Pathophysiological mechanisms in Parkinson’s disease related dementia: an MEG study

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Pathophysiological mechanisms in Parkinson’s disease related dementia: an MEG study

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geboren te Utrecht
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Chapter 1
Introduction
Parkinson’s disease (PD) is a slowly progressive neurodegenerative disease of unknown origin, 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 include brady-hypokinesia, tremor, rigidity and postural instability. In idiopathic Parkinson’s disease, dopaminergic neurons in the substantia nigra and the ventral tegmental area degenerate, leading to dopaminergic deficiency in the striatum and mesocorticolimbic areas. Neuropathologically, surviving neurons in PD patients are characterized by the presence of cytoplasmic inclusion bodies, referred to as Lewy-bodies after the American neurologist F.H. Lewy. The recognition of the nigrostriatal pathway and dopamine as the neurotransmitter of this system, led to the introduction of treatment with levodopa, a precursor of dopamine, in the late 1950’s. Dopaminergic replacement therapy still constitutes the mainstay of a therapy that is purely symptomatic. The degenerative process is not limited to dopaminergic structures and therefore PD is considered to be a multisystem disorder with degeneration of other neurotransmitter systems including the cholinergic system (the basal nucleus of Meynert), the noradrenergic system (locus coeruleus) and the serotonergic system (dorsal raphe nuclei). Furthermore, Lewy body-pathology can be demonstrated in many other areas such as the vagal motor nucleus, the amygdala, thalamus and neocortical areas. Indeed, unlike James Parkinson and others have described in the past, PD is not only characterized by the mostly dopaminergically related motor symptoms but importantly also by the appearance of non-motor symptoms. These symptoms comprise olfactory deficits, fatigue, sensory complaints, autonomic symptoms, sleep disturbances, depression, cognitive dysfunction/dementia and psychotic symptoms. Some of these symptoms, for instance depression and olfactory disturbances, can be present several years before the first motor symptoms of PD occur and are thought to be related to early degeneration of neurotransmitter systems other than the dopaminergic system. Subtle cognitive deficits can also be demonstrated in the earliest stages of disease, but the development of significant cognitive dysfunction and dementia, often accompanied by psychotic symptoms, is more characteristic of the late motor stages. In case of development of dementia before or within the first year of the appearance of motor symptoms, the condition is called dementia with Lewy Bodies (DLB), which is thought to be part of the same spectrum of Lewy body disease. The studies described in this thesis were focused on the effects of dementia in PD (PDD).
COGNITIVE DYSFUNCTION AND DEMENTIA IN PD

As mentioned above, subtle cognitive dysfunction, mainly consisting of disturbances of executive function, can be found in the earliest stages of disease. However, these subtle deficits do not interfere with daily activities, and often they can only be detected with specific neuropsychological tests. In a considerable number of PD patients dementia develops, mainly consisting of a severe dysexecutive syndrome with attentional deficits and fluctuating cognition, often accompanied by psychotic symptoms, mainly visual hallucinations. Unlike in Alzheimer’s disease (AD), agnosia, apraxia and aphasia are much less common. Prevalence and incidence numbers vary considerably among studies, probably depending on the methods used and the population studied. Cross-sectional studies have reported a prevalence of 10 to over 40%. Recent studies with follow up periods of up to 20 years have shown a cumulative incidence of up to 80 %. In the advanced stages of PD, dementia and psychotic symptoms are often more important in determining the quality of life of patients than the motor disturbances and constitute an important contributor to caregiver distress and nursing home placement. Independent of the severity of the motor symptoms, dementia and psychosis are associated with increased mortality.

Despite the growing attention for cognitive dysfunction and dementia in clinical studies in PD, the exact pathophysiology of cognitive dysfunction and dementia in PD is still poorly understood. Both subcortical and cortical changes are probably involved. Both dopaminergic deficiency in the caudate nucleus affecting the dorsolateral prefrontal loop as well as dopamine loss in mesocortical areas due to degeneration of neurons in the ventral tegmental area may be important and have been found to be associated with PDD. Degeneration of the noradrenergic locus coeruleus and serotonergic dorsal raphe nuclei may be involved as well. In addition, cholinergic deficiency is more prominent in PDD as compared to PD and this cholinergic deficit, which is also a prominent feature in AD and DLB, is correlated with cognitive impairment in PD. Finally, local degenerative changes in the cerebral cortex have been implicated in the pathophysiology of PDD. These changes may include both Lewy body and tau-pathology. While some authors point to the importance of Lewy-body pathology in PDD, this is opposed by other authors reporting comparable cortical Lewy body-pathology in both demented and non-demented PD patients. The relative importance of all of the above mentioned pathological changes in subcortical and cortical areas in the development of dementia in PD is still largely unknown. Further insight into the pathophysiological mechanisms involved in cognitive dysfunction and dementia in PD is warranted and is particularly important with respect to
the development of future treatment for dementia. To date, treatment with cholinesterase inhibitors is the only approved treatment for dementia in PD.

**NEUROPHYSIOLOGY AND BRAIN FUNCTION**

For normal brain function including cognition, coordinated activity within as well as the integration of activity between specialized brain areas is thought to be important. Furthermore, the brain must be able to dynamically adjust these interactions, depending on the demands of specific tasks. In recent years, it has become more and more apparent that one way for the brain to accomplish this is by synchronization of oscillatory brain activity within and between segregated areas.

Techniques such as electroencephalography (EEG) and magnetoencephalography (MEG) (see box 1) are especially suitable for exploring the way the brain accomplishes these dynamical spatial and temporal interactions and also for studying the changes in these interactions that occur as part of the pathophysiology of motor and cognitive dysfunction in neurodegenerative diseases. Recent evidence suggests that a number of networks of anatomical areas are active even during an awake, no task condition. This so-called resting state is thought to be important for normal cognition and appears to change during normal aging. Moreover, global efficiency of information processing in the resting state is found to be associated with level of intelligence. Therefore, it is likely that cognitive dysfunction and dementia in PD are associated with changes in spatial temporal patterns of resting state brain activity. Studying these changes may further help to understand the pathophysiological processes involved in cognitive dysfunction and dementia in PD. Furthermore, drugs that act on specific brain neurotransmitter systems, for instance cholinergic or anticholinergic drugs, can modulate resting state oscillatory brain activity in both animal and human studies, in this way presenting an additional way to explore the role of specific neurotransmitters in cognitive impairment in PD.

Oscillatory activity of neuronal populations and its synchronization within and between brain areas can be studied in several ways. First, a certain level of local synchronous neuronal activity is a prerequisite for recording of the EEG or MEG signal at the level of the skull. The degree of local synchrony can be measured by applying power spectral analysis by way of Fast Fourier Transformation (FFT). This results in decomposition of the measured EEG or MEG signal into several frequency bands which can be expressed in the absolute or relative power of the local oscillatory activity measured within a given frequency band. Second, the synchronization of brain activity between segregated, distinct areas can be measured by calculating the statistical interdependencies between
Magnetoencephalography (MEG)

Magnetoencephalography is a non-invasive imaging technique that measures the magnetic field that is generated by the continuously changing electrical activity produced by the activity of brain neurons. It is thought that the net effect of the activity of approximately 50,000 neurons is needed to detect a magnetic signal at the skull. The first measurements were conducted in 1968 with MEG equipment that consisted of a single sensor. Nowadays, devices with 150 or more sensors that provide whole head coverage are available. The magnetic field of the brain is very weak and considerably weaker than the magnetic field of the surroundings. Therefore, to measure the brain magnetic field, superconducting quantum interference devices (SQUIDS) have been developed. In addition, to reduce the noise of background magnetic fields, measurements are conducted inside a magnetically shielded room. Like EEG, MEG has excellent temporal resolution, in the order of milliseconds. Unlike EEG, the MEG signal is hardly disturbed by underlying tissues such as the skull or scalp and it does not require a reference electrode.
EEG AND MEG IN PARKINSON’S DISEASE

So far, there is little experience with EEG and in particular MEG in PD. Several authors have reported a slowing of background oscillatory activity in demented PD patients, but until recently, this had not convincingly been demonstrated in non-demented patients. Excessive synchronization in the beta frequency range in the basal ganglia thalamo-cortical circuitry is thought to be related to the motor symptoms of PD, and treatment with dopaminergic drugs or deep brain stimulation can normalize these pathological oscillations in conjunction which improvement in motor symptoms (for review see Hammond et al). Recently, it was demonstrated using EEG that a pathological increase of cortico-cortical beta synchronization is also associated with the degree of parkinsonism in advanced, treated patients and that this increase could also be attenuated by either dopamine replacement therapy or deep brain stimulation. Knowledge about the relationship between cognitive dysfunction in PD and synchronization of resting state brain activity within and/or between brain areas as well as the large scale network organization is limited. More specifically, it is largely unknown whether subtle cognitive deficits in PD are related to abnormal synchronization of neuronal activity or whether this relationship is more characteristic of dementia in PD. Furthermore, qualitative differences between demented and non-demented patients, possibly indicative of differential or additional pathophysiological mechanisms in PDD, nor the influence of degeneration of the various neurotransmitter systems in PD on synchronization of brain activity have been extensively studied.

AIMS AND OUTLINE

The main aim of this thesis was to explore the changes in resting state neuronal activity in PD, with special focus on dementia, using MEG as a research tool.

The following research questions were addressed:
Can changes in MEG resting state background oscillatory activity be demonstrated in non-demented PD patients or is this a feature of dementia in PD? If so, is there a quantitative or qualitative difference between the changes in demented and non-demented PD patients?
Does treatment with cholinergic drugs have a modulatory effect on MEG background oscillatory activity in demented PD patients?
Is dementia in PD characterized by changes in functional connectivity between brain areas and if so, how do these changes relate to disease related changes in non-demented PD?
Is dementia in PD characterized by changes in resting state large scale brain network organization compared to non-demented PD?
Are changes in resting state brain activity correlated with cognitive deficits in PD?

The outline of this thesis is as follows:
In chapter 2, an overview of cognitive dysfunction and dementia in PD and the possible underlying pathophysiological mechanisms is given. In chapter 3.1, local neuronal synchrony within separate brain regions was studied by applying frequency analysis. Relative spectral power was calculated in three study groups: demented PD patients, non-demented PD patients and healthy, elderly controls. In chapter 3.2, the results of the effects of cholinergic treatment in demented patients are presented. Relative spectral power was calculated before and after a mean of 29 weeks of treatment with rivastigmine, a cholinesterase inhibitor.
Changes in functional connectivity in non-demented as well as demented PD patients are described in chapter 4. First, in the study described in chapter 4.1, functional connectivity as measured with the synchronization likelihood (SL) was studied in early stage, drug-naive patients as well as in dopaminergically treated patients in different stages of disease, both compared to healthy controls. The results of a comparison of resting state functional connectivity between demented PD patients and non-demented PD patients, again using SL, are presented in chapter 4.2.
In chapter 5, the results are described of a study in which graph theoretical analysis was applied to study the characteristics of large scale brain network organization (based upon SL) in demented PD patients compared to non-demented patients.
Finally, in chapter 6, the results presented in the preceding chapters are summarized and discussed, and suggestions for future research are given.

All studies were performed with subjects in an eyes closed, resting state condition. In addition, to assess reactivity to eye opening, patients were also studied during an eyes open condition in one study (described in chapter 3.1). To minimize the effects of dopaminergic medication, the studies were conducted in the morning before patients had taken their first dose of dopaminergic medication. The exception to the use of this practically defined “OFF state”, was the study described in chapter 3.2, in which patients were allowed to take their regular antiparkinsonian medication in addition to rivastigmine, a cholinesterase inhibitor.
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Pathophysiological mechanisms in Parkinson's disease related dementia: an MEG study
Chapter 2
Cognitive dysfunction and dementia in Parkinson’s disease

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Journal of Neural Transmission 2004 111:1303–1315
Parkinson’s disease (PD) is a slowly progressive neurodegenerative disorder mainly characterized by degeneration of dopaminergic neurons in the substantia nigra and the ventral tegmental area, in combination with a varying loss of central noradrenergic ( locus coeruleus), cholinergic (nucleus basalis of Meynert) and serotonergic (dorsal raphe nuclei) integrity, leading to a multitude of motor and non-motor behavioural disturbances.

Apart from the clinical motor hallmarks, in the early stages of disease, subtle cognitive dysfunction might be seen comprising mainly executive dysfunction, with secondary visuospatial and mnemonic disturbances. In about 20-40% of patients, these problems may eventually proceed to dementia, which constitutes an important risk factor for caregiver distress, decreased quality of life and nursing home placement. Dementia in PD is typically characterized by a progressive dysexecutive syndrome with attentional deficits and fluctuating cognition, often accompanied by psychotic symptoms. It is thought to be the result of a combination of both subcortical and cortical changes. PD related dopaminergic deficiency in the nucleus caudatus and mesocortical areas (due to degeneration of projections from the substantia nigra and ventral tegmental area) and cholinergic deficiency in the cortex (due to degeneration of ascending projections from the nucleus basalis of Meynert), combined with additional Alzheimer-pathology and cortical Lewy bodies, may greatly contribute to dementia.

Current treatment of dementia in PD is based on compensation of the profound cholinergic deficiency. Recent studies with the cholinesterase inhibitors galantamine, donepezil and rivastigmine show promising results in improving cognition and ameliorating psychotic symptoms, which must further be confirmed in randomized controlled trials.
INTRODUCTION

Parkinson’s disease is a slowly progressive neurodegenerative disease, in which dopaminergic neurons in the substantia nigra and the ventral tegmental area degenerate, leading to dopaminergic deficiency in the striatum and mesocorticolimbic areas. In addition to degeneration of the dopaminergic system, in PD other ascending subcortical neurotransmitter systems are affected as well: the cholinergic system (nucleus basalis of Meynert), the noradrenergic system (locus coeruleus) and serotonergic system (dorsal raphe nuclei). The cardinal motor symptoms are brady(hypo-)kinesia, tremor, rigidity and postural instability. Non-motor symptoms mainly comprise autonomic disturbances, depression, cognitive dysfunction/dementia and psychotic symptoms. As a rule, these symptoms are often more important in determining the quality of life of patients and caregivers than the motor disturbances.

Dementia in PD constitutes not only an important factor for caregiver distress and nursing home placement, but is also associated with increased mortality (independent of severity of motor symptoms). As these symptoms are potentially treatable, identification is of major clinical importance both for the patients and their caregivers and may enable the Parkinson’s disease patient to maintain living at home for a longer period. Additionally, postponement of nursing home placement can lead to substantial reductions in healthcare costs.

COGNITIVE DEFICITS IN NON-DEMENTED PD PATIENTS

Cognitive impairment is often associated with PD, although deficits may be relatively subtle and not clinically apparent, or not overtly affect daily functioning. However, when compared to controls, subtle to prominent cognitive impairment is almost always found in PD patients. A wide variety of cognitive deficits has been described in non-demented PD patients, the most prominent of which is a deficit in executive function.

Executive function is a broad term used to describe a range of cognitive functions involved in the realisations of goal-directed, adaptive behaviour in response to new, challenging environmental situations, including attention, inhibition, task management, planning, monitoring and coding. A dysexecutive syndrome, resembling cognitive deficits found in frontal lobe patients, is thought to be at the heart of cognitive dysfunction and dementia in PD and is usually one of the earliest cognitive symptoms found in PD. Besides executive dysfunction, there is considerable evidence of deficits in visuospatial function in non-demented PD, even when tests contain few motor components. Most authors, however, believe visuospatial dysfunction to be the result of the high cognitive
demand that is usually required by such tasks. Indeed, with the possible exception of judgment of line orientation, it would appear that visuospatial dysfunction in PD can be readily explained by the demand of visuospatial tasks on executive functions such as planning and (shifting of) attention.12-16

Mnemonic dysfunction has also frequently been reported in PD. The most consistent findings in patients with PD are deficits of working memory17-21 and explicit memory.22-24 Working memory can be defined as the ability to hold internal representations in short-term memory and to manipulate this mnemonic information on line to enable adaptive behaviour to be based on these representations rather than on immediate stimuli.25 Most studies find preserved short-term memory in non-demented PD.26 The executive processes that operate on the contents of this memory, however, are often impaired.27 Therefore, most deficits in working memory can probably also be explained in terms of executive dysfunction. Defective explicit memory in PD can largely be remedied by semantic cueing or probing.28,29 This suggests that although new information is stored, it is not readily accessed, pointing to defective usage of stored information. In conclusion, quite analogous to visuospatial function, mnemonic function would for the most part be secondarily impaired, due to the reliance of its manifestation on executive functionality.

Several studies have also shown bradyphrenia in PD,30,31 although this is still a matter of much controversy in the literature. It has been suggested that the finding of reduced cognitive speed may very well have been caused by the inclusion of patients with mild dementia or depression.32 For an excellent and comprehensive review of the literature on cognitive deficits in PD, one is advised to read the chapter by Pillon and co-workers in the recent Handbook of Neuropsychology.33

DEMENTIA IN PD

The aforementioned cognitive deficits may eventually proceed to dementia in a number of patients. Prevalence and incidence vary considerably among studies, possibly due to differences in patient population, study design and criteria for diagnosing PD and dementia. In cross-sectional studies, prevalence of dementia in PD ranges from 10% to over 40%.34-36 Features associated with prevalence of dementia include age,34,35 age at onset of PD,34-36 disease severity,35 disease duration,34 depression34 and presence of atypical parkinsonian symptoms.34 The association with older age is of particular strength. Prospective studies have reported a cumulative incidence of 19 to 53% (the follow up period varied in these studies).4,37-39 Recently, in a prospective study with 8 years follow up, 78.2% of patients eventually developed dementia.40 Incidence rates vary from 31.4 to
122.5 cases per 1000 person years\textsuperscript{34,37-42} and the risk for developing dementia in PD patients is up to six times higher compared to age-matched control subjects.\textsuperscript{43} In these studies, factors associated with the risk of dementia were found to be age, age at onset of PD,\textsuperscript{41} disease severity,\textsuperscript{38,39,43,46} age at entry in the study,\textsuperscript{37,39} confusional state,\textsuperscript{45,46} early hallucinations and the mixed tremor/akinetic form of PD.\textsuperscript{40}

The neuropsychological profile of PD related dementia is characterized by a progressive dysexecutive syndrome, as described earlier. Essentially, the same types of deficits are found in non-demented patients,\textsuperscript{47} but are more severe in demented patients. From this perspective, it is not surprising that several recent studies have pointed to the predictive value of prodromal impairment of verbal memory (immediate and delayed recall) and especially executive function.\textsuperscript{44,48,49}

Memory deficits are present, but are less severe compared to Alzheimer’s disease (AD).\textsuperscript{50} Moreover, the quality of memory impairment differs from that seen in AD. In both conditions it is characterized by a deficit in free recall, but in PDD, as mentioned before, this can often be corrected by semantic cueing.\textsuperscript{28} Therefore, in PDD the problem seems to be of retrieval, and not of encoding. Indeed, recognition memory is often well preserved in demented PD patients.

Unlike in AD, instrumental disorders such as aphasia, apraxia or agnosia are not very common in PDD.\textsuperscript{51,52} Psychotic symptoms however, are especially common in PDD. Hallucinations (sensory percepts in the absence of an external stimulus\textsuperscript{53}), mainly visual, are the most frequent symptom. These are mostly non-threatening and often consist of vivid, colourful and sometimes fragmented figures of beloved (deceased) familiar persons and/or animals, which are described in detail.\textsuperscript{54} Insight is retained in the majority of occasions, but with reality testing deteriorating, the hallucinations may change and become more frightening, possibly inducing anxiety and panic attacks.\textsuperscript{55} Loss of insight is particularly seen in demented patients.\textsuperscript{56} Delusions (false beliefs based on incorrect inference about external reality\textsuperscript{53}) are less common than hallucinations and mainly of the paranoid type, dealing with persecution, spousal infidelity or jealousy.\textsuperscript{57}

Attentional deficits and fluctuating cognition are also very common in PDD. These features, together with parkinsonism and the above mentioned visual hallucinations, are the main characteristics of dementia with Lewy bodies (DLB), possibly accounting for 15-20\% of the dementias.\textsuperscript{58} Indeed, PDD and DLB share many clinical\textsuperscript{51,59,60} and pathological\textsuperscript{61} features and are often difficult to distinguish other than by the temporary onset of dementia and psychosis in relation to parkinsonism.\textsuperscript{62} Therefore, PD and DLB are often
considered to be part of the same disease spectrum, although this matter is still under considerable debate. It is suggested that similar pathological mechanisms may underlie the clinical symptoms, including dementia and psychotic symptoms.

PATHOPHYSIOLOGY OF COGNITIVE DEFICITS AND DEMENTIA IN PD

Based on his work in primates, Alexander described five parallel, segregated circuits interconnecting well-defined subregions of the basal ganglia to particular cortical fields via the thalamus. Disruptions at either basal ganglia or cortical points in such a circuit have been shown to produce similar behavioural effects. In one of the five circuits, the so-called dorsolateral prefrontal loop which is thought to be involved in executive behaviour, the dorsolateral part of the prefrontal cortex projects to the caudate nucleus. Hence projections lead through pallidum and thalamus back to the prefrontal cortex. It may therefore well be that degeneration of the dopaminergic nigrostriatal pathway affects executive function by causing a disruption at the level of the caudate nucleus, a notion supported by findings from imaging studies. This notion is further underlined by the fact that dopamine depletion in PD is greatest in the caudate’s most rostral portion, exactly the part that is most heavily interconnected to the dorsolateral region of the prefrontal cortex.

Alternatively, cognitive dysfunction in non-demented PD could be caused by dopamine depletion in the frontal cortex itself, resulting from degeneration of the mesocortical dopaminergic system mainly projecting from the ventral tegmental area. In any case, the exact contribution of dopaminergic deficiency to cognitive defects in Parkinson’s disease remains controversial, largely because the cognitive effects of dopaminomimetics appear heterogeneous. While some studies point to a positive effect on executive function, (working)memory and attention, others actually find deleterious effects, especially in the executive domain or show no effects at all. A recent longitudinal study showed the beneficial effect of dopaminergic medication to be particularly prominent in the very early stages of disease (for a review of the pertinent literature, read Kulisevsky et al). Also, cognitive function in PD seems to correlate with motor symptoms that show little response to dopaminergic treatment (axial symptoms and gait disturbances), but not with levodopa-responsive symptoms (akinesia and rigidity).

In conclusion, it would appear that dopaminergic medication improves or impairs cognitive performance depending on both the nature of the task and the basal level of dopaminergic function in underlying nigrostriatal and mesocortical circuitry.
The exact pathophysiology of dementia in PD is uncertain. A number of neuropathological and neurochemical changes in PD are thought to be involved. The aforementioned deterioration of the dopaminergic system is likely to contribute to the progression of cognitive deficits into more overt dementia. This is supported by the association between dementia and the loss of dopaminergic neurons in the medial part of the substantia nigra, projecting to the nucleus caudatus and mesocortical and mesolimbic areas, and in the ventral tegmental area, with ascending dopaminergic projections to mesocortical and mesolimbic areas. Still, dopaminergic deficiency by itself is not considered sufficient for the development of dementia. Non-dopaminergic systems are likely to be involved as well. As already mentioned, several neuromodulatory systems are affected to varying degree in PD, mainly the serotonergic, noradrenergic and cholinergic systems. Neuronal loss in locus coeruleus (LC) and noradrenergic deficiency in the cortex were reported to be associated with dementia in PD. However, in other studies, this relationship could not be found. Loss of serotonergic neurons in the dorsal raphe nucleus (DRN) has mainly been associated with depression, but demented and non-demented patients did not differ on neuronal counts in this area. Furthermore, Perry et al. could not establish a correlation between dementia in PD and diminished monoaminergic activity. Instead, they reported an association between cholinergic deficiency and dementia. Indeed, not only in Alzheimer’s disease, but especially in PDD and DLB, a cholinergic deficit has been implicated in the pathophysiology of cognitive impairment. In these patients, a definite and more pronounced deplet ion of cholinergic neurons is found in the nucleus basalis of Meynert compared to AD patients and non-demented patients, together with diminished cholinergic activity in the cortex. This nucleus in the basal forebrain, consisting for 90% of cholinergic neurons, provides major cholinergic projections to the amygdale and neocortex. The cholinergic deficit, possibly superposed on a normal age-related deterioration of the cholinergic system, is strongly correlated with cognitive impairment in both conditions and therefore, is likely to constitute an important mechanism in the development of dementia. This is further supported by the propensity of anticholinergic agents to elicit cognitive dysfunction in PD patients and the clinical beneficial results of cholinesterase inhibitors in disorders with associated dementia (see later). Beside these subcortical neuropathological changes in PDD, important cortical changes have been implicated in the etiology of dementia in PD. AD-pathology, especially AD-neurites, are more abundant in demented patients compared to non-demented PD.
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...correlating with the severity of PDD in a number of studies, though other authors point to the importance of cortical Lewy bodies as a neuropathological substrate for dementia in PD, independent of AD-pathology. This, however, is opposed by authors reporting comparable cortical LB distribution in demented and non-demented PD patients. Thus, this matter is still under considerable debate.

Overall, dementia in PD is thought to be the result of a combination of several subcortical and cortical pathological changes. Subcortical mechanisms include both dopaminergic deficiency in the nucleus caudatus and mesocortical areas, causing executive dysfunction, and cortical cholinergic deficiency, mainly due to degeneration of the nucleus basalis of Meynert. The latter may further deteriorate the patient’s executive functions by inducing attentional deficits (possibly together with noradrenergic deficiency). This effect might be increased by ageing. Additional AD-like changes and the presence of Lewy bodies in the cortex are likely to further compromise cognitive functions, with development of dementia as soon as a certain threshold is reached.

