. Such markers may serve to monitor disease progression and assess the effects of drug treatment.

In this thesis we demonstrated that slowing of oscillatory brain activity in PD carries a strong predictive value for the conversion to PDD. Therefore, neurophysiological measures hold great promise as risk markers for the early identification of PD patients at increased risk for conversion to dementia. The identification of high- and low-risk subgroups of PD patients is an essential step in the future development and evaluation of therapies to effectively treat cognitive decline and dementia in PD. In the short term, establishing a high risk for conversion to PDD at an early disease stage will provide patients and caregivers with better prognostic information that will help them to anticipate and recognize PDD-related problems such as depression and psychosis, and improve personal future planning.