TREATMENT OF DEMENTIA IN PD

Current pharmacological intervention in dementia is symptomatic and is based on compensation for the profound loss of cholinergic activity in the cortex. In AD, modest beneficial results have been reported with the cholinesterase inhibitors galantamine, rivastigmine and donepezil. These compounds might even prove to be more effective in PDD (and DLB) for several reasons. First, as mentioned above, the deterioration of ascending cholinergic projections from the nucleus basalis of Meynert is probably more pronounced in PDD and DLB compared to AD. Second, the cortex is thought to be relatively spared in PDD compared to AD. An early study with tacrine in PD reported a definite amelioration of psychotic behaviour, but this compound has been withdrawn from the market because of hepatotoxicity. Galantamine and donepezil are cholinesterase inhibitors that stimulate the nicotinic receptor. In open label studies with these compounds, cognition as well as hallucinations improved in PD patients with dementia without significant worsening of extrapiramidal features.

Open label studies with rivastigmine, a dual acetyl and butyryl cholinesterase inhibitor have yielded similar results in PDD as well as DLB. In these studies, extrapiramidal features did not worsen significantly and even tended to improve in one and worsened after withdrawal of rivastigmine in another. Given the possible parkinsonism inducing effects of cholinergic drugs, the improvement as seen in the motor scores of the
Parkinson’s disease patients treated with cholinesterase inhibitors is unexpected. In our own (unpublished) experience rivastigmine only tends to increase tremor in demented PD patients. The only randomized controlled trial with cholinesterase inhibitors in PDD so far, has been conducted with donepezil. The same beneficial results as in the previous open label studies have been reported, though the number of patients was small.\textsuperscript{124} A large, randomized, placebo controlled trial with rivastigmine in PDD, is currently being conducted.

The most frequent side effects reported with the use of cholinesterase inhibitors are nausea, vomiting and anorexia, sometimes causing problems with drug titration. Therefore, the drugs should be started at low dose with a gradual increase to the maximum tolerated dose.
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Pathophysiological mechanisms in Parkinson's disease related dementia: an MEG study
Chapter 3.1

Resting state oscillatory brain dynamics in Parkinson’s disease: an MEG study

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ABSTRACT

The pathophysiological mechanisms of cognitive dysfunction and dementia in Parkinson’s disease (PD) are still poorly understood. Altered resting state oscillatory brain activity may reflect underlying neuropathological changes. The present study using magnetoencephalography (MEG) was set up to study differences in the pattern of resting state oscillatory brain activity in groups of demented and non-demented PD patients and healthy, elderly controls.

The pattern of MEG background oscillatory activity was studied in 13 demented PD patients, 13 non-demented PD patients and 13 healthy controls. Whole head MEG recordings were obtained in the morning in an eyes closed and an eyes open, resting state condition. Relative spectral power was calculated using Fast Fourier Transformation in delta, theta, alpha, beta and gamma frequency bands.

In the non-demented PD patients, relative theta power was diffusely increased and beta power concomitantly decreased relative to controls. Gamma power was decreased in central and parietal channels. In the demented PD patients, a diffuse increase in relative delta and to lesser extent theta power and a decrease in relative alpha, beta and to lesser extent gamma power were found in comparison to the non-demented PD group. In addition, reactivity to eye opening was much reduced in the demented PD group.

Parkinson’s disease is characterized by a slowing of resting state brain activity involving theta, beta and gamma frequency bands. Dementia in PD is associated with a further slowing of resting state brain activity, additionally involving delta and alpha bands, as well as a reduction in reactivity to eye-opening.

The differential patterns of slowing of resting state brain activity in demented and non-demented PD patients suggest that, in conjunction with a progression of the pathological changes already present in non-demented patients, additional mechanisms are involved in the development of dementia in PD.
INTRODUCTION

Even in the early stages of Parkinson’s disease (PD), subtle cognitive deficits are found in the majority of patients.\(^1\) The most prominent of these deficits is executive dysfunction.\(^2\) Executive function is a broad term used to describe a range of cognitive functions involved in the realization of goal-directed, adaptive behaviour in response to new, challenging environmental situations, and includes attention, inhibition, task management, planning, monitoring and coding.\(^3\) In a number of patients, these deficits may evolve into overt dementia, defined as cognitive dysfunction in multiple domains interfering with activities of daily living. PD related dementia (PDD) is mainly characterized by executive dysfunction and memory problems with attentional deficits and fluctuating cognition. Furthermore, these symptoms in PDD are often accompanied by psychotic symptoms, especially visual hallucinations.\(^4\) Apraxia, aphasia and agnosia, often prominent in Alzheimer’s disease, are not very common in PD related dementia.\(^2,5\) Considerable differences in the prevalence of dementia in PD have been reported\(^6-8\) depending on the population studied and the criteria used for PD and dementia. A recent systematical review of relevant studies has resulted in an estimated prevalence of 25 to 30%, accounting for 3 to 4% of all dementia syndromes.\(^9\)

The pathophysiological mechanisms of subtle cognitive dysfunction and dementia in PD are still poorly understood. It has been suggested that both subtle cognitive dysfunction and dementia in PD are caused by progressive degeneration of dopaminergic neurons with loss of nigrostriatal (mainly at the level of the caudate nucleus) as well as mesocortical projections.\(^10-14\) However, there is some evidence to suggest that cognitive impairment in early stage and later stage dementia in PD do not share the same pathophysiological basis. According to a recently introduced neuropathological staging system for PD\(^15\), PD related changes first emerge in the dorsal vagal motor nucleus and olfactory bulb, eventually spreading into the cerebral cortex by way of the brain stem (including not only the substantia nigra, but also the locus coeruleus and dorsal raphe nuclei) and basal forebrain. At the early clinical stages of PD, involvement of noradrenergic and serotonergic corticopetal projection systems may therefore contribute to cognitive dysfunction. In demented PD patients, prominent additional pathological changes corresponding to stages five to six of the Braak staging system have been described: abundant cortical Lewy bodies\(^16-19\), Alzheimer-like pathology\(^20,21\) and severe degeneration of cholinergic projections from the basal forebrain\(^22,23\) with loss of cholinergic activity in the cortex.\(^24-26\)
As these changes correlate with increasing cognitive decline, a pathophysiological role in the development of PD dementia is quite likely.

In PD, motor deficits are associated with changes in oscillatory brain activity that can be reversed by the administration of dopaminergic agents or high frequency stimulation of the subthalamic nucleus. Considering the importance of (synchronized) oscillatory neuronal activity for normal brain function in general (see for review Schnitzler and Gross 2005), cognitive dysfunction and dementia in PD may also be associated with changes in oscillatory brain activity.

As a rule, non-demented PD patients do not show a pronounced slowing of resting state brain activity. In a single study, an actual increase in alpha power was found. Others have reported some slowing of the occipital background rhythm together with an increase of low frequency power compared to healthy controls using visual inspection or quantitative analysis of EEG recorded from one or more occipital leads. A recent EEG-study reported diffuse slowing in only one out of 11 non-demented PD patients. To date, experience with MEG in Parkinson’s disease is scarce. Timmermann et al reported coherence between Parkinsonian tremor measured with EMG and brain activity measured with MEG. In a recent MEG study, slowing of the dominant rhythm was found in PD patients compared to elderly controls. However, since the cognitive status of the patients in this study was apparently not documented, the observed slowing may well have been related to the presence of (incipient) PD dementia. Taken together, it is still largely unknown to what extent slowing of resting state oscillatory brain activity is a characteristic of non-demented PD patients.

However, in patients with PD related dementia, considerable diffuse slowing of resting state oscillatory brain activity has consistently been reported in comparison to both non-demented PD patients and healthy controls. EEG studies in demented patients generally show an increase in delta and theta activity and a decrease in alpha and beta activity. In one of these studies, the degree of EEG slowing positively correlated with the degree of cognitive decline.

In the present MEG study, power spectral analysis of oscillatory brain activity was used to study resting state brain oscillatory activity in groups of demented and non-demented PD patients compared to healthy, elderly controls. Differential patterns of slowing in the patient groups would support the hypothesis that differences exist in the pathophysiology of subtle cognitive dysfunction in PD and PD related dementia.
Subject characteristics

Three groups of subjects participated in this study: PD patients with dementia (PDD; N=13; 8♂/5♀), PD patients without dementia (PD; N=13; 6♂/7♀) and healthy, elderly controls (C; N=13; 8♂/5♀). All subjects underwent a full physical and neurological examination.

All PD patients fulfilled the United Kingdom Parkinson’s Disease Society Brain Bank (UK-PDSBB) clinical diagnostic criteria for probable Parkinson’s disease. Demented PD patients fulfilled DSM-IV-criteria for dementia and had a Mini Mental State Examination (MMSE) score of 24 or lower out of a maximum of 30 points, with lower scores indicating worse cognition. Blood examination and MR imaging were performed to exclude other potential causes of dementia.

Non-demented PD patients and healthy, elderly controls had MMSE scores of at least 28 out of 30 points. Healthy controls had no evidence of neurological or psychiatric disease in the past and on examination, nor did they experience difficulties with cognitive functioning in daily life. Further cognitive screening consisted of the cognitive section of the CAMDEX: the CAMCOG A total of 107 points can be scored on this test with higher scores indicating better cognition.

Disease stage and severity were assessed using the (modified) Hoehn & Yahr-scale (H&Y; range 0-5 with higher scores indicating more advanced disease stage) and the Unified Parkinson’s Disease Rating Scale (UPDRS; range 0-108 with higher scores indicating worse motor functioning), respectively. Further exclusion criteria for PD patients consisted of stereotactic surgery in the past and the use of anticholinergics, neuroleptics or cholinesterase inhibitors.

All patients were treated with a combination of levodopa and a decarboxylase inhibitor. Besides levodopa, eight demented patients and nine non-demented patients were also treated with a dopamine agonist.

The study protocol was approved by the medical ethical committee of the VU University Medical Center. After careful explanation of the procedures, all subjects gave written informed consent prior to participating.

MEG procedures

MEG data were acquired using a 151-channel whole head MEG system (CTF Systems Inc., Port Coquitlam, BC, Canada). Patients were seated in a magnetically shielded room (Vacuum-schmelze GmbH, Hanau, Germany). Compared to EEG, MEG provides a reference free method with higher spatial resolution. Furthermore, magnetic fields are much less disturbed by the skull compared to electric fields.
All MEG recordings were acquired in the morning. Patients were asked to come to the hospital without taking their first morning dose of dopaminergic medication (practically defined “OFF”). MEG registration took place in this OFF-state. MEG was recorded in an eyes closed resting state condition (EC) followed by an eyes open resting state condition (EO). In the latter condition, patients were asked to avoid ocular movements.

The recording pass band was 0-125 Hz with a sample rate of 312.5 Hz. A third-order software gradient (Vrba, 1996) was applied. Two approximately 13 s long artifact free epochs (sample rate 312.5 Hz; 4096 samples) were selected for further analysis. Epoch selection was always done by the same investigator (JLWB), who was blinded for group membership. MEG recordings were filtered offline with a band pass of 0.5-48 Hz. Relative band power was computed for the two 13 s epochs in the following frequency bands: 0.5-4 Hz (delta), 4-8 Hz (theta), 8-13 Hz (alpha), 13-30 Hz (beta) and 30-48 Hz (gamma). Results of the two epochs were averaged for each subject. Subsequently, the MEG channels were grouped into regions of interest corresponding to the major cortical areas (frontal, central, temporal, parietal and occipital) on the left and right side. The midline channels (Z) were left out of this clustering. A schematic distribution of these areas is shown in figure 1. Mean relative power for each of these groups of MEG channels was used in the statistical analysis. Of the original 151 channels, two channels (MLO41 en MZP02) were not available due to technical problems.

Figure 1
Schematic projection of MEG channels and head drawing to illustrate the groups into which the MEG channels were clustered based upon the approximate underlying cortical areas. Midline MEG channels were not used in the analyses. L or R = cortical area on the left (L) or right (R) side of the head C = central, F = frontal, O = occipital, P = parietal, T = temporal.
Statistical analysis

Differences between groups in the distribution of gender and (modified) Hoehn and Yahr scores were analyzed by means of chi-square tests. All analyses with regard to group differences in age, disease duration and UPDRS motor scores and CAMCOG scores, were analyzed by univariate analysis of variance (ANOVA). MEG relative power changes were analyzed by univariate analysis of variance with repeated measures with group as intersubject factor and cortical area as intrasubject factor. Greenhouse-Geisser corrected $p$-values were used. In case of a significant main effect of group, post hoc ANOVA was performed to detect differences between the three groups in each cortical area. In case of a significant effect, post hoc analysis between the different group pairs was performed. Only when this analysis yielded a significant result between different groups, estimates of effect size using eta squared ($\eta^2$) were calculated which represents the proportion of the total variability in the dependent variable (power) that is accounted for by the independent variable (group membership). Although the data did occasionally violate the assumption of normality, parametric testing was still maintained, since non-parametric testing only yielded additional significant differences. As a consequence, differences may have been underestimated.

Correlation analysis between clinical parameters and spectral power was performed by means of Pearson bivariate correlation. All analyses were performed at a significance level of .05 (two-tailed). Analysis was done using the SPSS 12.0 software package (SPSS inc., Chicago, USA).

RESULTS

Subject characteristics

General characteristics of the three study groups are listed in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>PDD</th>
<th>PD</th>
<th>C</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>SD</td>
<td>SD</td>
</tr>
<tr>
<td>Sex</td>
<td>M/F</td>
<td>8/5</td>
<td>6/7</td>
</tr>
<tr>
<td>Age</td>
<td>Years</td>
<td>74.4</td>
<td>4.9</td>
</tr>
<tr>
<td>Disease duration</td>
<td>Years</td>
<td>11.2</td>
<td>4.0</td>
</tr>
<tr>
<td>CAMCOG</td>
<td>Years</td>
<td>71.5</td>
<td>11.8</td>
</tr>
<tr>
<td>UPDRS III OFF</td>
<td></td>
<td>71.5</td>
<td>11.8</td>
</tr>
<tr>
<td>H&amp;Y stage OFF</td>
<td></td>
<td>71.5</td>
<td>11.8</td>
</tr>
</tbody>
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Table 1
Demographic characteristics

There were no significant differences in age or gender distribution between the three groups. Disease duration was not significantly different between demented and non-
demented patients. Demented patients had significantly higher UPDRS III motor scores compared to non-demented PD patients. Hoehn and Yahr scores did not differ between demented and non-demented patients, although a trend was found towards more advanced stages in demented patients. As expected, CAMCOG scores in the PDD group were significantly lower compared to the other groups. There were no statistical differences in mean CAMCOG score between the PD group and the group of healthy, elderly controls.

**Power spectral analysis**

**EYES CLOSED**

In the eyes closed condition, a significant general effect of Group (averaged over all cortical areas) was found for each frequency band (figure 2; see table 2 for details of the univariate analysis of variance with repeated measures and post hoc univariate analysis of variance). Figure 3A illustrates the effect sizes for each of the identified brain regions in which significant differences were found between controls and non-demented PD patients and between non-demented and demented PD patients. Relative to controls, non-demented patients displayed an increase in theta activity in all but the frontal channels as well as a decrease in relative beta power in posterior channels and gamma power in central and parietal channels. There were no significant differences in the delta and alpha frequency band.

![EYES CLOSED](image)

*Figure 2*

Relative power in the different frequency bands, averaged over all cortical areas, for each of the groups in the eyes close OFF state. Error bars indicate standard deviations.

PDD = Parkinson’s disease related dementia; PD = Parkinson’s disease without dementia; C = Healthy, elderly controls
Figure 3
Schematic representation of differences in relative power between groups. The individual MEG channels are clustered into groups according to the major cortical areas (frontal, central, temporal, parietal and occipital). The calculated eta squared, as an estimate of effect size, is illustrated in three color-coded categories of magnitude: 0-25%, 26-50% and >50% (from light to dark). An area is colored red when the mean power PDD>PD and PD>C; an area is colored blue if mean power PDD<PD and PD<C. Only those areas for which a significant group difference was found in the ANOVA and post hoc comparisons are color-coded.

PDD = Parkinson’s disease related dementia; PD = Parkinson’s disease without dementia; C = Healthy, elderly controls; L or R = cortical area on the left (L) or right (R) side of the head
C = central, F = frontal, O = occipital, P = parietal, T = temporal
Table 2
Mean relative power in the study groups in each cortical area and frequency band with statistical results of the main group effect in the univariate analysis of variance with repeated measures as well as the post hoc ANOVA.

PDD = Parkinson’s disease related dementia; PD = Parkinson’s disease without dementia; C = Healthy, elderly controls; M = MEG channel; L or R = cortical area on the left (L) or right (R) side of the head
C = central, F = frontal, O = occipital, P = parietal, T = temporal
Main group effect
\[
\begin{array}{c|c|c|c|c|c}
& F & p & F & p \\
\hline
\text{ANOVA} & & & & & \\
MLC & 0.150 & 0.296 & 0.377 & 21.375 & 0.000 \\
MRC & 0.166 & 0.310 & 0.381 & 17.745 & 0.000 \\
MLF & 0.127 & 0.246 & 0.257 & 11.701 & 0.000 \\
MRF & 0.144 & 0.261 & 0.269 & 8.868 & 0.001 \\
MLO & 0.090 & 0.156 & 0.235 & 14.563 & 0.000 \\
MRO & 0.103 & 0.159 & 0.247 & 15.673 & 0.000 \\
MLP & 0.093 & 0.201 & 0.320 & 21.856 & 0.000 \\
MRT & 0.108 & 0.181 & 0.235 & 12.552 & 0.000 \\
\hline
\text{Mean} & PDD & PD & C & PDD & PD & C \\
\hline
\text{MLC} & 0.168 & 0.322 & 0.381 & 16.416 & 0.000 \\
\text{MRC} & 0.170 & 0.331 & 0.382 & 14.907 & 0.000 \\
\text{MLF} & 0.137 & 0.265 & 0.252 & 7.935 & 0.001 \\
\text{MRF} & 0.145 & 0.271 & 0.264 & 7.877 & 0.001 \\
\text{MLO} & 0.099 & 0.205 & 0.249 & 12.345 & 0.000 \\
\text{MRO} & 0.109 & 0.204 & 0.275 & 14.722 & 0.000 \\
\text{MLP} & 0.117 & 0.258 & 0.356 & 21.782 & 0.000 \\
\text{MRT} & 0.110 & 0.207 & 0.253 & 12.920 & 0.000 \\
\end{array}
\]

Group x Area effect
\[
\begin{array}{c|c|c|c|c|c|c}
& F & p & F & p \\
\hline
\text{ANOVA} & & & & & & \\
MLC & 0.023 & 0.039 & 0.065 & 11.974 & 0.000 & \\
MRC & 0.027 & 0.043 & 0.065 & 10.748 & 0.000 & \\
MLF & 0.025 & 0.049 & 0.062 & 7.751 & 0.002 & \\
MRF & 0.028 & 0.053 & 0.065 & 8.030 & 0.001 & \\
MLO & 0.031 & 0.030 & 0.062 & 2.348 & 0.110 & \\
MRO & 0.038 & 0.032 & 0.062 & 2.613 & 0.087 & \\
MLP & 0.015 & 0.022 & 0.043 & 8.868 & 0.001 & \\
MRT & 0.027 & 0.032 & 0.063 & 4.463 & 0.019 & \\
\hline
\text{Mean} & PDD & PD & C & PDD & PD & C \\
\hline
\text{MLC} & 0.029 & 0.052 & 0.078 & 9.630 & 0.000 & \\
\text{MRC} & 0.030 & 0.052 & 0.077 & 9.067 & 0.001 & \\
\text{MLF} & 0.029 & 0.058 & 0.067 & 8.247 & 0.001 & \\
\text{MRF} & 0.029 & 0.057 & 0.074 & 12.066 & 0.000 & \\
\text{MLO} & 0.033 & 0.054 & 0.068 & 3.817 & 0.031 & \\
\text{MRO} & 0.041 & 0.056 & 0.082 & 3.832 & 0.031 & \\
\text{MLP} & 0.019 & 0.036 & 0.066 & 10.526 & 0.000 & \\
\text{MRT} & 0.028 & 0.043 & 0.066 & 9.735 & 0.000 & \\
\end{array}
\]

Compared to the non-demented patients, the demented group showed a general increase in delta and, to a lesser extent, theta activity, together with widespread decreases in alpha and, to lesser extent, beta activity. Significantly lower gamma power was found in frontal channels only. When directly comparing the demented group to the control group, the increases in delta and theta activity and the decreases in alpha, beta and gamma activity were even more pronounced (data not shown in figure 3).

In figure 4, individual power values for the occipital channels in the alpha and delta bands are shown. Limited overlap between power values in these frequency bands was found between non-demented and demented patients.

Table 2 continued
The interaction effect of Group x Area reached significance in the delta (F[2,36]=5,010; p<.001), theta (F[2,36]=3,971; p<.001), alpha (F[2,36]=8,415; p<.001) and beta band (F[2,36]=5,675; p<.001), but not the gamma band (F[2,36]=1,521; p=.199). Clearly, this means that in addition to differences in mean relative power between groups (as discussed earlier), there are differences in the distribution of power over the MEG channels between the groups. In general, distribution of power was much more homogenous in demented patients compared to the other two groups. For example, in the alpha band, mean spectral power in non-demented patients and controls was much higher in posterior areas compared to other regions, whereas demented patients exhibit a quite homogenous distribution of alpha power over cortical areas. In the gamma band, distribution of power over the MEG channels is rather similar in all three study groups. This may explain why the interaction effect of Group x Area did not reach significance for the gamma frequency band, in spite of lower mean power in most regions in patients with dementia (see above).

**EYES OPEN**

In the eyes open condition, alpha power in non-demented patients and especially healthy controls was much lower compared to the eyes closed condition (figure 5).
Figure 5
Schematic representation of results of the comparison between the eyes open and the eyes closed condition using a within group paired samples t-test. An area is colored red when a statistical significant increase of relative power in the eyes open condition is found; an area is colored blue when a significant decrease is found. The intensity of the colors indicates the magnitude of change (light = 0-25%; middle = 25-50%; dark = >50%). Marked alpha power decrease is found in controls and to lesser extent non-demented PD patients whereas alpha reactivity is virtually absent in demented PD patients.

PDD = Parkinson’s disease related dementia; PD = Parkinson’s disease without dementia; C = Healthy, elderly controls; L or R = cortical area on the left (L) or right (R) side of the head
C = central, F = frontal, O = occipital, P = parietal, T = temporal

This effect was most pronounced over posterior channels. In controls, the reduction in alpha power was accompanied by an increase in delta and gamma power, whereas in non-demented patients reduced alpha was combined with increased beta and gamma power. By contrast, in PDD patients, hardly any changes in relative spectral power were found compared to the eyes closed condition. Most noticeably, there was no significant difference in alpha power between the eyes open and eyes closed conditions. As a result, differences in alpha and delta power between demented patients and controls were not as prominent (figure 3B) as in the eyes closed condition (figure 3A). The other differences in relative power between groups were largely comparable to the eyes closed condition.

Correlations with clinical parameters
CAMCOG
In the demented group, no significant correlations were found at all between this cognitive test and spectral power values. In non-demented patients, a negative correlation
was found between theta power and CAMCOG score in bilateral occipital (r= -.603; p = .029 and r= -.588; p = .035 respectively) and right temporal (r= -.592; p = .033) channels in the eyes closed condition and in the left occipital channels (r= -.577; p = .039) in the eyes open condition.

**UPDRS III**

In non-demented PD patients, no correlations were found between the motor section score of the UPDRS and spectral power in any of the five frequency bands in the eyes closed condition as well as the eyes open condition.

In demented PD patients, no correlations were found in the eyes closed condition. In the eyes open condition, a negative correlation between UPDRS and alpha power was found in the right central (r= -.603; p = .029), right frontal (r= -.555; p = .049) as well the right parietal (r= -.599; p = .030) channels.

Tremor, as assessed with the UPDRS tremor scores, did not correlate with spectral power in any of the frequency bands in both patient groups, most importantly not with the theta band (the frequency band in which the influence of tremor might be expected).

**DISCUSSION**

In the present whole head MEG study, non-demented PD patients showed considerable slowing of resting state oscillatory brain activity compared to healthy controls, mainly consisting of a diffuse increase in theta power as well as a loss of beta power over the posterior channels and a loss of gamma power over central and parietal channels. In agreement with previous studies, demented PD patients showed more pronounced slowing of background oscillatory activity than non-demented PD patients compared to age matched controls, characterized by widespread increases in delta and theta power together with a diffuse decrease in alpha, beta and gamma power. The differential patterns of change in oscillatory brain activity between controls and non-demented PD patients on the one hand and between non-demented and demented PD patients on the other hand are suggestive of the involvement of additional pathophysiological mechanisms in PD related dementia compared to the pathophysiology of subtle cognitive dysfunction in PD.

A number of potential confounding factors have to be considered. First, Parkinsonian tremor generally has a frequency in the theta range (4-7 Hz). Timmermann et al reported coherence between tremor, measured with EMG, and several MEG oscillatory rhythms in the contralateral motor cortex. In theory, these area specific coherent oscillations could influence spectral power values. Since the observed theta power changes are widely
distributed, these differences can hardly be explained by MEG oscillatory activity that is coherent with tremor. Alternatively, tremor could diffusely influence the MEG signal in a direct way, influencing power in the theta band. However, in the present patient groups, UPDRS tremor scores did not correlate with MEG activity in any frequency band, importantly not in the theta band either. Furthermore, epochs were carefully selected for the absence of visible tremor. Taken together, we believe that it is highly unlikely that tremor would account for the differences between patients and controls observed in the theta band.

Second, an additional difference between patients and controls is the use of dopaminergic medication. To minimize the effects of antiparkinsonian medication on the results of the experiment, MEG recordings were obtained at least nine hours after the last dose of medication. Since it takes longer than this time window to eliminate dopamine agonists from the body, we can not fully rule out some medication effects on the power differences between patients and controls. Considering that, dopaminergic stimulation is believed to shift the frequency spectrum to higher frequencies. Therefore, medication effects could have led to an underestimation of the true degree of slowing in our patient groups. Lastly, several other factors can influence resting state brain activity. For instance, Barry et al reported a reduction in alpha power after a single dose of caffeine.\textsuperscript{46} Other factors might be dietary intake or anxiety level. We did not strictly control for these effects, although all subjects were inexperienced with the MEG setting and received a standard introduction to the MEG and MEG procedures. However, we have no reason to believe that these factors might have been unevenly distributed over the three subject groups. Therefore, we do not think that we have introduced any systematic errors that might have influenced our results.

Very few correlations between clinical parameters (UPDRS and CAMCOG) and MEG spectral data were found. Given the large number of relations that were analysed (five frequency bands with ten cortical areas in each band), these correlations were likely to be caused by coincidence. As a consequence, the reported correlations are regarded as spurious. Possibly, the numbers of subjects were too small to detect significant correlations.

So far, the presence of slowing of resting state brain activity in non-demented PD patients has been a controversial issue. Soikkeli et al reported an increase of theta activity and decrease of beta power in PD patients using spectral power analysis of EEG data obtained
with a single occipital electrode.\textsuperscript{37} Likewise, Neufeld et al found evidence of slowing of resting state brain activity in PD patients compared to healthy controls, but again only the occipital region was studied.\textsuperscript{36} By contrast, others did not find slowing of oscillatory background activity in non-demented PD patients\textsuperscript{32} or even reported an increase of alpha power.\textsuperscript{33} In an MEG study, a decreased frequency of the dominant alpha rhythm and an increase in low frequency power were found. Since the cognitive status of the PD patients in the latter study was not reported, the study may actually have involved cases with (incipient) PD related dementia.\textsuperscript{39} The results of the present MEG study clearly show that clinically non-demented PD patients are characterized by slowing of resting state oscillatory brain activity compared to healthy, elderly controls, characterized by a diffuse increase in theta as well as decrease in beta and gamma power.

In the early clinical stages of PD, corresponding to stages three and four of the recently introduced neuropathological staging system for PD,\textsuperscript{47} PD specific neuropathological changes are most prevalent in the brainstem, including dopaminergic, serotonergic and noradrenergic structures. It is tempting to speculate that (part of) the slowing of background activity in early stage PD is related to degeneration of the corticopetal projections originating from these brainstem nuclei. In support of this contention, stimulation of dopaminergic neurotransmission is associated with an increase in fast EEG activity in animal studies, whereas suppression of dopaminergic neurotransmission produces slowing of EEG activity.\textsuperscript{48,49} Little is known about the effect of changes in dopaminergic neurotransmission on resting state EEG activity in PD patients. Yaar reported an increase in power over all frequency bands after treatment with levodopa.\textsuperscript{50} An earlier study reported increased alpha and decreased theta activity in about 40% of the patients after dopaminergic treatment.\textsuperscript{51}

The noradrenergic system, arising in the brain stem locus coeruleus, may influence resting state oscillatory brain activity in a similar way. In animal studies, stimulation of the locus coeruleus induces a shift in the electroencephalogram towards higher frequencies\textsuperscript{52} and conversely, suppression of noradrenergic neurotransmission induces slow wave activity.\textsuperscript{53} Promotion of serotonergic activity, either by stimulation of the dorsal raphe nucleus or injection of serotonergic agonists into the cerebral cortex, is associated with the emergence of fast frequency cortical EEG activity in animal studies.\textsuperscript{54} On the other hand, lesions of this brain stem nucleus and therefore loss of the ascending serotonergic cortical projections have been found to result in a reduction or inhibition of this cortical activation.\textsuperscript{55,56}
Taken together, these data indicate that lesioning of the dopaminergic, noradrenergic and serotonergic systems, is associated with an increase in low frequency brain activity, at least in animal data. Early stage involvement of dopaminergic, noradrenergic and serotonergic systems in PD may therefore contribute to the observed slowing of resting state brain activity measured using MEG in non-demented PD patients.

In accordance with previous EEG studies, the present MEG study demonstrates that demented PD patients have a pronounced slowing of resting state oscillatory brain activity compared to age matched controls. In addition to widespread increases in delta and theta power, we observed a diffuse decrease in alpha, beta and gamma power. Moreover, in comparison to non-demented PD patients, demented patients have an increase in delta power and concomitant decreases in alpha and beta and gamma power. Considering this difference in the pattern of resting state brain activity between non-demented and demented PD patients, additional pathophysiological mechanisms must be involved in the development of PD related dementia.

Dementia in PD generally is a feature of the more advanced stages of the disease. The associated neuropathological changes in these stages are severe degeneration of the cholinergic basal nucleus of Meynert as well as abundant cortical Lewy body and tau pathology. The cholinergic system has a modulatory influence on cortical activity as evidenced by several animal as well as human studies. After cholinergic stimulation, a decrease of slow, mainly delta activity and an increase of fast EEG background activity has been found. By contrast, degeneration or lesioning of the cholinergic cortical projections from the basal nucleus of Meynert with a corresponding loss of cortical cholinergic activity as well as a blockade of postsynaptic cortical muscarinic receptors are associated with an increase in slow, mainly delta activity. Further support for importance of the cholinergic system comes from observations in dementia with Lewy bodies (DLB) and Alzheimer’s disease (AD) that are both characterized by severe degeneration of the cholinergic basal nucleus of Meynert and its ascending projections to the cortex. In DLB as well as AD, prominent slowing of posterior background rhythm has been reported (for review see Jeong 2004). Lastly, treatment with cholinesterase inhibitors in AD results in decreased low frequency and increased high frequency activity. Existing knowledge of the effects of the cholinergic system on resting state brain activity thus seems to indicate that degeneration of the cholinergic basal forebrain system in PD may play a role in the pronounced slowing of resting state brain activity in PD related dementia as demonstrated in the present MEG and previous EEG studies.
Another factor that may contribute to slowing of resting state brain activity is the extensive cortical Lewy body-pathology that occurs in advanced stage PD. According to a recent study, the extent of cortical Lewy body-pathology is the best correlate of the severity of PD related dementia.\textsuperscript{70} Neuropathologically, PD related dementia appears to be indistinguishable from DLB.\textsuperscript{18,47} Lastly, in PD related dementia, concomitant cortical AD-pathology is often found. This may also contribute to alterations in resting state brain activity.

In the present study, posterior alpha power in the demented PD patients was not decreased in the eyes open condition relative to the eyes closed condition. By contrast, in the control subjects and the non-demented PD patients, posterior alpha power was much reduced in the eyes open condition. Since this effect is well known in the literature, it seems appropriate to assume that the observed alpha power reduction is related to eye opening and that the increases in relative power in other frequency bands (mainly delta and gamma power in healthy subjects and beta and gamma power in non-demented patients; see figure 5) are at least partly secondary to the alpha power reduction. However, it can not be ruled out that increases in relative power outside the alpha range reflect actual changes in resting state brain activity after eye opening. Interestingly, the almost complete absence of reactivity of resting state brain activity to eye opening in the demented PD patients is reminiscent of the reduced alpha reactivity in Alzheimer’s disease.\textsuperscript{71}

In conclusion, our results demonstrate that a slowing of resting state brain activity is a feature of non-demented PD patients, possibly related to degeneration of ascending dopaminergic, noradrenergic and/or serotonergic projections arising in the brainstem. The pattern of slowing of resting state brain activity in demented PD patients is quite different, involving additional frequency bands, and may be related to cholinergic losses and/or cortical Lewy body- and AD-pathology. Based on the present data, it seems likely that pathological changes that are already present in non-demented PD patients, further progress in demented patients and that furthermore, additional pathophysiological mechanisms are probably involved in dementia in PD.
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Pathophysiological mechanisms in Parkinson's disease related dementia: an MEG study
Chapter 3.2
Cholinergic modulation of MEG resting state oscillatory activity in Parkinson’s disease related dementia

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ABSTRACT

EEG and MEG studies in Parkinson’s disease (PD) related dementia (PDD) have shown a slowing of resting state oscillatory activity compared to non-demented PD. Aim of the present study using MEG was to determine whether treatment with the cholinesterase inhibitor rivastigmine would reverse this slowing of resting state brain activity in PDD patients.

In eight PDD patients, whole head MEG was recorded in a resting state condition before and after treatment with rivastigmine. Relative spectral power was calculated in the delta, theta, alpha, beta and gamma frequency bands in fronto-central, parieto-occipital and temporal regions.

After treatment with rivastigmine, PDD patients demonstrated an increase in relative power in the alpha range in parieto-occipital and temporal regions together with a diffuse increase in beta power. Furthermore, a decrease of delta power in fronto-central and parieto-occipital regions was found.

Treatment with the cholinesterase inhibitor rivastigmine at least partly counteracts the slowing of resting state brain activity that is known to occur in PD related dementia. Our observations emphasize the prominent role of degeneration of the cholinergic system in the pathophysiology of dementia in PD. In the future, MEG might contribute to the selection of demented PD patients who may optimally benefit from cholinergic treatment.
INTRODUCTION

Dementia develops in up to 60% of patients suffering from Parkinson’s disease (PD)\(^1\) and contributes significantly to the impairment of the quality of life and to caregiver distress. Parkinson’s disease related dementia (PDD) mainly consists of a prominent dysexecutive syndrome together with memory complaints and is often accompanied by psychotic symptoms, mainly visual hallucinations.\(^2\) The pathophysiological mechanisms of cognitive dysfunction and dementia in PDD are still poorly understood. Although the loss of nigrostriatal and corticopetal dopaminergic (and serotonergic and noradrenergic) projections systems may contribute to the development of dementia in PD, it is generally believed that additional mechanisms are probably involved, most notably degeneration of cholinergic cortical projections and/or local cortical Lewy body- and tau-pathology (For review, see Bosboom 2004\(^3\)).

EEG studies have demonstrated a slowing of background oscillatory activity in PDD patients,\(^4-7\) correlating with the degree of mental impairment. Recently, using relative power spectral analysis of resting state magnetoencephalography (MEG) data, we found a qualitatively different pattern of slowing of background activity in demented compared to non-demented patients.\(^8\) Whereas in PD without dementia an increase in theta and a decrease of beta power were found compared to healthy controls, in PDD an additional increase of relative delta power and a decrease of alpha band power could be demonstrated relative to the non-demented patients, supporting the assumption that different or at least additional pathophysiological mechanisms are involved in the development of PDD.

Animal as well as human studies have demonstrated that the cholinergic system has a modulatory influence on cortical brain rhythms. Cholinergic stimulation mainly results in a shift in the powerspectrum towards faster frequencies, whereas interference with cholinergic function leads to an increase in slow wave activity.\(^9-16\) Considering the presence of a cholinergic deficit in PD, this would suggest that a hypofunctional cholinergic system might be responsible for the observed slowing of background oscillatory activity in PDD. Along this line of reasoning, it seems likely that treatment aimed at restoring cholinergic function would at least partly reverse the observed slowing of background activity in PDD.

To date, treatment with cholinesterase inhibitors is the only proven, albeit symptomatic treatment for PDD. The same holds for Alzheimer’s disease (AD), like PDD characterized by
prominent cholinergic deterioration. The action of cholinesterase inhibitors is aimed at increasing cholinergic brain activity by interfering with the function of the enzyme acetyl (and/or butyryl) cholinesterase, responsible for the breakdown of acetylcholine in the brain. In AD, several cholinesterase inhibitors such as rivastigmine, galantamine and donepezil, were found to be (equally) effective in the treatment of mild to moderate AD (for review see Birks 200617). In PDD, a number of open label studies and a randomized, placebo-controlled, multicenter study with rivastigmine have shown beneficial effects on cognitive function as well as on psychotic symptoms.18-23

In AD patients, treatment with cholinesterase inhibitors is associated with a decrease of low frequency EEG activity.24-29 In PDD, however, the effects of increasing cholinergic tone on changes in cortical rhythmic activity are largely unknown. In an EEG study, Fogelson et al found a diffuse increase in relative alpha amplitude after 12 weeks of treatment with rivastigmine, but significant changes in other frequency bands could not be demonstrated.30 MEG studies in patients are as of yet not available. In non-demented patients, we demonstrated slowing of resting state oscillatory activity already in the earliest31 as well as more advanced stages of disease,8 which has not been convincingly reported in previous EEG studies.4-7

The aim of this study was to study the effects of treatment with the cholinesterase inhibitor rivastigmine on spectral power distribution in PDD patients using MEG. Our hypothesis was that treatment with rivastigmine would result in (partial) reversal of the slowing of background oscillatory activity that is a characteristic of PDD.

MATERIALS AND METHODS

Subjects
A group of eight demented PD patients was studied immediately before and after a mean period of 29.3 weeks (range 19-48 weeks) of ongoing treatment with the cholinesterase inhibitor rivastigmine. Seven patients participated in a large double-blind, randomized, placebo-controlled trial with rivastigmine,18 one patient was treated in an open label setting. All patients underwent a full physical and neurological examination and fulfilled the United Kingdom Parkinson’s Disease Society Brain Bank (UK-PDSBB) clinical diagnostic criteria for probable Parkinson’s disease32 as well as the DSM-IV criteria33 for dementia. Each patient had a Mini Mental State Examination (MMSE) score34 of 24 or lower out of a maximum of 30 points, with lower scores indicating worse cognition. Blood examination
and MR imaging were performed to exclude other potential causes of dementia. Additional cognitive assessment consisted of the cognitive section of the CAMDEX: the CAMCOG. A total of 107 points is the maximal score on this test with higher scores indicating better cognition.

Disease stage and severity were assessed using the (modified) Hoehn & Yahr-scale (H&Y; range 0-5 with higher scores indicating more advanced disease stage) and the motor section of the Unified Parkinson’s Disease Rating Scale (UPDRS; range 0-108 with higher scores indicating worse motor functioning), respectively. Exclusion criteria for PD patients consisted of stereotactic surgery in the past and the use of anticholinergics, neuroleptics or cholinesterase inhibitors.

All patients were treated with a combination of levodopa and a decarboxylase inhibitor and six patients were treated with a dopamine agonist as well. The study protocol was approved by the medical ethical committee of the VU University Medical Center. After careful explanation of the procedures, all subjects gave written informed consent prior to participating.

**MEG procedures**

MEG data were acquired using a 151-channel whole head MEG system (CTF Systems Inc., Port Coquitlam, BC, Canada), with patients seated in a magnetically shielded room (Vacuum-schmelze GmbH, Hanau, Germany). Both the baseline and the follow up MEG recording were performed in a no task, eyes closed, resting state condition, approximately one hour after patients had taken their first morning dose of antiparkinsonian medication. The recording pass band was 0-125 Hz with a sample rate of 312.5 Hz. A third-order software gradient (Vrba, 1996) was applied. Two approximately 13 second long artifact-free epochs (sample rate 312.5 Hz; 4096 samples) were selected for further analysis. Epoch selection was always done by the same investigator (JLWB), who was blinded for the time of measurement (baseline or follow up). MEG recordings were filtered offline with a band pass of 1-48 Hz.

Relative band power of every MEG channel was computed for the two 13 second epochs in the following frequency bands: 1-4 Hz (delta), 4-8 Hz (theta), 8-13 Hz (alpha), 13-30 Hz (beta) and 30-48 Hz (gamma). Results of the two epochs were averaged for each subject.

To reduce the number of comparisons before and after treatment, the MEG channels were clustered into three regions of interest: fronto-central, parieto-occipital and temporal (figure 1). Mean relative spectral power in these clustered groups of MEG channels was used in the statistical analysis. The midline channels (Z) were left out of this
clustering. Of the original 151 channels, one channel (MLO41) was not available due to technical problems.

Figure 1
A. Schematic representation of the distribution of individual MEG sensors.
B. Schematic representation after clustering into three regions of interest, which were used in the statistical analysis.

STATISTICAL ANALYSIS

Demographics
Differences between the baseline and follow up measurement in the distribution of (modified) Hoehn and Yahr scores were analyzed by means of chi-square tests. Analyses with regard to within-subject changes from baseline to follow up in UPDRS motor scores and CAMCOG scores were analyzed using the Wilcoxon signed rank test.

Relative spectral power
For each frequency band, within subject changes from baseline to follow up in mean MEG relative spectral power in the three regions of interest (fronto-central, parieto-occipital and temporal) as well as averaged over all three regions, were analyzed using the Wilcoxon signed-rank test.

Correlation with clinical parameters
Correlations of changes in relative spectral power with changes in cognitive function (CAMCOG) were studied using Spearman’s rank correlation test. To achieve this, spectral power values in the various frequency bands as well as the CAMCOG scores at follow up, were subtracted from the baseline values and subsequently, the obtained value was divided by the baseline value, yielding a value between -1 and 1, expressing the percentage of change from baseline which could either be negative (a reduction after treatment) or positive (an increase after treatment). Furthermore, changes in CAMCOG scores were also correlated to baseline relative spectral power values.
All analyses were performed at a significance level of 5% (two-tailed) using the SPSS 15.0.0 software package (SPSS inc., Chicago, IL, U.S.A.).

RESULTS

Subject characteristics

In table 1, the general characteristics of the study group are listed. At baseline, mean age of the patients was 74.1 ± 5.3 years and disease duration 12.8 ± 2.6 years. All patients reached the maximum daily dose of 12 mg rivastigmine, but in one patient medication was discontinued because of side effects (nausea) after 19 weeks. The follow up MEG measurement in this patient was recorded before rivastigmine was discontinued. Patients had significantly higher mean CAMCOG and MMSE scores (corresponding with improved cognition) after cholinergic treatment: 77.4 ± 7.5 vs. 86.4 ± 8.7 (p=.01) and 21.6 ± 2.6 vs. 27.3 ± 1.9 (p=.02), respectively. The difference in MMSE scores is based on the results in seven patients since one MMSE score at follow up was missing. UPDRS motor score in the ON state increased during the study period, from 19.4 ± 4.2 at baseline to 22.1 ± 1.9 at follow up, but this difference was statistically not significant (p=.09).

<table>
<thead>
<tr>
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<tr>
<td>Age at baseline (years)</td>
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<tr>
<td>UPDRS ON (0-108)</td>
<td>Baseline 19.4 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>Follow up 22.1 ± 2.8</td>
</tr>
<tr>
<td>CAMCOG (0-107)</td>
<td>77.4 ± 7.5</td>
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<tr>
<td></td>
<td>86.4 ± 8.7</td>
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<tr>
<td>MMSE (0-30)</td>
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<td>27.3 ± 1.9</td>
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<td></td>
<td>0/0/2/2/4/0/0</td>
</tr>
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Table 1

PDD = Parkinson’s disease related dementia; UPDRS = Unified Parkinson’s Disease Rating Scale; CAMCOG = Cognitive section of the CAMDEX; MMSE = Mini Mental State Examination; H&Y = Hoehn & Yahr

Spectral power analysis

A schematic representation of the changes in relative spectral power between the baseline and follow up measurement is displayed in figure 2. Furthermore, in figure 3, the raw MEG signal of a number of occipital channels of two patients before and after rivastigmine treatment is shown.
Pathophysiological mechanisms in Parkinson’s disease related dementia: an MEG study

Figure 2
Schematic representation of the differences in resting state relative power before and after treatment with the cholinesterase inhibitor rivastigmine. Statistically significant increases in within subject relative power changes after treatment are coloured red, reductions are coloured blue. The intensity of the colours indicates the magnitude of the p-value (light = \( p < .05 \); middle = \( p < .01 \); dark = \( p < .001 \)).

- **PDD** = Demented Parkinson’s disease patients
- **FC** = Fronto-central
- **PO** = Parieto-occipital
- **LT** = Left Temporal
- **RT** = Right Temporal

Relative alpha power significantly increased in parieto-occipital and temporal areas (\( Z = -2.384; p = .017 \) and \( Z = -2.388; p = .017 \) respectively), as well as averaged over all regions of interest (\( Z = -2.201; p = .027 \)). Furthermore, there was a significant increase in beta power after treatment with rivastigmine. This change was found in all regions of interest: fronto-central (\( Z = -2.524; p = .012 \)), parieto-occipital (\( Z = -2.384; p = .017 \)) and temporal (\( Z = -2.410; p = .016 \)). Obviously, the average relative spectral power over all three regions reached significance as well (\( Z = -2.527; p = .012 \)). In contrast, there was a significant decrease in relative delta power in fronto-central and parieto-occipital areas (\( Z = -2.252; p = .024 \) and \( Z = -2.521; p = .012 \) respectively) as well as averaged over all areas (\( Z = -2.283; p = .017 \)). No significant differences were found in the theta and gamma band.

Figure 3
Raw MEG signal in part of the occipital region of two PDD patients before and after cholinergic treatment. Next to the raw signal, the corresponding power spectrum is demonstrated. In both patients, there is a shift of the power spectrum towards faster frequencies, which is also evident in the raw signals.
Correlations with clinical parameters

A negative correlation was found between changes in CAMCOG and baseline parieto-occipital ($\rho = -0.802; p = 0.017$) and total ($\rho = -0.714; p = 0.017$) delta power. Conversely, a positive correlation was found between the CAMCOG score and fronto-central beta as well as gamma relative power ($\rho = 0.708; p = 0.05$ and $\rho = 0.830; p = 0.011$ respectively). In other words, a greater beneficial effect of cholinergic treatment in our patients was associated with a smaller percentage of delta power and a greater amount of fronto-central beta and gamma power at baseline.

A positive correlation was found between changes in CAMCOG scores and changes in parieto-occipital ($\rho = 0.810; p = 0.015$) and total ($\rho = 0.802; p = 0.017$) spectral power in the delta band. Since in most patients, a reduction in delta power could be demonstrated (a negative value in the analysis), this means that a greater degree of cognitive improvement is correlated with a smaller reduction in relative delta power.

Conversely, the change in CAMCOG score was negatively associated with relative power changes in all three regions of interest in the theta band (fronto-central: $\rho = -0.833; p = 0.010$; parieto-occipital: $\rho = -0.719; p = 0.045$; temporal: $\rho = -0.755; p = 0.020$). Theta power increased in some patients with only a small change in CAMCOG score and decreased in patients with a more prominent increase in CAMCOG score, corresponding to improvement of cognition.

DISCUSSION

To our knowledge, this is the first MEG study comparing resting state spectral power in demented PD patients before and after treatment with cholinesterase inhibitors. We observed a shift of the frequency spectrum towards higher frequencies, consisting of an increase in parieto-occipital and temporal alpha power and a diffuse increase in beta power, together with a decrease in fronto-central and parieto-occipital delta power.

To date, the effect of cholinergic treatment on background oscillatory activity in PDD was largely unknown. An increase in alpha activity after twelve weeks of treatment with rivastigmine has been reported in an EEG study, but significant changes in other frequency bands could not be demonstrated. In the present MEG study, significant power increases in the alpha band occurred in parallel with changes in other frequency bands, i.e. a decrease in the delta band and an increase in the beta band. In a previous MEG study, we found an increase in delta and a decrease in alpha relative power in PDD compared to non-demented PD, together with a further increase of theta and decrease of beta power, which could already be demonstrated in non-demented patients compared to controls.
Thus, the changes after rivastigmine treatment in the present study are in the exact opposite direction of the relative spectral power changes we have recently reported in demented PD patients compared to non-demented patients\cite{8} and have been reported by others.\textsuperscript{4-7} The increase in alpha relative power was found in posterior regions, which is not surprising since the normal alpha distribution shows a posterior dominance, and the reductions in alpha power in PDD patients compared to non-demented PD patients mainly involve these regions.\textsuperscript{8} The changes in delta and beta power in the present study were more diffuse, possibly reflecting the widespread cholinergic innervation of the cerebral cortex.

Thus, the present MEG study demonstrates that cholinergic treatment can at least partly counteract the slowing of background oscillatory activity that is characteristic of dementia in PD. In other words, there is a shift in the power spectrum towards more normal background oscillatory activity. This finding further supports the notion that dementia in PD is not just caused by a progression of changes in neurotransmitter systems already present in non-demented PD, mainly the dopaminergic system, but that degeneration of the cholinergic system plays an important pathophysiological role.

The present results are in agreement with animal as well as human studies, in which a modulatory role of the cholinergic neurotransmitter system on cortical rhythms has previously been demonstrated. In these studies, a decrease of slow, mainly delta activity and an increase of fast EEG background activity have been found after enhancement of cholinergic function. Conversely, degeneration or lesioning of the cholinergic cortical projections from the basal nucleus of Meynert with a corresponding loss of cortical cholinergic activity as well as a blockade of postsynaptic cortical muscarinic receptors are associated with an increase in slow, mainly delta activity.\textsuperscript{9,12,13,15-17,38} Similar results have been obtained in studies with AD patients, who like PDD patients have significant though somewhat less prominent cholinergic losses and a slowing of background oscillatory activity (for review see Jeong 2004\textsuperscript{39}). In the majority of studies using various cholinesterase inhibitors, a decrease of low frequency power, mainly theta\textsuperscript{25-29} and delta power\textsuperscript{25,26,30} have been reported.

Using LORETA, Gianotti et al found that these effects were most prominent in (left) fronto-parietal and (para) hippocampal regions, as well as the posterior cingulate, areas involved in memory function.\textsuperscript{40}

Different from our findings in PDD, increases in alpha and beta power have seldom been reported, whilst even a reduction of higher frequency power has been found.\textsuperscript{25,27,41} However, follow up in the AD studies was one year, which is considerably longer than in
the present study. Therefore, an initial increase in higher frequency power may have become obscured by disease progression leading to decreased high frequency power. Furthermore, in the study by Babiloni et al, the alpha reduction was significantly lower in responders to cholinergic treatment compared to non-responders, supporting the promotion of high frequency oscillatory brain activity by effective cholinergic treatment in AD. This mechanism is further reflected in the reported increase in alpha to theta ratio in responders to cholinesterase treatment compared to non-responders.

We found a negative correlation between delta power at baseline and the magnitude of improvement on the CAMCOG, indicating that a better clinical response to rivastigmine is associated with lower baseline delta power. However, we also found an inverse relation between the magnitude of cognitive improvement as measured with the CAMCOG and decrease in delta power. Lastly, the change in CAMCOG score was negatively associated with relative power changes in the theta band. Since our study sample was small and only one patient showed a slight deterioration in CAMCOG score, the above mentioned correlations should be interpreted with caution. Future studies with a larger number of patients displaying a greater variety of response to treatment with cholinesterase inhibitors are needed to reliably study associations of spectral power changes with clinical parameters. In this way, by studying differences in baseline neurophysiological parameters between responders to treatment and non-responders, MEG (or EEG) might contribute to the selection of patients who may optimally benefit from cholinergic treatment.

In our study, treatment with rivastigmine constituted the major difference in study conditions between the baseline and follow up measurements. As a matter of fact, we carefully avoided other factors that may have influenced resting state MEG activity at the follow up measurement. First, the dosage of dopaminergic drugs was kept stable during the cholinergic treatment period, since stimulation of the dopaminergic system has been associated with an increase in fast EEG activity in animal studies as well as an EEG study in non-demented PD patients. Therefore, significant dopaminergic treatment effects are very unlikely. Second, the introduction of other drugs influencing spectral power activity, mainly the use of benzodiazepines that may increase beta band power, was avoided during the treatment period. Lastly, it could be argued that progressive degeneration of the dopaminergic system itself might have influenced cortical oscillatory brain activity. However, under a stable dopaminergic treatment regime, motor function only marginally (non significantly) worsened in PDD patients during the treatment period, indicative of only mild deterioration of dopaminergic function. More importantly,
dopaminergic deficiency is rather associated with a decrease of high frequency power and an increase in low frequency power activity.\textsuperscript{32,43,44} Taken together, it is unlikely that factors other than the intended cholinergic modulation have accounted for the observed changes in MEG background oscillatory activity in this study.

To reduce the number of statistical comparisons, MEG sensors were clustered into three regions of interest, corresponding to fronto-central, parieto-occipital and temporal cortical areas. Due to this clustering of sensors, possibly regional information may be lost. Furthermore, since we used relative spectral power, it is not exactly known which changes in spectral power are primary and which are secondary to changes in other frequency bands. However, since we were mainly interested in the general pattern of changes in spectral power after treatment with rivastigmine and we intended to relate the results to the changes demonstrated in PDD compared to PD in our previous study applying relative spectral power,\textsuperscript{8} we have chosen to use the current methodology.

In conclusion, our data show that the slowing of resting state oscillatory brain activity that is characteristic of dementia in PD can at least partly be counteracted by treatment with the cholinesterase inhibitor rivastigmine, strongly supporting the importance of degeneration of the cholinergic system in slowing of background oscillatory activity in and development of dementia in PD.
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Pathophysiological mechanisms in Parkinson's disease related dementia: an MEG study.
Chapter 4.1
Increased cortico-cortical functional connectivity in early stage Parkinson's disease: an MEG study

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We set out to determine whether changes in resting state cortico-cortical functional connectivity are a feature of early stage Parkinson’s disease (PD), explore how functional coupling might evolve over the course of the disease and establish its relationship with clinical deficits.

Whole head magnetoencephalography was performed in an eyes closed resting state condition in 70 PD patients with varying disease duration (including 18 recently diagnosed, drug-naive patients) in an “OFF” medication state and 21 controls. Neuropsychological testing was performed in all subjects. Data analysis involved calculation of three synchronization likelihood (SL, a general measure of linear and non-linear temporal correlations between time series) measures which reflect functional connectivity within (local) and between (intra- and interhemispheric) ten major cortical regions in five frequency bands.

Recently diagnosed, drug-naive patients showed an overall increase in alpha1 SL relative to controls. Cross-sectional analysis in all patients revealed that disease duration was positively correlated with alpha2 and beta SL measures, while severity of parkinsonism was positively correlated with theta and beta SL measures. Moderately advanced patients had increases in theta, alpha1, alpha2 and beta SL, particularly with regard to local SL. In recently diagnosed patients, cognitive perseveration was associated with increased interhemispheric alpha1 SL.

Increased resting state cortico-cortical functional connectivity in the 8-10 Hz alpha range is a feature of PD from the earliest clinical stages onwards that expands into neighbouring frequency bands with disease progression. These findings suggest that changes in functional coupling over the course of PD may be linked to the topographical progression of pathology over the brain.
INTRODUCTION

Synchronization of neuronal activity between distributed brain regions plays a key role in the integration of their activity.¹ This phenomenon can be studied by measuring statistical interdependencies between physiological signals derived from different brain regions over a certain time interval,² a concept aptly named functional connectivity.³ As functional integration is essential to normal brain function, clinical deficits in brain disorders may well be associated with changes in the synchronization of oscillatory brain signals,⁴,⁵ which might even be observed during a no task, resting state condition.⁵ The resting state is a far more stable and active condition than previously assumed⁶ and is characterized by activation within a series of functional–anatomic networks implicated in motor, sensory and cognitive functions.⁷ Each of these resting state networks appears to have a specific electrophysiological signature, that combines the involvement of different brain rhythms.⁸

Utilizing neurophysiological indices of functional connectivity, changes in cortico-cortical coupling during a resting state have now been demonstrated in diverse brain disorders; mild cognitive impairment,⁹-¹² Alzheimer’s disease,⁹,¹¹,¹³-¹⁹ multiple sclerosis,²⁰ brain tumour patients²¹,²² and schizophrenia.²³ In mild cognitive impairment and Alzheimer’s disease, changes were correlated with cognitive deficits.¹⁰,¹²,¹⁸

In a recent electroencephalography (EEG) study in advanced Parkinson’s disease patients, resting state cortico-cortical coupling in the ~10-35 Hz range was positively correlated with “OFF” treatment severity of parkinsonism.²⁴ Both dopaminergic therapy and deep brain stimulation led to a reduction in coupling in parallel to motor improvement. From these data, it would appear that at least in the advanced stages of Parkinson’s disease increased functional connectivity may play a role in the pathophysiology of parkinsonism. So far, it is unclear whether a similar phenomenon might occur in early stage Parkinson’s disease and whether it plays a role in Parkinson’s disease related cognitive dysfunction. Moreover, increased functional connectivity in Parkinson’s disease has never been demonstrated relative to controls.²⁵

The present study was undertaken to determine whether changes in resting state functional connectivity occur in the earliest clinical stages of Parkinson’s disease and to explore how functional coupling might evolve over the disease course. In addition, we investigated its relationship with clinical measures of motor and cognitive function. To this end, synchronization likelihood (SL, a general measure of linear and non-linear temporal correlations between time series) was calculated from whole head magnetoencephalography (MEG) recordings obtained during an eyes closed resting state condition in a group of 70 Parkinson’s disease patients with varying disease duration
(including 18 recently diagnosed, drug-naive patients) as well as in 21 healthy controls that were age-matched to the recently diagnosed patients.

**MATERIAL AND METHODS**

**Subjects**

A total of 70 patients with idiopathic Parkinson’s disease (disease duration 0-13 yrs) and 21 healthy controls were recruited and selected for analysis as described in a previous MEG study in the same subjects.\(^{26}\) Out of the 70 patients, two subgroups were constructed, i.e. a group of recently diagnosed, untreated patients (diagnosed in the last six months prior to participation in this study, disease duration of less than two years, never treated with anti-Parkinson medication, \(N = 18\)) and a group of moderately advanced patients (disease duration 9-13 years, \(N = 17\)). Controls were age-matched to the recently diagnosed patients.

**Subject characteristics**

Level of education was determined using the International Standard Classification of Education [ISCED-1997]\(^{27}\). The premorbid level of intelligence was measured using the National Adult Reading Test [NART]\(^{28}\). Global cognitive function was examined using the CAMCOG scale.\(^{29}\) Disease duration was based on the patients’ subjective estimate of the time of occurrence of the first motor symptoms. Side of onset was based on the body-half in which these symptoms occurred. Unified Parkinson’s disease Rating Scale motor scores [UPDRS-III]\(^{30}\) and modified Hoehn & Yahr stages\(^{31}\) were obtained by a trained physician. UPDRS tremor subscores were computed by summing scores on items 20 & 21. All subjects gave written informed consent to the research protocol, which was approved by the local medical ethical committee. Ethics review criteria conformed to the Helsinki declaration. Subject characteristics are listed in table 1.
Pathophysiological mechanisms in Parkinson’s disease related dementia: an MEG study

### Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls (N=21)</th>
<th>Recently diagnosed PD patients (N=18)</th>
<th>Moderately advanced PD patients (N=17)</th>
<th>All PD patients (n=70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, mean ± SD)</td>
<td>59,4 ± 7,3</td>
<td>59,4 ± 7,9</td>
<td>64,2 ± 5,8</td>
<td>62,1 ± 6,8</td>
</tr>
<tr>
<td>Sex (♂/♀)</td>
<td>11/10</td>
<td>12/6</td>
<td>8/9</td>
<td>40/30</td>
</tr>
<tr>
<td>Education (ISCED 0/1/2/3/4/5/6)</td>
<td>0/0/6/4/2/8/1</td>
<td>0/0/5/5/0/8/0</td>
<td>0/1/7/1/2/6/0</td>
<td>1/0/27/16/4/21/1</td>
</tr>
<tr>
<td>Verbal IQ (Dutch NART)</td>
<td>111,7 ± 9,4</td>
<td>109,2 ± 11,2</td>
<td>109,2 ± 12,9</td>
<td>107,7 ± 13,1</td>
</tr>
<tr>
<td>Global cognition (CAMCOG)</td>
<td>98,9 ± 4,2</td>
<td>98,2 ± 4,7</td>
<td>97,2 ± 5,1</td>
<td>97,0 ± 5,1</td>
</tr>
<tr>
<td>Disease duration (years ± SD)</td>
<td>n.a.</td>
<td>0,9 ± 0,5</td>
<td>13,3 ± 1,3</td>
<td>5,5 ± 3,7</td>
</tr>
<tr>
<td>Side of onset (left/right)</td>
<td>n.a.</td>
<td>4/14</td>
<td>10/7</td>
<td>33/37</td>
</tr>
<tr>
<td>H&amp;Y mod “OFF” (1/1,5/2/2,5/3)</td>
<td>n.a.</td>
<td>9/1/7/1/0</td>
<td>0/0/8/7/2</td>
<td>14/1/29/18/8</td>
</tr>
<tr>
<td>UPDRS III &quot;OFF&quot; (mean ± SD)</td>
<td>0,6 ± 1,4</td>
<td>13,1 ± 6,1</td>
<td>19,6 ± 5,4</td>
<td>17,1 ± 6,9</td>
</tr>
<tr>
<td>LEDD (mg)</td>
<td>n.a.</td>
<td>n.a.</td>
<td>795 ± 361</td>
<td>406 ± 423</td>
</tr>
</tbody>
</table>

**Table 1**

**Subject characteristics**

ISCED = International Standard Classification of Education, NART = National Adult Reading Test, CAMCOG = the cognitive part of the Cambridge Examination for Mental Disorders of the Elderly, H&Y mod = modified version of the Hoehn and Yahr rating scale, UPDRS-III = motor part of the Unified Parkinson’s Disease Rating Scale, LEDD = levodopa equivalent daily dose; n.a. = not applicable.

### Neuropsychological evaluation

Cognitive functions were assessed using a set of neuropsychological tasks as described previously. In short, six tasks from the Cambridge Neuropsychological Test Automated Battery (CANTAB eclipse 2.0, Cambridge Cognition, Cambridge, U.K.) as well as two tasks from the Vienna Test System version 6.0 (Dr. G. Shuhfried GmbH, Mödling, Austria) were administered.

### MEG data acquisition and pre-processing

MEG data acquisition and pre-processing were performed as described previously. In short, patients treated with levodopa were instructed to come to the hospital without taking their first morning dose of anti-Parkinson medication (practically defined “OFF”). We recorded using a 151-channel whole head radial gradiometer MEG system (CTF Systems Inc., Port Coquitlam, BC, Canada). Subjects were instructed to simply sit as still as possible with their eyes closed in the MEG apparatus and to try not to fall asleep during acquisition. Three approximately 13 second artefact free epochs per registration were selected off-line by two of the investigators, who were blinded to the clinical diagnosis and imported into the DIGEEGXP 2.0 software package (C.J. Stam, Amsterdam, The Netherlands). MEG data were digitally filtered in the following frequency bands: 0.5-4 Hz...
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(delta), 4-8 Hz (theta), 8-10 Hz (alpha1), 10-13 Hz (alpha2), 13-30 Hz (beta) and 30-48 Hz (gamma). MEG channels were grouped in sensor space into regions of interest (ROIs) roughly corresponding to ten major cortical areas (frontal, central, temporal, parietal and occipital) on both sides of the brain. The nine midline channels were left out of this clustering. Additionally, one channel above the occipital region was not available during all recordings because of technical problems, leaving a total of 141 channels divided over ten ROIs for analysis (figure 1A&B). As ROIs were based on the extra-cranial position of the MEG sensors, underlying cortical areas are to be considered as indicative.

Figure 1

A. Clustering of MEG sensors above the major cortical areas. Midline sensors (depicted in black) were excluded from synchronization likelihood (SL) analysis.

B. Schematic representation of regions of interest (ROIs) used for within and between ROI SL calculations.

C. Intrahemispheric SL

D. Interhemispheric SL. Arrows indicate between ROI SL measures used.

Synchronization likelihood

SL is a general measure of the temporal correlation between two time series sensitive to linear as well as non-linear statistical interdependencies (for a technical description, see Stam et al\textsuperscript{32}). Parameter settings used for SL computation were explicitly based on the frequency content of the data (for lags and embedding dimensions used, see\textsuperscript{33}). $P_{\text{ref}}$ was set at 0.01 for all frequency bands. Ten local SL measures were computed per epoch by averaging the SL values of all possible sensor pairs within each ROI (figure 1B). 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 1C). 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 1D). 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. Subsequently, SL values of the three epochs were averaged for each subject. Additionally, one or two channels showed artefacts of a technical nature during visual inspection of the
epochs in a small number of subjects (N = 13). Magnetic field strengths for the respective individual channels in these particular subjects, as well as for the aforementioned occipital channel in all subjects, were substituted by zero in all epochs, ensuring that the averaged synchronization in the ROI containing the bad channel was only minimally distorted (for the location of bad channels, see Stoffers et al\textsuperscript{26}).

**Statistical analysis**

Differences between groups in the distribution of sex and education level were analyzed by means of chi-square tests. Analyses with regard to group differences in age, premorbid IQ and CAMCOG scores were performed by means of univariate general linear model (GLM) testing. To increase statistical power, we attempted to normalize SL values using inverse transformation. Although this yielded very good results, a few SL measures could still not be normalized sufficiently by means of this transformation to pass Kolmogorov-Smirnov tests of normality. However, serious non-normality was only observed for delta band SL measures. In view of the fact that delta band functional connectivity can easily be confounded by movement artefacts, we excluded this band from further analyses. As an exploratory analysis suggested abnormal functional connectivity even in the earliest stages of disease, we chose to first compare functional connectivity in recently diagnosed, drug-naive Parkinson’s disease patients with age-matched controls to study changes in early stage disease (analysis A) and, subsequently, to explore the relation of functional connectivity with disease duration and disease (motor) severity within the whole group of Parkinson’s disease patients (analysis B). Since analysis A demonstrated changed functional connectivity over a limited frequency range in early stage Parkinson’s disease, and analysis B suggested these changes might well expand into neighbouring frequency bands with disease progression, we then compared functional connectivity in those frequency bands between moderately advanced Parkinson’s disease patients and controls to further explore changes in functional connectivity in later stages of disease (analysis C). The analysis of the relation between functional connectivity and cognitive performance was limited to recently diagnosed, drug-naive patients to exclude confounding effects of medication on task performance (analysis D). Relations between cognitive performance and functional connectivity were only analyzed for parameters that showed differences between recently diagnosed Parkinson’s disease patients and controls, in this way diminishing the likelihood of type-I statistical errors.

All analyses were performed at a significance level of 5% (two-tailed) using the SPSS 15.0.1.1 software package (SPSS inc., Chicago, IL, U.S.A.). Potential confounders that were included in the initial analysis were considered relevant if at least a trend involving the confounder (P below 10%) was observed, otherwise they were excluded from final
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Partial eta squared (\(\eta^2\)) was calculated when performing GLM analyses, which represents the proportion of the total variability in the dependent variable that is accounted for by the relevant factor/covariant, when controlling for all (other) covariants in the GLM.

**ANALYSIS A**
Differences in SL between recently diagnosed, drug-naive Parkinson’s disease patients \((N = 18)\) and controls \((N = 21)\) were analyzed by means of three univariate GLM analyses per frequency band using each of the overall SL measures (local, intra- and interhemispheric SL) as dependent and group as well as any relevant confounders as determinants.

**ANALYSIS B**
The relation of SL with disease parameters in Parkinson’s disease patients \((N = 70)\) were analyzed by means of three univariate GLM analyses per selected frequency band using each of the SL measures as dependent and both disease duration and UPDRS motor score as well as any relevant confounders as determinants. A disease parameter was maintained in the final analysis if at a minimum a trend involving the disease parameter \((P < 10\%)\) was observed. The relation with UPDRS motor subscores was analysed using stepwise linear regression analysis \((0.05 \text{ probability of } F \text{ for entry, } 0.10 \text{ for removal of a determinant from the regression equation})\) using four major UPDRS motor subscores (tremor, rigidity, bradykinesia and axial involvement) as well as age and sex in the initial regression equation.

**ANALYSIS C**
Differences in SL between moderately advanced Parkinson’s disease patients \((N = 17)\) and controls \((N = 21)\) involved three univariate GLM analyses per frequency band using each of the SL measures as dependent and group as well as any relevant confounders as determinants.

**ANALYSIS D**
The number of cognitive measures was reduced by means of principal component analysis (PCA) with varimax rotation and Kaiser normalization. For details, see Stoffers et al.\(^{26}\) This analysis yielded four separate components which were attributed to four executive functions: strategy/analysis, set-shifting, planning/spatial memory and perseveration. Analyses with regard to differences in cognitive performance between recently diagnosed Parkinson’s disease patients \((N = 18)\) and controls \((N = 21)\) were performed by means of univariate GLM analyses using each of the cognitive components as dependent and group as well as any relevant confounders as determinants. The relation of SL with cognitive performance in Parkinson’s disease patients \((N = 18)\) were analyzed by means of
univariate GLM analyses using an SL measure as dependent and a cognitive component from the PCA as well as any relevant confounders as covariants.

**RESULTS**

**Subject characteristics and confounders**

There were no significant differences in the distribution of sex or education level between groups, nor were there differences in age, premorbid IQ (NART) or global cognitive function (CAMCOG). Since age and sex could well be modifiers of functional connectivity, they were nonetheless added as covariants in all initial analyses of SL. Since age and premorbid IQ are well known modifiers of cognitive performance, they were initially added as covariants in all analyses of cognitive performance, as was sex.

**ANALYSIS A: EFFECT OF EARLY STAGE, UNTREATED DISEASE**

In recently diagnosed Parkinson’s disease patients, local ($p=0.001$, $\eta^2 = 26.4\%$), intra- ($p=0.013$, $\eta^2 = 15.9\%$) as well as interhemispheric ($p=0.026$, $\eta^2 = 13.1\%$) alpha1 SL were increased relative to controls. For each frequency band, means and SD’s of the SL measures are listed in table 2, a detailed illustration of changes in within ROI and between ROI SL measures can be found in figure 2.

![Figure 2](image)

_Schematic overview of specific synchronization likelihood (SL) measures that have mainly contributed to the differences in local, intra- and interhemispheric SL measures found in univariate testing between recently diagnosed, drug-naive Parkinson’s disease patients ($N = 18$) and controls ($N = 21$) in analysis A. Specific SL measures have been included if they showed a trend towards higher synchronization or better ($P < 0.10$) in post hoc testing. Note the relatively diffuse increase in functional connectivity, both with regard to within region of interest (ROI) as well as between ROI SL._

**ANALYSIS B: RELATION WITH DISEASE DURATION AND MOTOR FUNCTION**

Analyses in the full group of Parkinson’s disease patients showed positive associations of disease duration with local, intra- and interhemispheric alpha2 SL (figure 3A) and with
local beta SL (figure 3B), as well as positive associations of UPDRS motor score with local, intra- and interhemispheric theta SL (figure 4A) and with interhemispheric beta SL (figure 4C). Intrahemispheric beta SL was positively associated with both disease duration (figure 3B) and UPDRS motor score (figure 4A), but only in the absence of the other disease parameter in the GLM. When both were maintained, neither reached significance and effects were roughly comparable (p≈0.15). To study the relation of SL with tremor in more detail, the analysis was repeated using the tremor sub-score instead of the total motor score of the UPDRS. Analyses showed positive associations of tremor sub-score with local, intra- and interhemispheric theta SL (figure 5).
Figure 3
Scatterplots showing SI measures in the alpha2 (A) and beta (B) frequency band set out against the subjective disease duration in years in all PD patients (N = 70) in analysis B. Used covariates, significance level (P) and percentage of explained variance (η²) are indicated in the top left corner of each plot.
Figure 4

Scatterplots showing SL measures in the theta (A) and beta (B) frequency band set out against the total UPDRS motor score in all PD patients (N = 70) in analysis B. Used covariates, significance level (P) and percentage of explained variance (η²) are indicated in the top left corner of each plot.
Figure 5
Scatterplots showing SL measures in the theta (A) and beta (B) frequency band set out against the UPDRS sub-score that was maintained in stepwise linear regression analyses which initially contained all four UPDRS subscores (tremor, rigidity, bradykinesia and axial involvement) as well as age and sex in all PD patients (N = 70) in analysis B. Maintained covariates, significance level (P) and percentage of explained variance (η²) are indicated in the top left corner of each plot.
ANALYSIS C: EFFECT OF MODERATELY ADVANCED DISEASE:

In moderately advanced Parkinson’s disease patients, increases were found relative to controls in local (p=.002, \(\eta^2=23.2\%\)) intra- (p=.022, \(\eta^2=14.2\%\)) and interhemispheric (p=.025, \(\eta^2=13.3\%\)) theta SL, local (p=.003, \(\eta^2=22.5\%\)) and intrahemispheric (p=.017, \(\eta^2=15.3\%\)) alpha1 SL, local (p=.025, \(\eta^2=13.2\%\)) and interhemispheric (p=.045, \(\eta^2=10.7\%\)) alpha2 SL, and local (p=.044, \(\eta^2=11.1\%\)) beta SL. For each frequency band, means and SD’s of the SL measures are listed in table 2.

<table>
<thead>
<tr>
<th>Frequency band</th>
<th>SL Measure</th>
<th>Controls (N=21)</th>
<th>Recently diagnosed PD patients (N=18)</th>
<th>Moderately advanced PD patients (N=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theta (4-8Hz)</td>
<td>Local</td>
<td>0.1283 ± 0.0120</td>
<td>0.1342 ± 0.0096</td>
<td>\textbf{0.1434 ± 0.0174}</td>
</tr>
<tr>
<td></td>
<td>Intrahemispheric</td>
<td>0.0227 ± 0.0054</td>
<td>0.0228 ± 0.0029</td>
<td>\textbf{0.0279 ± 0.0090}</td>
</tr>
<tr>
<td></td>
<td>Interhemispheric</td>
<td>0.0350 ± 0.0115</td>
<td>0.0366 ± 0.0079</td>
<td>\textbf{0.0425 ± 0.0127}</td>
</tr>
<tr>
<td>Alpha1 (8-10Hz)</td>
<td>Local</td>
<td>0.1321 ± 0.0094</td>
<td>\textbf{0.1444 ± 0.0125}</td>
<td>0.1409 ± 0.0109</td>
</tr>
<tr>
<td></td>
<td>Intrahemispheric</td>
<td>0.0305 ± 0.0036</td>
<td>0.0345 ± 0.0061</td>
<td>\textbf{0.0325 ± 0.0032}</td>
</tr>
<tr>
<td></td>
<td>Interhemispheric</td>
<td>0.0381 ± 0.0039</td>
<td>\textbf{0.0421 ± 0.0064}</td>
<td>0.0390 ± 0.0040</td>
</tr>
<tr>
<td>Alpha2 (10-13Hz)</td>
<td>Local</td>
<td>0.1240 ± 0.0063</td>
<td>0.1238 ± 0.0068</td>
<td>\textbf{0.1314 ± 0.0128}</td>
</tr>
<tr>
<td></td>
<td>Intrahemispheric</td>
<td>0.0253 ± 0.0030</td>
<td>0.0243 ± 0.0020</td>
<td>0.0277 ± 0.0065</td>
</tr>
<tr>
<td></td>
<td>Interhemispheric</td>
<td>0.0327 ± 0.0038</td>
<td>0.0319 ± 0.0021</td>
<td>0.0370 ± 0.0069</td>
</tr>
<tr>
<td>Beta (13-30Hz)</td>
<td>Local</td>
<td>0.1230 ± 0.0077</td>
<td>0.1225 ± 0.0041</td>
<td>\textbf{0.1280 ± 0.0072}</td>
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<tr>
<td></td>
<td>Intrahemispheric</td>
<td>0.0208 ± 0.0029</td>
<td>0.0199 ± 0.0012</td>
<td>0.0219 ± 0.0030</td>
</tr>
<tr>
<td></td>
<td>Interhemispheric</td>
<td>0.0329 ± 0.0048</td>
<td>0.0324 ± 0.0028</td>
<td>0.0354 ± 0.0050</td>
</tr>
<tr>
<td>Gamma (30-48Hz)</td>
<td>Local</td>
<td>0.0964 ± 0.0042</td>
<td>0.0954 ± 0.0022</td>
<td>0.0987 ± 0.0071</td>
</tr>
<tr>
<td></td>
<td>Intrahemispheric</td>
<td>0.0164 ± 0.0010</td>
<td>0.0161 ± 0.0006</td>
<td>0.0171 ± 0.0025</td>
</tr>
<tr>
<td></td>
<td>Interhemispheric</td>
<td>0.0202 ± 0.0022</td>
<td>0.0196 ± 0.0015</td>
<td>0.0208 ± 0.0021</td>
</tr>
</tbody>
</table>

Table 2

Means ± SDs of synchronization likelihood (SL) measures in five major frequency bands.
Significant differences between patients and controls are indicated in bold; PD = Parkinson’s disease.

ANALYSIS D: RELATION WITH COGNITIVE DYSFUNCTION IN EARLY STAGE, UNTREATED DISEASE:

Recently diagnosed Parkinson’s disease patients had a lower capacity for planning/spatial memory (p=.031, \(\eta^2=13.3\%\)) and an increased tendency to cognitive perseveration (p=.029, \(\eta^2=13.6\%\)) relative to controls. No significant differences in performance were found with regard to strategy/analysis or set-shifting. Within the group of recently diagnosed patients, analyses showed a positive association of the level of perseveration with interhemispheric alpha1 SL (p=.007, \(\eta^2=39.9\%\)).
DISCUSSION

This is the first study to demonstrate widespread increases in alpha1 band functional connectivity in early stage Parkinson’s disease. With increasing disease duration and severity of parkinsonism, there appear to be higher levels of functional coupling in the theta, alpha2 and beta frequency bands. This results in significantly increased theta, alpha2 and beta band functional connectivity in moderately advanced Parkinson’s disease patients, in addition to the widespread increases in the alpha1 band that are already present in early stage Parkinson’s disease. In the group of early stage Parkinson’s disease patients, interhemispheric alpha1 coupling was positively associated with one of the earliest signs of cognitive dysfunction in Parkinson’s disease, i.e. an increased tendency to perseveration.

Excessive cortico-cortical coupling in Parkinson’s disease was first suggested by a study using coherence analysis of EEG data recorded from patients who had undergone stereotactic implantation of macroelectrodes in the subthalamic nucleus (STN) for deep brain stimulation. In these patients, resting state coherence in the ~10-35 Hz range was positively correlated with “OFF” treatment severity of parkinsonism. Moreover, reductions of coherence in this frequency range following either dopamine replacement therapy or high frequency STN stimulation were associated with the degree of motor improvement. The presently observed positive correlation between beta band functional connectivity and severity of parkinsonism, as well as the broad-band increases in functional connectivity demonstrated in our most advanced patient group are in line with results from the study by Silberstein and colleagues. Interestingly, a recent study combining resting state functional MRI with concurrent EEG recording has shown the resting state network implicated in motor function to be predominantly correlated with beta band oscillatory activity, further supporting the pathophysiological role of resting state activity in this frequency band in parkinsonism. From our data, it would seem that increased functional connectivity over a broad frequency range does not emerge until more advanced stages of disease, at least at the cortical level. The first increases in coupling in the earliest clinical stages of Parkinson’s disease appear to be restricted to the 8-10 Hz frequency range and do not seem to increase further with disease progression. We should note that due to the use of relatively global measures of functional connectivity to handle the multiple comparison problem, very localized increases in other frequency bands may have gone unnoticed.

In parallel to the present study, matched groups of demented and non-demented Parkinson’s disease patients were studied using the same techniques as used in the current study. Preliminary analyses have demonstrated a loss of resting state functional
connectivity in the alpha band in the fronto-temporal areas of demented relative to non-
demented patients.\textsuperscript{34} This pattern of changes is completely different from that observed in the present study. Taken together, the results of our MEG studies and the EEG study by Silberstein emphasize that changes in resting state cortico-cortical functional connectivity in Parkinson’s disease seem to evolve as disease progresses. Clearly, this assumption needs to be substantiated by longitudinal studies.

According to an influential neuropathological Parkinson’s disease staging system,\textsuperscript{35} brain pathology evolves following a predictable pattern over the course of Parkinson’s disease. In the earliest stages of this system, neuropathological changes are most prevalent in the brainstem, including dopaminergic, serotonergic and noradrenergic brainstem nuclei. To a lesser extent, also the forebrain cholinergic system is affected. In more advanced stages pathology ascends to include more forebrain structures, eventually spreading into the neocortex in stages associated with dementia. Considering this pattern of progression of neuropathological involvement, the observed changes in functional connectivity over the disease course may well be linked to the topographical progression of pathological changes over the brain. Along this line of reasoning, it is likely that there will be several distinct patterns of resting state functional connectivity that each characterize a specific disease stage.

In the present study, increased theta band functional connectivity correlated with both total UPDRS motor scores as well as tremor subscores. Parkinsonian tremor generally has a frequency in the theta range (4-7 Hz). Consequently, tremor could directly influence measured theta band functional connectivity through movement artefacts. We should note, however, that epochs were carefully selected for the absence of visible tremor and UPDRS tremor scores did not correlate with spectral power in the theta band in a previous study in the same subjects.\textsuperscript{26} Alternatively, tremor might be related to SL in a more indirect way. Timmermann and co-workers have reported coherence between tremor, measured with electromyography, and MEG oscillatory rhythms at tremor (and double tremor) frequencies in the contralateral motor cortex.\textsuperscript{36} In theory, these area-specific coherent oscillations could well have influenced our global SL measures. Given the associations between theta band coupling and UPDRS tremor score in the present study, differences in SL between controls and patients may indeed be explained by MEG oscillatory activity that is coherent with tremor. Taken together, increased functional coupling in the theta band may well play a role in the pathophysiology of Parkinson’s disease related tremor. Future studies comparing patients with tremor-dominant Parkinson’s disease to those with the hypokinetic-rigid variety might shed more light on the pathophysiological role of the theta band in Parkinson’s disease, particularly when
combining the recording of oscillatory brain rhythms with mechanical or electromyography recording of tremor.

In a previous study using power spectral analysis on the same resting state MEG datasets used in the present study, we found diffuse increases in theta and alpha1 relative spectral power in addition to decreases in beta and gamma power in early stage as well as more advanced Parkinson’s disease patients.26 Theoretically, estimates of statistical interdependencies between channels can be influenced by differences in signal power. Assuming a constant level of measurement/background noise, signals with lower power are likely to have a lower signal-to-noise ratio, resulting in biased lower values of functional connectivity. However, it is unlikely that our results were influenced in this way. Firstly, the previously reported pattern of increases in low frequency power in conjunction with decreases in higher frequency bands would not explain the presently observed broad-band increases in cortico-cortical functional connectivity. Furthermore, in our previous study spectral power was (largely) independent of disease duration and severity, and hardly influenced by dopaminomimetic treatment, whereas the present results demonstrate that increased coupling is a dynamic phenomenon that evolves as disease progresses. Therefore, the assessment of functional connectivity provides information that appears to be independent from signal power and more likely reflects true functional interactions between brain regions.

Neuropsychological studies have shown cognitive perseveration to be a very common deficit in Parkinson’s disease (e.g.37,38), which can be observed at the earliest clinical stages of disease.39 Perseverative tendencies are thought to be the result of attentional deficits, especially with regard to switching of internally driven response generation strategies. We were able to confirm the presence of these deficits in a different sample of early stage Parkinson’s disease patients and showed them to be positively correlated with increased interhemispheric alpha1 coupling. In a previous study, increased alpha1 spectral power in central and parietal ROIs was associated with cognitive perseveration.26 The role of the alpha band in attention is further underlined by the results of the aforementioned study combining functional MRI and EEG, which showed the alpha rhythm to be negatively correlated with activity in the dorsal attention resting state network.8 As effective cognition probably requires the constant changing of synchronous neural cell assemblies, enabling the rapid formation and decay of functional networks,40 increased resting state functional connectivity may be a sign of this dynamic process becoming overly static, in this way reducing cognitive flexibility. Whether increased spectral power and coupling are primary mechanisms that induce cognitive deficits or, instead, reflect a compensatory mechanism for another as yet unidentified pathophysiological mechanism remains to be
established. More insight may be gained by future methodological improvements that facilitate the study of rapidly changing sequential network configurations and by studying task-related changes in functional connectivity.

In conclusion, this study is the first to demonstrate increased resting state functional connectivity in Parkinson’s disease patients relative to a control group. In early stage Parkinson’s disease, increases were confined to the 8-10 Hz range, but appeared to expand with disease progression to cover a broad 4-30 Hz range in moderately advanced Parkinson’s disease patients. We were able to confirm the correlation between beta band coupling and severity of parkinsonism and found some evidence for a similar correlation between functional connectivity in the theta band and motor symptoms, in particular tremor. Cognitive perseveration in early stage Parkinson’s disease was positively correlated with increased interhemispheric functional connectivity in the 8-10 Hz range. The results of the present study suggest that changes in functional connectivity over the disease course in Parkinson’s disease may be linked to the topographical progression of pathology over the brain.
REFERENCES


Pathophysiological mechanisms in Parkinson’s disease related dementia: an MEG study
Chapter 4.2
MEG resting state functional connectivity in Parkinson’s disease related dementia

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D. Stoffers
E.Ch. Wolters
C.J. Stam
H.W. Berendse

ABSTRACT

Parkinson's disease (PD) related dementia (PDD) develops in up to 60% of patients, but the pathophysiology is far from being elucidated. Abnormalities of resting state functional connectivity have been reported in Alzheimer’s disease (AD). The present study was performed to determine whether PDD is likewise characterized by changes in resting state functional connectivity.

MEG recordings were obtained in 13 demented and 13 non-demented PD patients. The synchronization likelihood (SL) was calculated within and between cortical areas in six frequency bands. Compared to non-demented PD, PDD was characterized by lower fronto-temporal SL in the alpha range, lower intertemporal SL in delta, theta and alpha1 bands as well as decreased centro-parietal gamma band synchronization. In addition, higher parieto-occipital synchronization in the alpha2 and beta bands was found in PDD. The observed changes in functional connectivity are reminiscent of changes in AD and may reflect reduced cholinergic activity and/or loss of cortico-cortical anatomical connections in PDD.
INTRODUCTION

Dementia develops in up to 60% of patients suffering from Parkinson’s disease (PD)\(^1\) and importantly contributes to the impairment of the quality of life and to caregiver distress. The mechanisms of PD related dementia (PDD) are still poorly understood. Although the loss of nigrostriatal and corticopetal dopaminergic (and serotonergic and noradrenergic) projection systems may contribute to the development of dementia in PD, it is generally believed that additional mechanisms must be involved, most notably degeneration of cholinergic cortical projections and/or local cortical Lewy body- and tau-pathology.

Most normal cognitive processes require dynamic coordination of activity within and between specialized brain areas.\(^2\) The way the brain accomplishes such functional coupling has received growing attention in recent years. Synchronization of oscillatory neuronal activity within as well as between brain regions is thought to be a possible mechanism, which can be studied by measuring statistical interdependencies between oscillating neurophysiological signals.\(^3\) Using this approach, non invasive neurophysiological studies have demonstrated that synchronization of neuronal activity is associated with a variety of cognitive processes, for example working memory and processing of stimuli (For reviews see Schnitzler et al, Fries P, Uhlhaas et al, Stam CJ\(^4\text{-7}\)). Patterns of functional connectivity can also be studied in the resting state and may be relevant to our understanding of neurodegenerative disorders.\(^8\) Changes in resting state functional connectivity have been demonstrated using EEG and MEG in several brain disorders, including multiple sclerosis,\(^9\) brain tumours,\(^10\) mild cognitive impairment (MCI)\(^11\text{-13}\) and Alzheimer’s disease (AD).\(^12\text{-21}\)

Changes in functional connectivity have also been reported in non-demented PD patients at several different stages of disease. Using MEG and a general measure of synchronization, the synchronization likelihood (SL),\(^22\) in early stage, untreated PD patients, we recently demonstrated increased synchronization for both local and long distance connections in the alpha\(_1\) frequency range compared to healthy controls.\(^23\) In advanced, but non-demented PD patients, higher levels of cortico-cortical synchronization in the 10-35Hz frequency range were correlated with more severe parkinsonism and could be attenuated by treatment with levodopa or deep brain stimulation of the subthalamic nucleus, in parallel with clinical motor improvements, suggesting an association between increased synchronization and impaired motor function.\(^24\) Several other studies also suggest that increased (mainly beta) synchronization in basal ganglia-thalamo-cortical...
circuits may play an essential pathophysiological role in the development of motor symptoms in PD (For review see Hammond et al\textsuperscript{25}).

To date, studies of functional coupling in patients with PD related dementia are not available and it is therefore fully unknown whether dementia in PD is also characterized by changes in synchronization and if so, whether these changes consist of a progression of changes already present in early stage PD (without dementia) or whether the pattern is more like the changes described in AD.

Recently, using power spectral analysis of MEG data, we found a qualitatively different pattern of slowing of background activity in demented compared to non-demented patients.\textsuperscript{26} Whereas in PD without dementia an increase of theta and a decrease of beta power were found compared to healthy controls, in PDD an additional increase of delta relative power and a decrease of alpha band power could be demonstrated relative to the non-demented patients. This raises the question whether changes in resting state functional connectivity in demented PD patients, if present, likewise exhibit a qualitatively different pattern from that observed in non-demented patients, suggesting the involvement of different or at least additional pathophysiological mechanisms.

The aim of this study was to analyze resting state cortico-cortical functional connectivity in non-demented and demented PD patients using the synchronization likelihood as a general measure of synchronization.

Our research questions were:
Is PD related dementia characterized by changes in resting state functional connectivity compared to PD without dementia?
Do the changes in functional connectivity in PDD, if present, reflect a progression of the changes observed in non-demented PD patients or is the pattern similar to the changes recently reported for AD?

MATERIALS AND METHODS

Subjects
Two groups of subjects were studied: PD patients with dementia (PDD; N=13; 8♂/5♀) and PD patients without dementia (PD; N=13; 6♂/7♀). All PD patients underwent a full physical and neurological examination and fulfilled the United Kingdom Parkinson’s Disease Society Brain Bank (UK-PDSBB) clinical diagnostic criteria for probable Parkinson’s disease.\textsuperscript{27} Demented PD patients additionally fulfilled DSM-IV-criteria\textsuperscript{28} for dementia and
had a Mini Mental State Examination (MMSE) score of 24 or lower out of a maximum of 30 points, with lower scores indicating worse cognition. Blood examination and MR imaging were performed to exclude other potential causes of dementia. Non-demented PD patients did not experience difficulties with cognitive functioning in daily life and did not display any signs of dementia on clinical as well as neuropsychological examination. Disease stage and severity were assessed using the (modified) Hoehn & Yahr-scale (H&Y; range 0-5 with higher scores indicating more advanced disease stage) and the motor section of the Unified Parkinson’s Disease Rating Scale (UPDRS; range 0-108 with higher scores indicating worse motor functioning), respectively. Exclusion criteria for PD patients consisted of stereotactic surgery in the past and the use of anticholinergics, neuroleptics or cholinesterase inhibitors.

All patients were treated with a combination of levodopa and a decarboxylase inhibitor. Eight demented patients and nine non-demented patients were also treated with a dopamine agonist.

The study protocol was approved by the medical ethical committee of the VU University Medical Center. After careful explanation of the procedures, all subjects gave written informed consent prior to participating.

**MEG procedures**

MEG data were acquired using a 151-channel whole head MEG system (CTF Systems Inc., Port Coquitlam, BC, Canada), with patients seated in a magnetically shielded room (Vacuum-schmelze GmbH, Hanau, Germany). All MEG recordings were acquired in the morning. Patients were asked to come to the hospital without taking their first morning dose of dopaminergic medication (practically defined “OFF”). MEG registration took place in this OFF-state in a no task, eyes closed, resting state condition. At the beginning and end of the measurement, head position was recorded by leading small currents through three position coils situated at the left and right pre-auricular points and the nasion.

The recording pass band was 0-125 Hz with a sample rate of 312.5 Hz. A third-order software gradient (Vrba, 1996) was applied. Two approximately 13 second long artifact free epochs (sample rate 312.5 Hz; 4096 samples) were selected for further analysis. Epoch selection was always done by the same investigator (JLWB), who was blinded for group membership. MEG recordings were filtered offline with a band pass of 0.5-48 Hz.

**Synchronization likelihood**

A technical description of SL can be found elsewhere and is summarized briefly here. We assume two dynamic systems, for instance neural networks designated X and Y. From
both systems, time series $x_i$ and $y_i$, for instance using EEG or MEG signals, are recorded. The general problem is to infer functional interactions between $X$ and $Y$ from $x_i$ and $y_i$. Usually it is assumed that the more $x_i$ and $y_i$ “resemble” each other, the stronger $X$ and $Y$ interact. This “resemblance” can be quantified, for instance, by the cross-correlation. When this is done as a function of frequency, the coherence is determined, which is the most commonly used tool for this purpose. However, it has been shown that $X$ and $Y$ can interact even when $x_i$ and $y_i$ do not “resemble” each other in a simple way. This more general concept, called generalized synchronization, implies that the state of $Y$ is a function of the state of $X$. The SL is a way to quantify this “generalized synchronization.” Recently it has been shown that parameters $L$ and $m$ should take into account the low and high frequency filter settings rather than using fixed values of these parameters. Therefore, in the present study the choice of $L$ and $m$ was based explicitly on the frequency content of the data, which resulted in the following lags ($L$) and embedding dimensions ($m$): delta: $L=20$, $m=20$; theta: $L=10$, $m=9$; alpha1: $L=8$, $m=6$; alpha2: $L=6$, $m=6$; beta: $L=3$, $m=9$; low gamma: $L=2$, $m=6$; $P_{ref}$ was set at .01 for all frequency bands.

The synchronization likelihood was computed for the two 13 second epochs in the following frequency bands: 0.5-4 Hz (delta), 4-8 Hz (theta), 8-10 Hz (alpha1), 10-13 Hz (alpha2), 13-30 Hz (beta) and 30-48 Hz (gamma; cut off at 48 Hz to exclude the line artifact of 50 Hz). For each frequency band, average SL was calculated within and between a number of regions of interest (ROI) corresponding to the major cortical areas (frontal, central, temporal, parietal and occipital) on the left and right side. The midline channels ($Z$; $N=9$) were left out of this clustering and one channel was not available for analysis due to technical problems, leaving a total of 141 MEG channels to be analyzed. A schematic distribution of these ROIs and SL measures is shown in fig 1. Short distance (local) functional connectivity was calculated by averaging the SL values of all possible sensor pairs within each ROI. Long distance connectivity was calculated by averaging the SL values of all possible sensor combinations between two ROIs. Long distance functional connectivity measures included eight intrahemispheric SL parameters (four for each hemisphere) and five interhemispheric SL parameters. Finally, for each of the SL measures, the results of the two epochs were averaged for each subject.
Pathophysiological mechanisms in Parkinson’s disease related dementia: an MEG study

**Figure 1**

A. **Schematic representation of the distribution of individual MEG sensors.**
B. **Schematic representation of the major cortical areas after clustering of MEG sensors.**
C. **Interhemispheric connections studied with SL: interfrontal, intercentral, intertemporal, interparietal and interoccipital.**
D. **Intrahemispheric connections studied with SL: fronto-temporal, fronto-parietal, temporo-occipital and parieto-occipital, in both hemispheres.**

**STATISTICAL ANALYSIS**

**Demographics**

Differences between groups in the distribution of gender and (modified) Hoehn and Yahr scores were analyzed by means of chi-square tests. Student’s t-tests were used to analyze differences between the groups in age, disease duration and UPDRS motor scores.

**Synchronization likelihood**

To increase statistical power we attempted to normalize SL parameters by means of a transformation that is commonly used when trying to normalize relative spectral power, a neurophysiological variable that also varies between 0 and 1; \( \log^{10}\frac{x}{(1-x)} \). Unfortunately, several parameters could not be sufficiently normalized by means of this transformation to pass Kolmogorov-Smirnov tests of normality. However, the linear regression technique we used for *post hoc* analyses of SL in the current study is rather robust when it comes to violations of the assumption of normality as long as there are (roughly) more than ten observations and no substantial non-normality that leads to outliers in the X-Y data. In that case, skewed distributions, light-tailedness as well as heavy-tailedness have little effect on linear regression statistics. As our smallest group still contains 13 observations per parameter and SL does not result in extreme outliers, we chose, in the interest of uniformity, to report parametric analyses of log-transformed SL values only.
For each frequency band, three separate ANOVA’s with repeated measures were performed, using Greenhouse-Geisser corrected $p$-values when appropriate. For short distance synchronization, the repeated measures factor had ten levels (left and right frontal, central, temporal, parietal and occipital SL); for long distance intrahemispheric synchronization the repeated measures factor had eight levels (left and right fronto-temporal, fronto-parietal, parieto-occipital and temporo-occipital SL); for long distance, interhemispheric synchronization the repeated measures factor had five levels (interfrontal, intercentral, intertemporal, interparietal and interoccipital SL). The between-subjects factor had two levels (non-demented PD and demented PD).

In case of a significant main effect of group or an interaction effect of group with the repeated measure, subsequent post hoc analyses with regard to differences in SL between non-demented PD patients and demented PD patients were performed by means of linear regression using each of the SL measures as dependent and group membership (effect of dementia) as determinant.

**Correlation with clinical parameters**

To study the correlation of SL values with clinical parameters of motor (UPDRS) and cognitive (MMSE) function, three separate univariate analyses of variance with repeated measures were performed: one with the UPDRS OFF in the PD group, one with the UPDRS OFF in the PDD group and finally one with the MMSE in the PDD group as independent variable, all three analyses with SL as dependent variable. In case of a significant main effect of the UPDRS or MMSE or an interaction effect with SL, post hoc linear regression analyses were conducted.

Partial eta squared ($\eta^2$) was calculated when performing ANOVA with repeated measures. Coefficients for relevant determinants when performing regression analysis were standardised and subsequently squared ($\beta^2$). Both $\eta^2$ as well as $\beta^2$ represent the proportion of the total variability in the dependent variable (SL) that is accounted for by the relevant determinant and, throughout the paper, are expressed as a percentage of the total variance.

All analyses were performed at a significance level of 5% (two-tailed) using the SPSS 15.0.0 software package (SPSS Inc., Chicago, IL, U.S.A.).

**RESULTS**

**Subject characteristics**

In table 1, the general characteristics of the study groups are listed. Demented patients had a mean MMSE score of 20.9 ± 3.3. Age was not significantly different between
demented and non-demented patients (74.4 ± 4.9 and 71.7 ± 5.1 years, respectively; p=.180). Demented patients had significantly higher UPDRS OFF motor scores compared to non-demented PD patients (23.9 ± 6 and 16.2 ± 3 respectively; p<.001).

<table>
<thead>
<tr>
<th></th>
<th>PDD</th>
<th>PD</th>
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<tbody>
<tr>
<td>Sex (♂/♀)</td>
<td>8/5</td>
<td>6/7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>74.4</td>
<td>4.9</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>11.2</td>
<td>4.0</td>
</tr>
<tr>
<td>MMSE (0-30)</td>
<td>20.9</td>
<td>3.3</td>
</tr>
<tr>
<td>UPDRS III OFF (0-108)</td>
<td>23.9</td>
<td>5.6</td>
</tr>
<tr>
<td>H&amp;Y stage OFF (2/2.5/3/4)</td>
<td>0/5/7/1</td>
<td>4/6/3/0</td>
</tr>
</tbody>
</table>

Table 1

Patient characteristics

PDD = Parkinson’s disease patients with disease related dementia
PD = Parkinson’s disease patients without disease related dementia
H&Y = Hoehn & Yahr
UPDRS = Unified Parkinson’s Disease Rating Scale
MMSE = Mini Mental State Examination

Synchronization likelihood

Mean SL values as well as the values after logarithmic transformation are displayed in table 2. Significant differences between the study groups are graphically represented in figure 2 and are discussed below.
### DELTA

<table>
<thead>
<tr>
<th></th>
<th>Regional SL</th>
<th>Intrahemispheric SL</th>
<th>Interhemispheric SL</th>
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</thead>
<tbody>
<tr>
<td><strong>Main group effect</strong></td>
<td>3.667 ,0.067</td>
<td>0.988 ,0.330</td>
<td>0.821 ,0.374</td>
</tr>
<tr>
<td><strong>Interaction effect</strong></td>
<td>2.169 ,0.068</td>
<td>3.444 ,0.021</td>
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</tbody>
</table>

<table>
<thead>
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<th>Intrahemispheric SL</th>
<th>Interhemispheric SL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main group effect</strong></td>
<td>1.055 ,0.562</td>
<td>0.237 ,0.631</td>
<td>0.371 ,0.546</td>
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<tr>
<td><strong>Interaction effect</strong></td>
<td>1.691 ,0.148</td>
<td>2.064 ,0.044</td>
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</table>

### THETA

<table>
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<th>Interhemispheric SL</th>
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</thead>
<tbody>
<tr>
<td><strong>Main group effect</strong></td>
<td>1.568 ,0.023</td>
<td>4.673 ,0.041</td>
<td>4.662 ,0.005</td>
</tr>
<tr>
<td><strong>Interaction effect</strong></td>
<td>2.181 ,0.063</td>
<td>5.171 ,0.179</td>
<td>1.14 ,0.739</td>
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</tbody>
</table>

### ALPHA1

<table>
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</thead>
<tbody>
<tr>
<td><strong>Main group effect</strong></td>
<td>0.360 ,0.064</td>
<td>0.230 ,0.636</td>
<td>3.203 ,0.034</td>
</tr>
<tr>
<td><strong>Interaction effect</strong></td>
<td>2.001 ,0.084</td>
<td>5.122 ,0.002</td>
<td>0.628 ,0.436</td>
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</table>

### ALPHA2

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<th>Interhemispheric SL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main group effect</strong></td>
<td>0.138 ,0.713</td>
<td>2.380 ,0.136</td>
<td>3.339 ,0.023</td>
</tr>
<tr>
<td><strong>Interaction effect</strong></td>
<td>1.515 ,0.193</td>
<td>4.507 ,0.006</td>
<td>0.317 ,0.578</td>
</tr>
</tbody>
</table>

### BETA

<table>
<thead>
<tr>
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<th>Regional SL</th>
<th>Intrahemispheric SL</th>
<th>Interhemispheric SL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main group effect</strong></td>
<td>0.117 ,0.88</td>
<td>0.123 ,0.86</td>
<td>0.140 ,0.84</td>
</tr>
<tr>
<td><strong>Interaction effect</strong></td>
<td>1.113 ,0.193</td>
<td>4.507 ,0.006</td>
<td>0.317 ,0.578</td>
</tr>
</tbody>
</table>

### GAMMA

<table>
<thead>
<tr>
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<th>Interhemispheric SL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main group effect</strong></td>
<td>0.643 ,0.431</td>
<td>0.744 ,0.397</td>
<td>3.249 ,0.036</td>
</tr>
<tr>
<td><strong>Interaction effect</strong></td>
<td>3.353 ,0.024</td>
<td>0.697 ,0.412</td>
<td></td>
</tr>
</tbody>
</table>

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Pathophysiological mechanisms in Parkinson’s disease related dementia: an MEG study
In the delta band, a significant result for the interaction between group and long distance, interhemispheric SL was found (p=.021; η²=13%). Post hoc linear regression showed that demented patients had significantly lower intertemporal synchronization compared to the non-demented group (p<.001; β²=43%; figure 2). There were no significant main or interaction effects for long distance intrahemispheric or short distance regional synchronization.

Likewise, in the theta band, a significant interaction effect was found between group and interhemispheric SL (p=.044; η²=11%). As for the delta band, linear regression demonstrated lower synchronization between temporal regions in PDD compared to PD (p<.001; β²=42%; figure 2). For intrahemispheric and regional SL, no significant main group or interaction effects were found.

In the alpha1 band, a significant main group effect was found for long distance intrahemispheric synchronization (p=.041; η²=16%). Post hoc linear regression showed lower fronto-temporal SL values in demented patients in both hemispheres (p=.001; β²=37% and p=.012; β²=22%) as well as lower fronto-parietal SL values on the left side (p=.046; β²=16%; figure 2). Furthermore, the interaction effect between group and long distance interhemispheric synchronization also reached significance (p=.005; η²=16%). Comparable to the delta and theta bands, post hoc regression analysis showed lower intertemporal SL in demented patients (p<.001; β²=41%; figure 2).

A significant interaction effect between group and intrahemispheric SL was found in the alpha2 frequency band (p=.002; η²=18%). In the post hoc analysis, significantly lower right and left fronto-temporal SL was found in demented patients (p=.004; β²=29% and p=.021; β²=23% respectively), as well as higher left parieto-occipital SL (p=.046; β²=16%; figure 2). For interhemispheric and regional synchronization, no significant results were found.

In the beta band, a significant interaction effect was found between group and both intrahemispheric (p=.006; η²=16%) and interhemispheric synchronization (p=.023; η²=12%). In the post hoc analysis of intrahemispheric SL, demented patients displayed lower right fronto-temporal synchronization (p=.031; β²=18%) together with higher left temporo-occipital (p=.040; β²=16%) as well as parieto-occipital synchronization (p=.021; 

(Previous page)

Table 2

<table>
<thead>
<tr>
<th>Group SL-values before and after logarithmic transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Significant differences in SL between the groups are in bold</strong></td>
</tr>
<tr>
<td>FP = fronto-parietal, FT = fronto-temporal, PO = parieto-occipital, TO = temporo-occipital</td>
</tr>
<tr>
<td>PDD = Parkinson’s disease with dementia, PD = Parkinson’s disease without dementia</td>
</tr>
<tr>
<td>C = central, F = frontal, O = occipital, P = parietal, T = temporal</td>
</tr>
</tbody>
</table>

In the delta band, a significant result for the interaction between group and long distance, interhemispheric SL was found (p=.021; η²=13%). Post hoc linear regression showed that demented patients had significantly lower intertemporal synchronization compared to the non-demented group (p<.001; β²=43%; figure 2). There were no significant main or interaction effects for long distance intrahemispheric or short distance regional synchronization.

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Post hoc analysis did not show any differences for individual interhemispheric measures. Short distance effects could not be demonstrated (figure 2).

Lastly, in the gamma band, a significant interaction effect between group and interhemispheric as well as regional SL was found ($p=.036; \eta^2=12\%$ and $p=.024; \eta^2=12\%$ respectively). Post hoc regression analysis revealed lower interparietal SL in demented patients ($p=.005; \beta^2=28\%$) together with lower SL in left ($p=.006; \beta^2=28\%$) and right parietal ($p=.001; \beta^2=37\%$) as well as left central ($p=.018; \beta^2=21\%$) local synchronization (figure 2).

Figure 2
Schematic representation of the differences in resting state synchronization between demented and non-demented patients. Statistically significant higher SL values in demented patients compared to non-demented patients are coloured red, lower values blue. The intensity of the colours indicates the magnitude of the $\beta$ squared in the post hoc linear regression (light = 10–20%; middle = 20–30%; dark = 30%).

Decreased fronto-temporal SL as well as increased left sided posterior synchronization is seen in the alpha and beta bands in demented patients. Intertemporal decrease of SL in demented patients is found in the 0, 5–10 Hz range. Lastly, decreased gamma band SL is demonstrated in demented patients in local and interhemispheric centro-parietal areas.

PDD demented Parkinson’s disease patients, PD non-demented Parkinson’s disease patients
LF left frontal, LC left central, LT left temporal, LP left parietal, LO left occipital, RF right frontal, RC right central, RT right temporal, RP right parietal, RO right occipital
Correlations with clinical parameters

**MMSE**

In demented patients, the only significant result in the ANOVA with repeated measures was found for the main effect of MMSE for intrahemispheric SL in the delta band ($p=.040; \eta^2=33\%$). Post hoc linear regression showed that for right parieto-occipital and temporo-occipital SL, lower MMSE scores (meaning worse cognition) correlated with higher synchronization ($p=.018; R^2=41\%$ and $p=.042; R^2=32\%$, respectively).

**UPDRS**

No significant correlations were found in any of the frequency bands between UPDRS OFF-motor scores and SL parameters in demented or non-demented PD patients in the ANOVA with repeated measures.

**DISCUSSION**

To our knowledge, this is the first MEG study comparing resting state functional connectivity between demented and non-demented PD patients. Our main findings are a reduction in long distance intrahemispheric, predominantly bilateral fronto-temporal synchronization in the alpha1 and alpha2 bands in demented patients together with a reduction in intertemporal synchronization in the 0.5-10 Hz frequency range. In addition, local and interhemispheric gamma band synchronization in centro-parietal regions is lower in demented PD patients, whereas left sided parieto-occipital synchronization in the alpha2 and beta band is higher in the demented patients.

Changes in functional connectivity have been reported in non-demented PD patients in several stages of disease. In very early stage, untreated, non-demented patients, we recently found increased alpha1 synchronization.\(^{23}\) In moderately advanced patients, the increase in functional connectivity involved a more extended frequency range, also including the theta, alpha2 and beta bands.\(^{23}\) In advanced stage non-demented PD patients receiving deep brain stimulation, Silberstein et al found a correlation between higher cortico-cortical coupling in the beta band and more impaired motor function.\(^{24}\) In the present study, we report a completely different pattern of changes in demented PD patients in comparison to non-demented patients, mainly consisting of reductions in long distance fronto-temporal and intertemporal functional connectivity as well as in short distance functional connectivity in several frequency bands.
Combining the results of the present and previous studies, there appear to be differential patterns of change in functional connectivity when comparing between groups of PD patients in different stages of disease and between PD patients and controls. It is therefore tempting to speculate that there are stage-specific patterns of change in synchronization in PD. According to the neuropathological staging system for PD, the earlier stages of PD are mainly characterized by degeneration of dopaminergic (and serotonergic and noradrenergic) ascending pathways. This would suggest that changes in these neurotransmitter systems might be involved in the increases of functional connectivity observed in early stage non-demented patients. Indeed, the results of the study by Silberstein et al seem to point to a modulatory role of dopaminomimetic treatment on cortico-cortical synchronization. The qualitatively completely different pattern of changes in dementia in PD we report in this study, however, suggests that these changes are not just related to progression of the above mentioned degeneration of neurotransmitter systems already involved in non-demented PD, but that additional mechanisms are involved.

In AD, using coherence and more recently, the synchronization likelihood, reductions of general synchronization as well as loss of functional connectivity in the alpha and gamma bands have been demonstrated in patients compared to healthy controls in EEG as well as MEG studies. In several studies, the decrease of synchronous activity was correlated with worse cognition (lower MMSE scores). Interestingly, this pattern of changes reported in AD is very similar to the loss of long distance fronto-temporal and intertemporal resting state functional connectivity we demonstrate in the present study in demented PD patients. Recently, in dementia with Lewy bodies (DLB), a disease considered to be part of the same disease spectrum as PDD, a reduction of long distance intrahemispheric functional coupling in the alpha frequency range, as measured with coherence, has been reported in an MEG study.

Given the similarities in the pattern of reduction of synchronization in AD, DLB and PDD, common pathophysiological mechanisms may be underlying changes in these conditions. A possible common candidate accounting for the loss of synchronization could be the profound loss of cortical cholinergic projections from the basal nucleus of Meynert, since this is a characteristic of AD, DLB and PDD. Involvement of the cholinergic system is supported by an animal study, in which lesioning of the cholinergic system resulted in a reduction of long distance intrahemispheric as well as interhemispheric coherence. Furthermore, even in young and elderly healthy subjects, a reduction in interhemispheric
EEG and MEG coherence can be demonstrated after the administration of the anticholinergic drug scopolamine, which has been shown to be able to cause temporary cognitive deficits in healthy subjects.

In addition to a decrease of long distance intrahemispheric and interhemispheric SL, we found a loss of short range gamma synchronization in centro-parietal regions in PDD. Interestingly, in AD, loss of gamma band synchronization has also been demonstrated using MEG. Since cholinergic activity is often associated with a shift of the powerspectrum to faster frequencies as well as with induction of coherence in the high frequency range, it could well be that the decrease of gamma synchronization in central and parietal areas also reflects loss of cholinergic activity.

Given the suspected pathophysiological significance of degeneration of the cholinergic system in PD related dementia, it would be extremely interesting to see whether cholinesterase inhibitors are able to (partly) reverse the changes in functional connectivity.

In addition to the cholinergic deficit, especially in relation to the decrease of long distance synchronization, other pathophysiological mechanisms may be involved. It seems obvious that loss of anatomical connections between brain areas may lead to a reduction of functional coupling. In AD, atrophy of the corpus callosum has been shown to be associated with loss of lower interhemispheric coherence. Furthermore, in multiple sclerosis, associated with widespread degeneration of the white matter and therefore loss of anatomical connections, a strong reduction in interhemispheric connectivity has been reported. Especially in demented PD patients cortical atrophy can be found, including atrophy of the temporal lobes. Therefore, cortical atrophy as well as pathological changes in the surviving cortex, such as Lewy body- and/or tau-pathology, may be associated with the loss of functional coupling we report in the present study.

The last observation in the present study is an increase in left posterior synchronization in demented patients in the alpha2 and beta frequency range. Interestingly, a similar posterior increase of synchronization levels has recently also been demonstrated in mildly affected AD patients. The similarity in these observations might suggest that these changes in functional connectivity might be associated with cognitive impairment. An alternative, but speculative explanation might be that increased synchronization constitutes a compensatory mechanism in relatively healthy networks for the loss of functional connectivity in other more damaged networks.
In the present study, we found hardly any correlation between cognition, as measured with the MMSE, and SL parameters. Several factors might explain the absence of significant correlations. First, the MMSE is a global screening tool for cognitive dysfunction. Impairments in specific cognitive domains that might possibly be related to changes in synchronization are not specifically assessed with this measure. Second, the variance of MMSE scores in our demented PD group was relatively small. Lastly, our study sample was relatively small and, therefore, correlations might not have reached significance because of a lack of power.

In the future, studies using more specific measures of different cognitive domains, for instance executive dysfunction, in a larger group of PD patients are needed to further address the relationship between cognitive dysfunction and changes in functional connectivity.

Some possible limitations of our study have to be considered. First, demented patients had significantly higher UPDRS motor scores compared to the non-demented patients. Therefore, it might be argued that our results are partly related to differences in motor function between patient groups. However, for the UPDRS OFF scores, there were no significant results nor even a trend towards significance for any of the SL parameters in the ANOVA with repeated measures in both the demented and non-demented PD group. Furthermore, previous studies have shown that impaired motor function in early as well as more advanced stage, non-demented PD patients is associated with increases in synchronization. Since, in the present study, we mainly report significant reductions in the demented patients, it seems highly unlikely that our results can be explained by worse motor function in our demented patients. To the contrary, worse motor function in the demented PD patients might have even partly masked reductions in SL.

Second, MEG correlations between signals from nearby sensors could be due to common sources rather than true interactions. This is the well known problem of volume conduction that may give rise to spurious correlations in sensor space. One possible solution is to estimate correlations between signals from reconstructed sources (source space) rather than the actually recorded signal (signal space). However, no unique way exists to reconstruct the sources, and the source reconstruction algorithm used could influence the interdependencies between the sources. Therefore, in the present study, we used a pragmatic approach, restricting the analysis to signal space. Although volume conduction may influence SL values in this way, it seems unlikely that this can explain major group differences in SL between PDD en PD. Furthermore, the majority of our main
results involve changes in long distance interactions which are less likely to be affected by volume conduction.

CONCLUSION

In conclusion, dementia in PD is characterized by a decrease of alpha fronto-temporal as well as low frequency intertemporal resting state functional connectivity, together with a loss of local gamma band connectivity. This pattern of changes is different from the changes in functional connectivity in non-demented PD patients but, in contrast, very similar to the abnormalities seen in AD and may reflect degeneration of the cholinergic system as well as local cortical changes, such as atrophy and Lewy body- and tau-pathology. Future studies, addressing the modulatory effects of cholinesterase inhibitors as well as dopaminergic drugs, should clarify the exact contribution of these neuropathological changes.
REFERENCES

Pathophysiological mechanisms in Parkinson's disease related dementia: an MEG study
Chapter 5
Pathological brain network organization in Parkinson’s disease related dementia

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ABSTRACT

EEG and MEG studies have shown that dementia in Parkinson’s disease (PD) is characterized by a slowing of rhythmic oscillatory brain activity, and decreases as well as increases in functional connectivity between brain areas. The effect of these changes on the large scale organization of functional brain networks in these patients is still unknown. Functional brain networks in healthy subjects demonstrate ‘small world’ features, characterized by an optimal combination of high local connectedness and global integration. We hypothesized that PD related dementia would be associated with a pathological organization of brain networks away from these optimal network features, in particular involving a loss of global integrative properties.

Whole head MEG recordings were obtained in 13 demented and 13 non-demented PD patients in an eyes closed, resting state condition. For the main frequency bands, correlations between all possible pairwise MEG sensors were calculated using the synchronization likelihood. Subsequently, graph theoretical analysis was applied to the resulting synchronization matrices. We then calculated the weighted clustering coefficient $C_{\hat{w}}$, a measure of local connectedness of networks, and the weighted path length $L_{\hat{w}}$, a measure of global integration.

The normalized clustering coefficient $C_{\hat{w}}$ was higher in demented than in non-demented PD patients in all frequency bands. Furthermore, the normalized path length $L_{\hat{w}}$ was increased in demented patients in all but the delta frequency band. The differences for both network characteristics reached significance in the theta band.

In conclusion, large scale brain network organization in PD related dementia is different from that in non-demented patients, involving a shift away from global integration toward more local information processing. The observed changes may result from the interplay between the ongoing degeneration of ascending monoaminergic neurotransmitter systems, contributing to increased local clustering, on the one hand, and cholinergic deficits and/or cortical Lewy-body and tau-pathology, leading to loss of global integrative properties, on the other hand.
INTRODUCTION

Dementia eventually develops in up to 80% of patients suffering from Parkinson’s disease (PD)\(^1\)\(^,\)\(^2\) and contributes significantly to impaired quality of life and caregiver distress.\(^3\)\(^,\)\(^4\) The mechanisms of dementia in PD are still poorly understood. Ongoing loss of corticopetal dopaminergic, serotonergic and noradrenergic projections systems, as well as degeneration of cholinergic cortical projections and/or local cortical Lewy body- and tau-pathology may all contribute to the development of dementia in PD.

Normal brain function relies on specialization of specific brain areas but also on integration of function of these specialized regions.\(^5\) In recent years, it has become increasingly apparent that this integration is achieved by synchronization of oscillatory rhythms in different frequency bands, a mechanism referred to as functional connectivity.\(^6\)\(^,\)\(^7\) Functional connectivity can be studied by measuring statistical interdependencies between oscillating neurophysiological signals.\(^8\) Several measures are available to describe functional connectivity: for instance, coherence is a measure of linear interdependencies between signals whereas the synchronization likelihood (SL) or phase coherence are sensitive to linear as well as non linear interdependencies.\(^9\)

Changes in the strength of functional connectivity in the resting state have been demonstrated in non-demented\(^10\)\(^,\)\(^11\) as well as demented PD patients.\(^12\) In non-demented patients, functional connectivity is increased from the earliest stages of disease onward\(^10\) and progressively involves other frequency bands in more advanced stages of disease.\(^10\)\(^,\)\(^11\)

In contrast, PD related dementia is characterized mainly by reductions in long distance interhemispheric and intrahemispheric functional connectivity in several frequency bands. In addition, short distance functional connectivity was increased in parieto-occipital connections in the beta band and decreased in centro-parietal connections in the gamma band.\(^12\)

Recent studies have emphasized that, in addition to the strength of functional connectivity, also the large scale spatial organization of brain networks is vital to normal brain function.\(^13\) To study spatial network organization, Watts and Strogatz mathematically represented networks as graphs, consisting of points (a vertex) in a network and the connection (an edge) between these points.\(^14\) A graph can be characterized by the clustering coefficient \(C\), a measure of local connectivity, and the path length \(L\), a measure of global integration. Different types of possible networks are known (see figure 1).
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Figure 1 Schematical representation of different types of networks:

A. A regular network, characterized by a high clustering coefficient and high path length: vertices are only connected to their neighbours.

B. A small world network with a high C but lower L: a few connections are rewired randomly to allow long distance connections.

C. A random network with low C and L: there are many long range connections, but no more local ones.

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A regular network is characterized by a high degree of local connectivity (C is high), but a very long average path length connecting any two vertices. A random network has both a low clustering coefficient and a very short path length. A “small world” network is a network with a high C, close to that of an ordered network, and a low L, closer to that of a random network. Several social and biological networks have small world features. Studies using EEG and MEG have shown that functional brain networks in healthy subjects are also characterized by small world features; (for review see Reijneveld et al, Bullmore et al). Changes in the large scale functional organization of brain networks have recently been reported in several neurological diseases, including patients with brain tumors, schizophrenia, epilepsy, attention deficit hyperactivity disorder, and Alzheimer’s disease (AD).

It is tempting to speculate that dementia in PD is associated with an alteration in brain network architecture compared to non-demented PD patients; in particular we expected a loss of global integration. Therefore, in this study we compared the topological network features of demented PD patients and non-demented PD patients, based on the SL.
MATERIALS AND METHODS

Subjects
Two groups of subjects were studied: PD patients with dementia (PDD; N=13; 8♂/5♀) and PD patients without dementia (PD; N=13; 6♂/7♀). MEG data from these groups of patients have been used in previous studies using different analysis techniques. All subjects underwent a full physical and neurological examination and fulfilled the United Kingdom Parkinson’s Disease Society Brain Bank (UK-PDSBB) clinical diagnostic criteria for probable Parkinson’s disease. Demented PD patients additionally fulfilled DSM-IV-criteria for dementia and had a Mini Mental State Examination (MMSE) score of 24 or lower out of a maximum of 30 points, with lower scores indicating worse cognition. Blood testing and MR imaging were performed to exclude other potential causes of dementia. Non-demented PD patients had MMSE scores of at least 28 out of 30 points and did not experience difficulties with cognitive functioning in daily life. Further cognitive screening consisted of the cognitive section of the CAMDEX: the CAMCOG. A total of 107 points can be scored on this test with higher scores indicating better cognition. Disease stage and severity were assessed using the (modified) Hoehn & Yahr-scale (H&Y; range 0-5 with higher scores indicating more advanced disease stage) and the Unified Parkinson’s Disease Rating Scale (UPDRS; range 0-108 with higher scores indicating worse motor functioning), respectively. Exclusion criteria consisted of stereotactic surgery in the past and the use of anticholinergics, neuroleptics or cholinesterase inhibitors.

All patients were treated with a combination of levodopa and a decarboxylase inhibitor. Eight demented patients and nine non-demented patients were also treated with a dopamine agonist. The study protocol was approved by the medical ethical committee of the VU University Medical Center. After careful explanation of the procedures, all subjects gave written informed consent prior to participating.

MEG procedures
MEG data were acquired using a 151-channel whole head MEG system (CTF Systems Inc., Port Coquitlam, BC, Canada). Patients were seated in a magnetically shielded room (Vacuum-schmelze GmbH, Hanau, Germany). All MEG recordings were acquired in the morning. Patients were asked to come to the hospital without taking their first morning dose of dopaminergic medication (practically defined “OFF”). MEG registration took place in this OFF-state in a no task, eyes closed, resting state condition. The recording pass band was 0-125 Hz with a sample rate of 312.5 Hz. A third-order software gradient was applied. Two approximately 13 second long artifact free epochs
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(sample rate 312.5 Hz; 4096 samples) were selected for further analysis. Epoch selection was always done by the same investigator (JLWB), who was blinded for group membership. MEG recordings were filtered offline with a band pass of 0.5-48 Hz.

**Functional connectivity**

Synchronization likelihood (SL) was used as a measure of functional connectivity sensitive to linear as well as non-linear temporal statistical interdependencies between brain signals.\(^{38}\) A mathematical description can be found in Appendix A to this paper. In summary, we assume two dynamic systems, for instance neural networks designated X and Y. From both systems, time series \(x_i\) and \(y_i\) are recorded (the MEG signals). Both time series are then divided into short segments containing a few cycles of the dominant frequency, referred to as “patterns”. The SL is the chance that a recurrence of the pattern of times series of system \(y_i\) coincides with pattern recurrence of time series \(x_i\). In the present study, system X and Y are every possible combination of MEG channel pairs and time series \(x_i\) and \(y_i\) are the recorded MEG signals of these channels. The SL ranges between \(P_{\text{ref}}\), a chosen number close to zero, in case of completely independent time series and 1 in case of completely synchronous signals.

SL was computed for the two 13 second epochs in the following frequency bands: 0.5-4 Hz (delta), 4-8 Hz (theta), 8-10 Hz (alpha1), 10-13 Hz (alpha2), 13-30 Hz (beta) and 30-48 Hz (gamma). Results of the two epochs were averaged for each subject.

**Graph analysis**

Graphs can be represented as points in a network (vertices) and the connections between them (edges). They can be represented as unweighted graphs (an edge between two vertices either exists or not) or weighted graphs (an edge has a value or ‘weight’ between 0 and 1). We calculated weighted graphs with 149 vertices corresponding to each MEG channel (two channels were not available) and used the matrix of the SL values as the weighted edges between all possible vertices. Subsequently, we calculated the clustering coefficient, a measure of the local connectivity of a network, and the path length, a measure of more global integrative network properties.

The clustering index \(C_i\) of a vertex \(i\) generally represents the likelihood that other vertices \(j\) that are connected to the vertex \(i\) will also be connected to each other. This notion can be adopted for use with weighted graphs in various ways. The following is mostly based on the approach in Stam et al.\(^{29}\) We propose a definition, closely related to the proposal of Onnela et al.\(^{39}\) which only requires symmetry \((w_{ij} = w_{ji})\) and that \(0 \leq w_{ij} \leq 1\) holds. Indeed, both conditions are readily fulfilled when using SL as weight definition. The (weighted) clustering index of vertex \(i\) is then defined as
Notice that in all sums in (1) terms with \(k = i, l = i,\) or \(l = k\) are skipped. In the special case in which \(w_{ij}\) equals either 0 or 1, this definition is equivalent to the classical definition for unweighted graphs.\(^{14}\) For isolated vertices, i.e. vertices that do not have any connections, all weights \(w_{ij}\) vanish, and the clustering index is defined as \(C_i=0.40\) The mean clustering coefficient of the entire network can be determined via (1) as

\[
C_w = \frac{1}{N} \sum_{i=1}^{N} C_i
\]

The path length \(L\) was originally defined for unweighted graphs.\(^{14}\) We extend this definition to weighted graphs building on the approach of Latora and Marchiori.\(^{41}\) In detail we define the length of an edge as the inverse of the aforementioned edge weight, i.e. \(L_{ij} = 1/w_{ij}\) if \(w_{ij} \neq 0,\) and \(L_{ij} = +\infty\) if \(w_{ij} = 0;\) recall that \(w_{ij}\) is positive because we use the SL as edge weight. The length of a weighted path between two vertices is then defined as the sum of the lengths of the edges of this path. The shortest path \(L_{ij}\) between two vertices \(i\) and \(j\) is the path between \(i\) and \(j\) with the shortest length. Analogously to definition (2) the average weighted path length of the entire graph is computed as

\[
L_w = \frac{1}{N(N-1)} \sum_{i=1}^{N} \sum_{j \neq i}^{N} 1/L_{ij}
\]

Notice that instead of the arithmetic mean we here employed the harmonic mean (see Newman et al\(^{40}\)), so that we can handle infinite path lengths between disconnected edges, i.e. \(1/\infty \rightarrow 0.\)

To correct for effects of network size as well as functional connectivity values, the calculated weighted values of the clustering coefficient \((C_w)\) and path length \((L_w)\) were compared to theoretical random networks generated from the same MEG data, following
the procedure described by Sporns and Zwi. In this way, the weighted C and L of 50 random networks were calculated and averaged, \( C_{w-s} \) and \( L_{w-s} \).

In this study we report the normalized clustering coefficient \( C_\text{w} \) \( (C_w/C_{w-s}) \) and path length \( L_\text{w} \) \( (L_w/L_{w-s}) \) for each frequency band.

**STATISTICAL ANALYSIS**

**Demographics**

Differences between groups in the distribution of gender and (modified) Hoehn and Yahr scores were analyzed by means of chi-square tests. Student’s t-tests were used to analyze differences between groups in age, disease duration, UPDRS motor scores and CAMCOG scores.

**Graph analysis**

Average normalized \( C_\text{w} \) and \( L_\text{w} \) values of the two study groups in each frequency band were compared using a non-parametrical Mann Whitney test. A p-value of 0.05 was used.

**Correlation with clinical parameters**

In both patient groups, network properties were correlated with the UPDRS motor score and the CAMCOG, using the Spearman correlation test.

**RESULTS**

**Subject characteristics**

In table 1, general characteristics of the study groups are listed. There were no significant differences in age or gender distribution between the two groups. Demented patients had significantly higher UPDRS III (motor) scores compared to non-demented PD patients (23.9 ± 5.6 and 16.2 ± 3.4 respectively; p<.001). As expected, CAMCOG scores in the PDD group were significantly lower compared to the non-demented group (71.5 ± 11.8 compared to 96.0 ± 4.7 respectively, p<.001).
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### Table 1

**Patient characteristics**

<table>
<thead>
<tr>
<th></th>
<th>PDD</th>
<th>PD</th>
<th>statistic</th>
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<td>Sex (♂/♀)</td>
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<td>6/7</td>
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<td>Age (years)</td>
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<td>Disease duration (years)</td>
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<td>4.0</td>
<td>t=-0.923</td>
<td>0.365</td>
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<tr>
<td>CAMCOG (0-107)</td>
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<td>11.8</td>
<td>t=6.938</td>
<td>0.000</td>
</tr>
<tr>
<td>UPDRS III OFF (0-108)</td>
<td>23.9</td>
<td>5.6</td>
<td>t=-4.266</td>
<td>0.000</td>
</tr>
<tr>
<td>H&amp;Y stage OFF (2/2.5/3/4)</td>
<td>0/5/7/1</td>
<td>4/6/3/0</td>
<td>$\chi^2$=6.691</td>
<td>0.082</td>
</tr>
</tbody>
</table>

**Graph analysis**

The average normalized clustering coefficient $C_\text{w}$ was higher in the PDD group in all frequency bands (figure 2). In the theta frequency range, this difference reached statistical significance ($p=.033$). In the gamma band, there was a trend towards significance ($p=.091$).

The average normalized path length $L_\text{w}$ was higher in the PDD group in every frequency band but the delta band (figure 2). Again, a significant difference was found in the theta band ($p=.009$).

**Figure 2**

*Values of the normalized clustering coefficient $C_\text{w}$ and path length $L_\text{w}$ in demented PD patients (PDD) and non-demented PD patients (PD).*
**Correlation with clinical parameters**

In the demented patients, a negative correlation was found between the CAMCOG score and $C_{\beta}$ in the beta band ($p=0.019$). There were no correlations with the UPDRS motor score. In the non-demented patients, a positive correlation was found between the UPDRS motor score and $C_{\alpha_1}$ in the alpha1 band ($p=0.038$). There were no correlations with the CAMCOG score.

**DISCUSSION**

To our knowledge, this is the first study addressing brain network structure in PD related dementia using modern graph theory. In this resting state MEG study, differences in the large scale network structure were found between demented and non-demented PD patients. Overall, dementia in PD was characterized by a higher normalized clustering coefficient as well as a longer normalized path length. In the theta band, these differences reached significance. In other words, the network structure in demented patients was shifted towards an organization with more local clustering and less global integration.

So far, most EEG/MEG-work in PD has focused on changes in strength of resting state functional connectivity. In the earliest stages of disease, even before initiation of dopamine replacement therapy, increases in functional connectivity have been found in the alpha1 band.\textsuperscript{10} These increases are also present in more advanced stage, treated patients where they occur over a much broader frequency range.\textsuperscript{10,11} At the large scale level of functional brain network organization, preliminary MEG results in non-demented PD patients using graph theoretical analysis are indicative of an increase in the clustering coefficient compared to controls, whereas the path length remains unchanged.\textsuperscript{43} These changes were observed in the alpha1 and theta bands. In the present study, a significantly higher clustering coefficient was found in PDD patients relative to non-demented PD patients in the theta band. Disease severity, measured using the UPDRS motor score, was higher in the PDD patients than in the non-demented patients. Therefore, this observation suggests that the early changes in local clustering in PD continue to progress with advancing disease stage also in demented PD patients.

Several ascending neurotransmitter systems, including dopaminergic, serotonergic and noradrenergic projections, are greatly affected already in the early pathological stages of PD.\textsuperscript{44} These systems continue to degenerate as the disease progresses and dementia develops. It is therefore tempting to speculate that the ongoing degeneration of these
ascending cortical projections contributes to the changes in local network organization we report between demented and non-demented patients. This notion is supported by the loss of small world network properties in a patient with brain stem ischemia, obviously leading to disruption of cortical projections.\textsuperscript{45} The effects of the serotonergic and noradrenergic systems on neural network properties are unknown, whereas experience with the effects of dopaminergic modulation is sparse. Recently, a decrease of global and local efficiency of brain networks of healthy subjects was found in an fMRI study after a single gift of sulpiride, a dopamine antagonist.\textsuperscript{46} In a very recent study in attention deficit hyperactivity disorder (ADHD), a disorder in which a hypofunctional state of the dopaminergic system is thought to represent an important pathophysiological mechanism,\textsuperscript{47} an increase in local connectedness was found in conjunction with a less prominent decrease of global integration.\textsuperscript{27}

Studies using functional MRI or PET have revealed that several cortical areas that are normally not involved in motor or cognitive tasks in healthy subjects become activated in non-demented PD patients (for an overview see Dagher et al\textsuperscript{48}). These observations support the idea that cortical dopaminergic neurotransmission reduces unwanted cortical activity\textsuperscript{49} and leads to focusing of cortical activity.\textsuperscript{50} Although these studies compared activity during a task condition to the baseline, it is possible that dopamine has the same focusing influence in the resting state. Indeed, a recent resting state functional MRI study based on graph theory exploring functional connectivity in a network involved in motor function, has revealed disturbances in PD as compared to controls. This pattern of changes could be normalized by dopamine treatment.\textsuperscript{51}

Along this line of reasoning, it can be hypothesized that a loss of the ascending dopaminergic influence on cortical activity (via the thalamus or direct mesocortical projections), which is one of the hallmarks of PD, may lead to a less efficient local spatial network organization. In demented PD patients, the ongoing deterioration of these systems might be responsible for a further increase of the clustering coefficient relative to non-demented patients, as reported in the present study.

In a previous study in PD, we observed that long range fronto-temporal and intertemporal functional connectivity in several frequency bands was lower in demented as compared to non-demented PD patients.\textsuperscript{12} We hypothesized that this might reflect either local cortical Lewy body and/or tau pathology leading to degeneration of long distance cortico-cortical connections, or the loss of subcortical input from the cholinergic basal nucleus of Meynert, which is thought to affect mainly long range synchronization.\textsuperscript{52} Quite possibly,
the same mechanisms may be responsible for the increase in the normalized path length in demented PD patients, indicative of decreased global integration, observed in the present study. This notion is supported by the results of resting state PET studies in PD showing cortical hypometabolism, mainly in frontal and parietal cortical areas in a brain network that is related to cognition. This hypometabolism was independent of motor function or dopaminergic treatment, rendering dopaminergic mechanisms underlying the changes less plausible. Furthermore, in a PET study using the acetylcholine analogue MPA4A, the cholinergic system was found to be more heavily affected in demented as compared to non-demented patients in approximately the same cortical areas.

In AD, a disorder that is also characterized by prominent local cortical pathology as well as cholinergic deficits, several studies using structural or functional imaging techniques have been conducted. He et al constructed unweighted graphs based on a structural measure, the cortical thickness, in 92 AD patients and compared their networks to the networks of 97 control subjects. AD patients turned out to have both a higher clustering coefficient and a longer path length, a pattern that is similar to the present observations in PDD. In contrast, using functional MRI data, Supekar et al reported a lower clustering coefficient in AD patients compared to healthy controls. In the only MEG study in AD so far, opposite to the results obtained using structural imaging data, a lower clustering coefficient as well as a shorter path length was found in AD patients in the alpha1 and beta band. Our results in PDD thus seem to be in line with the structural imaging data and in contrast with the functional imaging (MEG and fMRI) data in AD. In the future, direct comparisons between AD and PD using the same imaging techniques may further clarify the similarities and differences in large scale network changes between these two neurodegenerative conditions.

In computing brain network structure, we have to take into account differences in strength of functional connectivity between study groups. For instance, lower SL values may result in a lower estimate of the clustering coefficient as well as a longer path length. As mentioned before, we previously demonstrated decreases in interhemispheric as well as intrahemispheric SL in demented patients compared to non-demented patients. Therefore, since we used SL to perform graph analysis it could be argued that the longer path length we report in the present study for demented PD patients might directly result from decreases in SL. Therefore, to avoid a direct influence of changes in SL strength on path lengths and clustering coefficients, we calculated normalized clustering coefficients and path lengths by comparing them with clustering coefficients and path lengths of
random control networks. In this way, the present results are corrected for overall changes in SL strength. Thus, the observed changes in large scale brain network structure cannot be accounted for by changes in overall SL strength.

In conclusion, the large scale spatial organization of resting state brain networks in demented PD patients is different from that of non-demented PD patients and is characterized by both increased local connectedness as well as decreased global integration. We hypothesize that the observed changes may result from the interplay between the ongoing degeneration of ascending monoaminergic neurotransmitter systems, contributing to increased local clustering, on the one hand, and cholinergic deficits and cortical Lewy-body and tau-pathology, leading to loss of global integration, on the other hand. Future studies should address the effects of pharmacological intervention with dopaminergic and/or cholinergic drugs on the large scale brain network structure in PD related dementia.

APPENDIX A

Mathematical Details of Computation of SL

The SL is a measure of the “generalized synchronization” between two dynamical systems X and Y.\(^{38}\) Generalized synchronization\(^ {58}\) that exists between X and Y of the state of the response system is a function of the driver system: \(Y = F(X)\). The first step in the computation of the SL is to convert the time series \(x_i\) and \(y_i\) recorded from X and Y as a series of state space vectors using the method of time delay embedding:\(^ {59}\)

\[
X_i = (x_i, x_{i+L}, x_{i+2L}, x_{i+3L}, \ldots, x_{i+(m-1)L})
\]

where \(L\) is the time lag and \(m\) the embedding dimension. From a time series of \(N\) samples, \(N - (m\ 3\ L)\) vectors can be reconstructed. State space vectors \(Y_i\) are reconstructed in the same way. SL is defined as the conditional likelihood that the distance between \(Y_i\) and \(Y_j\) will be smaller than a cutoff distance \(r_y\), given that the distance between \(X_i\) and \(X_j\) is smaller than a cutoff distance \(r_x\). In the case of maximal synchronization, this likelihood is 1; in the case of independent systems, it is a small, but nonzero number, namely, \(P_{\text{ref}}\). This small number is the likelihood that two randomly chosen vectors \(Y\) (or \(X\)) will be closer than the cutoff distance \(r\). In practice, the cutoff distance is chosen such that the likelihood of random vectors being close is fixed at \(P_{\text{ref}}\), which is chosen the same for X and Y. To understand how \(P_{\text{ref}}\) is used to fix \(r_x\) and \(r_y\), we first consider the correlation integral:
\[ C_r = \frac{2}{N(N-w)} \sum_{i=1}^{N} \sum_{j=i+w}^{N-w} \theta(r - \left| X_i - X_j \right|) \quad (2) \]

Here the correlation integral \( C_r \) is the likelihood that two randomly chosen vectors \( X \) will be closer than \( r \). The vertical bars represent the Euclidean distance between the vectors. \( N \) is the number of vectors, \( w \) is the Theiler correction for autocorrelation, and \( h \) is the Heaviside function: \( h(X) = 0 \) if \( X > 0 \) and \( h(X) = 1 \) if \( X < 0 \). Now, \( r_x \) is chosen such that \( C_{rx} = P_{ref} \) and \( r_y \) is chosen such that \( C_{ry} = P_{ref} \). The SL between \( X \) and \( Y \) can now be formally defined as:

\[ SL = \frac{2}{N(N-w)P_{ref}} \sum_{i=1}^{N} \sum_{j=i+w}^{N-w} \theta(r_x - \left| X_i - X_j \right|) \theta(r_y - \left| Y_i - Y_j \right|) \quad (3) \]

SL is a symmetric measure of the strength of synchronization between \( X \) and \( Y \) (\( SL_{XY} = SL_{YX} \)). In equation (3), the averaging is done over all \( i \) and \( j \); by doing the averaging only over \( j \), SL can be computed as a function of time \( i \). From equation (3), it can be seen that in the case of complete synchronization \( SL = 1 \); in the case of complete independence \( SL = P_{ref} \); in the case of intermediate levels of synchronization \( P_{ref} < SL < 1 \).

Recently it has been shown that parameters \( L \) and \( m \) should take into account the low and high frequency filter settings rather than using fixed values of these parameters. Therefore, in the present study the choice of \( L \) and \( m \) was based explicitly on the frequency content of the data, which resulted in the following \( L \) and \( m \): delta: \( L=20, m=20 \); theta: \( L=10, m=9 \); alpha1: \( L=8, m=6 \); alpha1: \( L=6, m=6 \); beta: \( L=3, m=9 \); low gamma: \( L=2, m=6 \); \( P_{ref} \) was set at \( .01 \) for all frequency bands.
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Pathophysiological mechanisms in Parkinson’s disease related dementia: an MEG study

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Pathophysiological mechanisms in Parkinson's disease related dementia: an MEG study
Chapter 6
Summary and general discussion
Parkinson’s disease (PD) is a true multisystem disorder, characterized by degenerative changes in many neurotransmitter systems and brain areas, including the cerebral cortex, and leading to the classical motor as well as a variety of non-motor symptoms, such as olfactory disturbances, autonomic dysfunction, depression, sleep disorders and importantly also cognitive deficits and dementia, often accompanied by psychotic symptoms. This thesis aims to contribute to our knowledge of the pathophysiology of cognitive dysfunction and especially dementia in PD, using magnetoencephalography (MEG) as a research tool.

In chapter 2, a review is given of cognitive dysfunction and dementia in PD and the possible pathophysiological mechanisms involved. A recent study has confirmed that impairments on neuropsychological tests are present in most PD patients in the earliest stages of disease, especially disturbances in executive function. In about 20 percent of patients, impairment is more prominent in at least one cognitive domain, which some authors refer to as mild cognitive impairment in PD (PD-MCI), defined as a deficit of at least 1.5 standard deviations below the expected age corrected mean score on a neuropsychological test loading on a specific domain. In a considerable number of patients dementia develops, generally consisting of a prominent dysexecutive syndrome with attentional deficits, fluctuating cognition and memory disturbances, often accompanied by psychotic symptoms, mainly visual hallucinations. At the time of publication of our review, dementia was thought to occur in 25 to 40% of PD patients. These figures were based on cross-sectional data. More recent publications based upon studies with extensive follow up periods, have provided data on the cumulative incidence of dementia in PD. Buter et al found that 60% of PD patients had become demented after 12 years of follow up. In a study with a follow up period of 20 years, Hely et al established that dementia had developed in 75% of patients before they died. In early stage PD, disease-specific pathology is concentrated in the brain stem involving among others the locus coeruleus, dorsal raphe nuclei and substantia nigra. Hence, dopaminergic, noradrenergic and serotonergic ascending cortical (and striatal) projections degenerate and the loss of these projections might be involved in the subtle cognitive dysfunction present in the early clinical stages of PD. In advanced stage PD patients, monoaminergic corticopetal projection systems continue to degenerate but other systems are also prominently involved, including the cholinergic system as well as the cerebral cortex. Local cortical Lewy body-pathology and concomitant tau-pathology have been demonstrated in PD. The exact pathophysiological mechanisms that contribute to the development of dementia in PD, however, are still largely unknown and it is still a subject
of debate to what extent PD related dementia results from progression of pathological changes that can already be demonstrated in non-demented patients, and to what extent additional mechanisms are necessary for dementia to develop. Identifying the mechanisms that lead to dementia is important for the development of novel symptomatic treatment and, ideally, treatments that protect patients from developing dementia. Currently, the symptoms of dementia can only be treated to some extent with drugs that enhance cholinergic function, the cholinesterase inhibitors (and the often accompanying psychotic symptoms additionally with atypical neuroleptics).9-14

Oscillatory neuronal synchrony, both within as well as between brain areas is considered to be important for normal cognitive function. Therefore, it is likely that cognitive dysfunction and dementia in PD are associated with disturbances of synchronization within and/or between brain areas. To gain insight in the pathophysiological mechanisms involved in the development of cognitive dysfunction and dementia in PD, we studied changes in synchronized oscillatory brain activity in non-demented and demented PD patients, using MEG as a research tool. The results of these studies are described in chapters 3-5.

**LOCAL NEURONAL SYNCHRONY**

First, in chapter 3.1, local oscillatory brain activity, measured using MEG, was compared between three groups: 13 healthy subjects, 13 non-demented PD patients and 13 demented PD patients. Relative spectral power was calculated in the delta (0-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz) and gamma (30-48 Hz) frequency bands.

The first interesting finding, which had not been consistently reported before,15-21 was the presence of a diffuse slowing of the background oscillatory brain activity in non-demented PD patients compared to controls, consisting of increased relative theta activity and decreased beta activity. In a subsequent study by our group we were able to establish that these changes are present from the earliest clinical stages of PD onward.22 In the latter study, slowing of oscillatory activity was largely independent of disease duration, disease severity and was hardly influenced by dopaminergic treatment, suggesting that these changes may not be related to degeneration of dopaminergic neurons, but possibly to changes in non-dopaminergic mechanisms for instance involving noradrenergic and serotonergic projections. Cholinergic involvement in these early stage changes seems less likely, since the cholinergic basal nucleus of Meynert is only mildly involved in the early stages of disease.8 Furthermore, a lesion of the cholinergic system would more likely be expected to produce an increase of delta activity,23,24 which we did not observe in our study.
Consistent with previous data,\textsuperscript{17,18,20,21} demented PD patients had a much more pronounced slowing of brain activity than non-demented PD patients. Furthermore, posterior reactivity of the alpha rhythm to eye opening was much reduced in the demented patients. Interestingly, the pattern of slowing in demented patients relative to the non-demented patients was qualitatively very different from the slowing observed in non-demented patients compared to controls. In addition to a further increase in theta and a decrease in beta activity, we found an additional increase in delta and a decrease in alpha band power in demented PD patients. These qualitative changes in the pattern of slowing going from non-demented to demented PD patients, suggest that the alterations in local oscillatory brain activity in PD related dementia do not merely reflect a progression of the pathological changes that characterize the non-demented stages of PD, i.e. mainly dopaminergic, noradrenergic and serotonergic deficiencies related to brain stem pathology. Instead, different or at least additional mechanisms would seem to be involved. According to the current pathological concept of PD,\textsuperscript{8,25} dementia develops in the advanced stages of PD. In these stages, degeneration of the basal nucleus of Meynert is a prominent pathological feature. Therefore, degeneration of cholinergic cortical projections with resultant diminished cortical cholinergic activity is a likely candidate to explain the changes in local oscillatory MEG activity in PDD. This hypothesis finds support in animal as well as human studies using spectral power analysis, which have shown that degeneration or lesioning of the cholinergic system results in a shift toward slower frequencies in the powerspectrum.\textsuperscript{23,24,26-31} Further support comes from studies in Alzheimer’s disease (AD) and dementia with Lewy bodies (DLB), both characterized by prominent cholinergic deficits, in which profound slowing of background activity is reported.\textsuperscript{32,33}

In some of these animal studies and also in several studies in AD, promotion of cholinergic activity produced a shift of the powerspectrum towards higher frequencies.\textsuperscript{34-39} Consequently, one would anticipate a similar change in the powerspectrum in demented PD patients when treated with drugs that enhance cholinergic function. This effect of cholinergic modulation on relative spectral power in PDD could indeed be demonstrated in the study described in chapter 3.2. In this study, relative spectral power was calculated from the MEG recordings in eight demented PD patients before and after a period of treatment with the cholinesterase inhibitor rivastigmine (patients participated in a multicenter, randomized, placebo-controlled trial\textsuperscript{11}). After a mean treatment period of 29 weeks, an increase in relative alpha and beta power and a decrease of delta power could be demonstrated. In other words, there was a shift in the powerspectrum towards higher frequencies. When taken together with the results of chapter 3.1, the characteristic
slowing of oscillatory activity in demented PD patients could partly be reversed by treatment with rivastigmine. Moreover, in parallel to the changes in recorded brain activity, cognitive function improved in all but one patient after treatment. It is tempting to speculate that responders to treatment might be characterized by a specific pattern of baseline spectral power parameters and that, in this way, neurophysiological measures might help to select patients who could benefit from treatment. Studies including a larger number of patients, both responders and non-responders to treatment, are needed to further address baseline neurophysiological parameters in relation to treatment response.

In addition to cholinergic deficits, advanced stage PD is associated with widespread neuropathological changes in the cerebral cortex, involving both cortical Lewy body- and concomitant tau-pathology. Since these changes are often demonstrated to be more pronounced in PDD than PD \(^{40-45}\) and the extent of cortical Lewy body-pathology was the best correlate of the severity of dementia in one study, \(^{46}\) it can be hypothesized that Lewy body- and tau-pathology may also contribute to the slowing of resting state local oscillatory activity in PD related dementia.

In summary, the results presented in chapters 3.1 and 3.2 indicate that it is unlikely that dementia in PD is merely caused by a progression of changes in neurotransmitter systems already affected in non-demented PD, including the dopaminergic system. Instead, it seems plausible that additional mechanisms, notably cholinergic loss and possibly local cortical Lewy body and tau pathology, play an important pathophysiological role.

**FUNCTIONAL CONNECTIVITY**

In chapter 4, the results are described of two studies in which neuronal synchronization between brain areas was studied using the synchronization likelihood (SL). Rather than measuring the average local power at each separate MEG sensor, this technique measures linear as well as non-linear statistical interdependencies between MEG sensors, \(^{47}\) which represents a measure of functional connectivity between brain areas. In the first study, described in chapter 4.1, SL was calculated in 70 non-demented PD patients in different stages of disease and compared to 21 healthy controls. A diffuse increase of 8-10 Hz alpha1 synchronization was demonstrated in very early stage, drug naive PD patients. In more advanced patients, increases in SL additionally involved the theta and beta frequency bands. In agreement with the results of an EEG study in advanced stage PD patients, \(^{48}\) the increases we observed in theta and beta band synchronization in non-demented PD patients were positively correlated with the severity of parkinsonism. Therefore, the increases in functional connectivity in these frequency bands in non-demented patients might especially be related to motor symptoms.
In the subgroup of early stage, untreated PD patients, increased alpha1 synchronization between both hemispheres correlated with increased perseveration, a quite common and early cognitive deficit in PD.\textsuperscript{49-51} Synchronization in the alpha band is thought to be related to cognitive function, in particular attentional processes.\textsuperscript{52} Possibly, the increased alpha1 synchronization in non-demented PD patients reflects a disturbance of the dynamical balance of functional connectivity with a resultant loss of flexibility. Therefore, increased alpha synchronization may well reflect the very early cognitive deficits known to occur in early stage PD.

\textbf{Chapter 4.2} describes the results of a study in which SL values were compared between 13 demented PD patients and 13 non-demented PD patients (the same patient groups as described in chapter 3.1). In contrast to the increases in synchronization demonstrated in chapter 4.1 for the non-demented PD patients, PD related dementia was associated with reductions in long range intrahemispheric and interhemispheric synchronization in several frequency bands, mainly in fronto-temporal regions. In addition, there were minor increases in posterior alpha2 and beta synchronization. As frontal and temporal regions are known to be important for memory and executive function, it is tempting to speculate that the observed loss of fronto-temporal functional connectivity reflects cognitive dysfunction in PDD, although we were not able to find correlations between reduced synchronization and measures of cognitive function to support this. The reductions in fronto-temporal and intertemporal functional connectivity we observed in PD related dementia are strikingly similar to the changes in functional connectivity found in AD\textsuperscript{53} and DLB.\textsuperscript{54} Interestingly, an increase in posterior synchronization was also reported in an MEG study in AD.\textsuperscript{53} These observations in PD and AD may suggest that increased synchronization may also be related to cognitive dysfunction. This would seem to be supported by the fact that worse cognition in the demented PD group was associated with higher levels of synchronization. Alternatively, increases in synchronization may constitute a compensatory mechanism in relatively healthy networks. Considering that increases in SL were also found in non-demented PD patients relative to controls (chapter 4.1), the increases in the PDD patients may merely reflect disease progression. The latter possibility is supported by the fact that PDD patients had higher UPDRS motor scores than the non-demented patients.

The resemblance in the pattern of changes in functional connectivity with AD and DLB, disorders characterized by prominent cholinergic deficits, suggests that, much like the changes in spectral power (chapter 3), the reductions in functional connectivity in PD may also be related to the degeneration of the cholinergic system. This notion is further supported by the demonstration of reductions of intrahemispheric as well as
interhemispheric functional connectivity after a decrease in cholinergic tone in an animal study and following the administration of the anticholinergic drug scopolamine to healthy subjects.

In addition to the involvement of the cholinergic system, a loss of structural connections, much the same as in AD and DLB, may also contribute to the reported changes in functional connectivity between brain areas. In AD, an association was found between fMRI coherence and cortical atrophy. However, since we did not combine MRI and MEG data in our study, this relationship remains to be explored. However, cortical changes including cortical atrophy are more prominent in demented in comparison to non-demented PD patients, and therefore, a contribution of local cortical changes to the alterations in functional connectivity seems likely.

In summary, in both non-demented and demented PD patients, there are disturbances of functional connectivity between brain areas. In contrast to the increases in synchronization reported in non-demented PD patients, which may result from degeneration of ascending brainstem monoaminergic projections, a quite different pattern of changes is found in demented patients, mainly consisting of reductions in synchronization. Very much like the changes in spectral power, the loss of functional connectivity may be caused by cholinergic degeneration, possibly together with cortical Lewy body- and/or tau-pathology.

LARGE SCALE NETWORK ORGANIZATION

Finally, changes in the large scale spatial organization of brain networks in PD were studied in chapter 5. In the study described in this chapter, modern graph theoretical analysis was applied to matrices of synchronization likelihood values. Networks of 13 non-demented and 13 demented PD patients were characterized by the clustering coefficient, a measure of local connectedness, and the path length, a measure of global integration. Compared to non-demented patients, PDD was characterized by a higher clustering coefficient in all frequency bands as well as a longer path length in all but the delta band. Although these differences reached significance only for the theta band, the overall pattern of change in the organization of networks in PDD was a loss of global integrative properties and increased local interconnectedness.

Preliminary results of a comparison between early stage, non-demented PD patients and controls also show increased local interconnectedness in several frequency bands, including the theta band. However, in these non-demented PD patients there were no changes in path length. Therefore, it is tempting to suggest that the increase in local interconnectedness observed in both demented and non-demented PD patients reflects...
the same pathophysiological mechanism. Obviously, loss of dopaminergic tone is the most likely candidate mechanism. Dopamine is thought to reduce unwanted activity during a task and at rest, and also leads to focusing of activity. Based upon these observations, we hypothesize that the degeneration of the dopaminergic system in PD leads to reduced focusing of cortical activity which is expressed in a pathological increase in local connectedness.

The increase in path length appears to be a characteristic of dementia in PD and, therefore, this more likely reflects the pathophysiological mechanisms underlying dementia in PD. Indeed, a recent fMRI study has emphasized the relationship between a longer path length and a lower intelligence quotient. As discussed above for other changes in brain activity in PDD, this may involve cholinergic dysfunction and/or local cortical pathology. This assumption is supported by resting state PET studies in PD, in which cortical hypometabolism that was independent of motor function or dopaminergic treatment could be demonstrated in a network of brain areas related to cognitive function. In another PET study, the cholinergic system was more severely affected in demented PD patients than in non-demented patients in approximately the same areas. Therefore, the loss of cholinergic projections and/or local cortical changes may lead to the loss of global integrative properties of brain networks we observed in PDD.

Our results are in agreement with structural network data in AD, as measured by the cortical thickness. In contrast, the clustering coefficient is reported to be lower in an fMRI study. In a recent MEG study, both a lower clustering coefficient and shorter path length were demonstrated in AD. Therefore, our results seem to be in contrast with functional network data in AD. It remains to be clarified whether these differences are related to the use of different methodologies in these studies or whether they reflect differences in pathophysiological mechanisms. Future studies directly comparing PDD and AD are needed to clarify the similarities and differences in large scale network organization between these two neurodegenerative conditions.

**CONCLUSION**

Taken together, the studies in this thesis contribute to a better understanding of changes in brain activity in PD. Our results have shown that resting state brain activity in PD related dementia is characterized by a prominent and diffuse slowing of rhythmic oscillatory brain activity, reductions in fronto-temporal and intertemporal functional connectivity, and a shift in large scale network organization from global integration to more local connectedness. This pattern of changes is qualitatively very different from the pattern of changes that occurs in non-demented PD patients. Based on this qualitative difference,
the development of dementia in PD entails more than a quantitative progression of disease pathology in brain areas already affected in non-demented patients, but rather the involvement of additional pathophysiological mechanisms. Given the effects of cholinergic modulation on brain activity and the fact that the changes in brain activity observed in PDD in many aspects resemble those reported for AD, these pathophysiological mechanisms most likely include the degeneration of the cholinergic system. In addition, local cortical Lewy body- and tau-pathology may play an additional role. In the future, the differential patterns of altered brain activity in demented and non-demented PD patients may prove useful to identify patients at risk for developing dementia.

Our findings also demonstrate that modulatory effects of drugs on resting state brain activity can be measured using MEG. In this way, studying pre- and post-treatment resting state brain activity may prove to be useful as a monitoring instrument of the effects of potential future treatments for dementia.

Based on our results, several recommendations for future research can be made:

**LONGITUDINAL DATA**
Longitudinal studies in PD patients combining MEG data and neuropsychological follow up may further help to clarify the natural course of changes in resting state brain activity in PD and their relation to cognitive dysfunction and the development of dementia. In this way, patients at risk for developing dementia (and possibly also psychosis), may be identified. Furthermore, the modulatory effects of for instance cholinergic and dopaminergic drugs may further clarify the contribution of the different neurotransmitter systems to changes in resting state brain activity.

**TASK-RELATED CHANGES IN BRAIN ACTIVITY**
The changes in brain activity in our study groups were all demonstrated in the resting state. Possibly, differences between the groups may be even more pronounced when subjects perform a cognitive task during the MEG recording. Therefore, studies applying task-specific conditions may further address the relation of oscillatory brain activity and cognitive function.

**ADDITIONAL ANALYSIS TECHNIQUES**
To reduce the number of statistical comparisons in our studies, we used fairly general measures of analysis, possibly at the cost of detailed local information. Future research might focus more in detail on specific areas that might be involved in cognitive dysfunction and dementia in PD, possibly in combination with other imaging modalities.
For example, co-registration with structural MRI may allow the assessment of the relationship between changes in functional neurophysiological parameters of brain activity and cortical atrophy. Similarly, special techniques such as diffusion tensor imaging (DTI) may provide the link between the loss of connecting fibers and changes in functional connectivity. Studies combining MEG and fMRI may yield important information on the relationship of metabolic changes as a measure of neuronal activity with changes in electromagnetic fields induced by neuronal activity.

Furthermore, different time series analysis techniques may further help to unravel the neurophysiological changes in PD. For instance, techniques that take into account volume conduction in EEG or MEG, such as the phase lag index (PLI) may be useful.

**Prediction of response to (cholinergic) treatment**

Finally, by studying differences in baseline neurophysiological parameters in a larger number of both responders and non-responders to treatment, MEG (or EEG) might contribute to the selection of patients who may optimally benefit from cholinergic treatment. Furthermore, MEG or EEG may be useful to monitor the effects of other potential treatment strategies.
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Pathophysiological mechanisms in Parkinson's disease related dementia: an MEG study


Pathophysiological mechanisms in Parkinson’s disease related dementia: an MEG study
Pathophysiological mechanisms in Parkinson's disease related dementia: an MEG study
Chapter 7
Appendices

Nederlandse samenvatting
List of publications
Curriculum Vitae
Dankwoord
De ziekte van Parkinson is een multisysteem-aandoening, waarin er veranderingen optreden in diverse neurotransmittersystemen en hersengebieden, waaronder de hersenschors. De ziekte wordt dan ook niet alleen gekenmerkt door motorische verschijnselen, gezamenlijk parkinsonisme genoemd, maar in belangrijke mate ook door niet-motorische verschijnselen zoals reukstoornissen, autonome disfunctie, depressie, slaapstoornissen en cognitieve disfunctie/dementie, vaak gepaard gaande met psychotische verschijnselen. Dit proefschrift heeft als doel om bij te dragen aan de kennis van de pathofysiologie van cognitieve disfunctie en dementie, waarbij gebruik is gemaakt van magneto-encephalografie (MEG) als onderzoeksinstrument.

In hoofdstuk 2 wordt een overzicht gegeven van cognitieve disfunctie en dementie bij de ziekte van Parkinson en de mogelijke pathofysiologische mechanismen. Subtiele cognitieve stoornissen komen al heel vroeg in de ziekte voor bij een groot deel van de patiënten, vooral stoornissen van de executieve functies. Ook ontwikkelt er zich bij een aanzienlijk deel van de patiënten met de ziekte van Parkinson een dementie. De geschatte percentages variëren van ongeveer 25-40% ten tijde van de publicatie van het overzichtsartikel tot meer dan 60% in recentere studies met een langere follow-up.

In de eerste pathologische stadia van de ziekte van Parkinson worden vooral afwijkingen gevonden in hersenstamstructuren zoals de locus coeruleus, dorsale raphe kernen en de substantia nigra met als gevolg een verlies van serotonerge, noradrenerge en dopaminerge projecties naar de hersenschors. Deze veranderingen spelen mogelijk een rol bij het ontstaan van vroege cognitieve stoornissen bij de ziekte van Parkinson. In de meer gevorderde stadia van de ziekte blijft degeneratie van deze systemen voortschrijden, maar andere systemen en structuren vertonen nu ook pathologische veranderingen, zoals het cholinerge systeem en de hersenschors, waarin Lewy- en tau-pathologie kunnen worden aangetoond.

De exacte mechanismen die verantwoordelijk zijn voor dementie bij de ziekte van Parkinson zijn echter nog grotendeels onbekend. Er is nog veel discussie of voortgaande degeneratie van de al in vroege fasen betrokken systemen verantwoordelijk is voor de ontwikkeling van dementie of dat andere of in ieder geval additionele mechanismen hiervoor verantwoordelijk zijn. Kennis van de betrokken mechanismen is essentieel voor de ontwikkeling van nieuwe symptomatische therapieën en in de toekomst voor medicatie die mogelijk beschermt tegen het ontwikkelen van dementie gerelateerd aan de ziekte van Parkinson. Momenteel kunnen de dementie-verschijnselen deels behandeld
worden met cholinesterase-remmers (en eventueel met neuroleptica in het geval van psychotische symptomen).


**LOKALE SYNCHRONISATIE**

In *hoofdstuk 3.1* werd synchronisatie van oscillatoire hersenactiviteit binnen hersengebieden vergeleken tussen 13 dementerende Parkinson-patiënten, 13 niet-dementerende Parkinson-patiënten en 13 gezonde controles. Relatieve spectraal power werd berekend in de delta, theta, alfa, beta en gamma frequentiebanden. De eerste bevinding was dat reeds bij niet-dementerende patiënten een vertraging van het achtergrondpatroon van hersenactiviteit ten opzichte van gezonde controles kon worden. In een andere studie door onze groep konden deze veranderingen al in de allervroegste klinische fasen van de ziekte worden aangetoond. Deze waren onafhankelijk van ziekte-ernst en dopaminerge medicatie, wat suggereert dat niet-dopaminerge systemen mogelijk betrokken zijn bij deze vertraging, zoals het serotonerge en noradrenerge systeem. Betrokkenheid van het cholinerge systeem lijkt minder waarschijnlijk omdat er dan vooral een toename van delta power verwacht mag worden, iets wat wij in onze studie niet gerapporteerd hebben. De vertraging bij Parkinson-patiënten was, zoals verwacht, nog veel meer uitgesproken. De vertraging toonde echter een kwalitatief heel ander karakter bij demente ten opzichte van niet-demente patiënten in vergelijking met die bij niet-demente patiënten ten opzichte van gezonde controles. Dit kwalitatief verschillende patroon suggereert dat de locale veranderingen van oscillatoire hersenactiviteit niet slechts het gevolg zijn van progressie van pathologische veranderingen zoals die al bij niet-demente patiënten te vinden zijn maar dat er additionele processen bij betrokken zijn. Hierbij kan vooral gedacht worden aan het cholinerge systeem omdat dit neurotransmittersysteem bij demente patiënten meer uitgesproken is aangedaan. Dit wordt ondersteund door het feit dat in dierexperimentele studies, waarbij er een laesie is aangebracht in het cholinerge systeem, soortgelijke veranderingen van hersenactiviteit worden gevonden. Bovendien is er ook bij patiënten met de ziekte van Alzheimer en dementie met Lewy...
lichaampjes, beide gekarakteriseerd door prominente cholinerge degeneratie, een zelfde soort vertraging van de hersenactiviteit aangetoond.

In de eerder genoemde dierstudies kon er een versnelling van het achtergrondpatroon bewerkstelligd worden wanneer het cholinerge systeem gestimuleerd werd. Dit valt dan ook te verwachten bij dementerende Parkinson-patiënten. Dit werd onderzocht in hoofdstuk 3.2, waarin relatieve spectrale power werd berekend in een groep van acht dementerende Parkinson-patiënten voor en na een periode van behandeling met rivastigmine, een cholinesterase remmer. Inderdaad werd er na een behandelperiode van gemiddeld 29 weken een verschuiving van het power spectrum naar hogere frequenties gevonden ofwel, er werd een versnelling van het achtergrondpatroon in de rusttoestand waargenomen.

In combinatie met de resultaten van hoofdstuk 3.1 kan geconstateerd worden dat de karakteristieke vertraging van het achtergrondpatroon van oscillatoire hersenactiviteit bij demente patiënten deels weer tegengegaan kan worden door middel van cholinerge stimulatie. Bij zeven van de acht patiënten ging dit ook gepaard met een klinische verbetering van het cognitief functioneren. Het is verleidelijk om te speculeren dat patiënten met een goede behandelrespons gekarakteriseerd worden door een specifiek patroon van veranderingen in locale synchronisatie en dat op deze manier patiënten geselecteerd kunnen worden die mogelijk goed reageren op de behandeling. Toekomstige studies met grotere aantallen patiënten en ook de inclusie van patiënten die geen gunstig effect hebben op de medicatie (non-responders) zijn aan te raden ten einde hier meer inzicht in te krijgen.

FUNCTIONELE CONNECTIVITEIT

In hoofdstuk 4 worden twee studies beschreven waarin synchronisatie van oscillatoire hersenactiviteit tussen hersengebieden werd bestudeerd. Hiervoor werd de “synchronization likelihood” (SL) gebruikt, een uitkomstmaat die de functionele interactie tussen hersengebieden weerspiegelt, ook wel functionele connectiviteit genoemd.

In hoofdstuk 4.1 werd bij niet-demente Parkinson-patiënten die nog niet behandeld werden met dopaminerge medicatie (de novo) een globale toename van alfa SL gevonden in vergelijking met gezonde controles. Bij meer gevorderde patiënten werden ook nog toenames gevonden in andere frequentiebanden (theta en beta). Deze toenames waren gecorreleerd met meer gevorderde motorische verschijnselen, wat suggereert dat de toenames in SL mogelijk gerelateerd zijn aan de motorische verschijnselen van de ziekte.
Verder werd in een subgroep van patiënten een relatie gevonden tussen toegenomen alfa SL en toegenomen perseveratie, een stoornis die vaak al in de zeer vroege fasen van de ziekte van Parkinson gedemonstreerd kan worden. Mogelijk reflecteert de toegenomen functionele connectiviteit in de alfa frequentieband een verlies aan cognitieve flexibiliteit.

In hoofdstuk 4.2 werden opnieuw 13 demente Parkinson-patiënten vergeleken met 13 niet-demente patiënten met de SL als uitkomstmaat. In tegenstelling tot de toenames van synchronisatie bij de niet-demente patiënten ten opzichte van controles, zoals beschreven in hoofdstuk 4.1, werd er bij demente patiënten voornamelijk een afname van synchronisatie binnen en tussen de hersenhemisferen gevonden, in het bijzonder tussen frontale en temporale gebieden. Verder werd er nog een minder uitgesproken toename van posterieure connectiviteit gevonden. Aangezien frontale en temporale hersengebieden belangrijk zijn voor het geheugen en executieve functies, is het verleidelijk om aan te nemen dat het verlies van fronto-temporale connectiviteit bij demente patiënten cognitief disfunctioneren reflecteert. De veranderingen bij demente Parkinson-patiënten lijken sterk op de veranderingen zoals die bij de ziekte van Alzheimer en dementie met Lewy lichaampjes gerapporteerd worden. Dit geldt opvallend ook voor de posterieure toename van synchronisatie. Het laatste suggereert dat ook deze toename van synchronisatie een relatie heeft met cognitieve disfunctie, wat gesteund wordt door de bevinding van een positieve correlatie tussen deze toename en cognitieve disfunctie in de bestudeerde dementerende groep. Een alternatieve verklaring kan zijn dat de toename van synchronisatie een compensatiemechanisme behelst in nog relatief onaangedane hersengebieden.

De overeenkomsten tussen de veranderingen van functionele interactie tussen hersengebieden bij de ziekte van Parkinson, de ziekte van Alzheimer en dementie met Lewy lichaampjes, alle drie gekarakteriseerd door prominent verlies van cholinerge verbindingen, suggereert opnieuw dat het cholinerge systeem hierbij mogelijk een belangrijke rol speelt. Deze gedachte wordt verder ondersteund door de resultaten van een dierexperimentele studie, waarin een laesie van het cholinerge systeem gepaard ging met een afname van functionele connectiviteit, zowel binnen als tussen hersengebieden. Hetzelfde effect kon bewerkstellig worden door bij gezonde personen scopolamine, een anticholinerg middel, toe te dienen.

Een verlies aan structurele corticale verbindingen speelt uiteraard mogelijk ook een belangrijke rol. Bij de ziekte van Alzheimer is er een verband gevonden tussen functionele connectiviteit en corticale atrofie. Wij hebben dit niet onderzocht bij de ziekte van Parkinson.
Parkinson, maar het is echter wel bekend dat demente Parkinson-patiënten meer corticale atrofie hebben dan niet-demente patiënten.

Samenvattend wordt er bij niet-demente Parkinson-patiënten een toename van functionele connectiviteit gevonden, mogelijk gerelateerd aan het verlies van dopaminerge, serotonerge en noradrenerge verbindingen, terwijl bij demente patiënten een volledig ander patroon van veranderingen wordt gevonden, voornamelijk bestaand uit een afname van synchronisatie. Analoog aan de veranderingen in spectraal power is deze reductie van functionele connectiviteit mogelijk gerelateerd aan bijkomende cholinerge tekorten en/of corticale veranderingen.

**NETWERK ORGANISATIE**

In *hoofdstuk 5* werd de ruimtelijke organisatie van hersennetwerken bestudeerd. “Graph analysis” werd toegepast op matrices van SL waarden. De netwerken van 13 demente en 13 niet-demente Parkinson-patiënten werden gekarakteriseerd door middel van de “clusteringcoëfficiënt”, een netwerkmaat voor dichteit van lokale verbindingen, en de “padlengte”, een maat voor globale netwerkintegratie. In vergelijking met de niet-demente patiënten hadden demente patiënten een hogere clusteringcoëfficiënt en een langere padlengte. Ondanks dat deze resultaten alleen statistische significantie bereikten in de theta frequentieband, was er bij de demente patiënten een algemene tendens zichtbaar van een verschuiving van globale integratieve kenmerken naar meer toegenomen lokale netwerkverbindingen.

In een andere (nog niet gepubliceerde) studie werd bij patiënten in een zeer vroege fase van de ziekte ook een hogere clusteringcoëfficiënt gevonden in vergelijking met controles terwijl de padlengte geen verschillen toonde. Mogelijk dat deze toename van clustering in zowel de demente als niet-demente patiënten door hetzelfde mechanisme wordt veroorzaakt. Het dopaminerge systeem is hiervoor de meest logische kandidaat. Dopaminerge activiteit leidt waarschijnlijk tot een reductie van ongewenste activiteit tijdens een taak en waarschijnlijk ook tot meer gefocuste activiteit. Wij hypothetiseren dat verlies van dopaminerge verbindingen leidt tot een afname van de focus van activiteit van de hersenschors, wat zich uit in een hogere maat voor clustering. In een PET studie is eerder hypometabolisme onafhankelijk van de motorische verschijnselen en dopaminerge behandeling gedemonstreerd in een netwerk dat betrokken is bij cognitie, terwijl in een andere studie het cholinerge systeem meer uitgesproken aangedaan was in ongeveer dezelfde hersengebieden. Cholinerge degeneratie speelt daarom mogelijk een rol bij het verlies van globale integratie van hersennetwerken bij de ziekte van Parkinson. Deze
resultaten zijn in overeenstemming met studies bij de ziekte van Alzheimer waarbij structurele maten werden gebruikt, maar in een functionele MRI studie werd een lagere clusteringcoëfficiënt gemeten. MEG studies bij de ziekte van Alzheimer demonstreren zowel een toegenomen clusteringcoëfficiënt als padlengte. Voorspelling is het onduidelijk of deze verschillen met de ziekte van Parkinson te verklaren zijn door methodologische verschillen dan wel door een daadwerkelijk verschil in pathofysiologische mechanismen. Toekomstige studies waarin de ziekte van Parkinson en Alzheimer direct vergeleken worden zullen hier meer duidelijkheid in kunnen scheppen.

CONCLUSIE

Onze studies dragen hopelijk bij aan een beter begrip van de veranderingen van hersenactiviteit bij de ziekte van Parkinson. Activiteit in de rusttoestand bij demente patiënten wordt gekenmerkt door een forse diffuse vertraging van locale oscillerende hersenactiviteit, een reductie van functionele verbindingen tussen hersengebieden en een verschuiving van de organisatie van hersennetten werken van globale integratie naar meer locale onderlinge verbindingen. Bij niet-demente patiënten kan juist een kwalitatief compleet ander patroon van veranderingen gedemonstreerd worden. Dit ondersteunt de gedachte dat dementie bij de ziekte van Parkinson niet slechts wordt veroorzaakt door een voortgaande degeneratie van systemen die al vroeg betrokken zijn in de ziekte, maar dat er mogelijk additionele mechanismen in belangrijke mate bij betrokken zijn. Gezien de effecten van cholinerge modulatie en de belangrijke gelijkenissen tussen dementie bij de ziekte van Parkinson en de ziekte van Alzheimer, beiden gekarakteriseerd door belangrijke cholinerge tekorten, ligt een belangrijke betrokkenheid van degeneratie van het cholinerge systeem voor de hand, mogelijk in combinatie met pathologische veranderingen in de hersenschors.

Onze resultaten laten ook zien dat veranderingen in hersenactiviteit door medicamenteus ingrijpen gemeten kunnen worden met MEG. MEG kan daarom in de toekomst mogelijk een instrument zijn om de effecten van eventuele nieuwe behandelingen voor dementie te monitoren.

Toekomstig onderzoek kan zich richten op het verzamelen van longitudinale data, het gebruik van nieuwe analyse-technieken en het combineren van MEG met andere beeldvormende technieken zoals fMRI of DTI.
LIST OF PUBLICATIONS (PEER REVIEWED AND BOOK CHAPTERS)

JLW Bosboom, D Stoffers, CJ Stam, HW Berends, ECH Wolters

JLW Bosboom, D Stoffers, ECH Wolters, CJ Stam, HW Berendse
MEG resting state functional connectivity in Parkinson’s disease related dementia.

D Stoffers, JLW Bosboom, ECH Wolters, CJ Stam, HW Berendse

D Stoffers, JLW Bosboom, JB Deijen, ECH Wolters, CJ Stam, HW Berendse

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Resting state oscillatory brain dynamics in Parkinson’s disease: an MEG study.
**JLW Bosboom, D Stoffers, CJ Stam, HW Berendse, ECh Wolters**

Cognitive disfunction and dementia in Parkinson’s disease: EEG/MEG Experience.

In: Mental Disfunction in Parkinson’s Disease III, ECh Wolters, HW Berendse, CJ Stam.


**CJ Stam, JLW Bosboom, D Stoffers, BW van Dijk, J Verbunt, HW Berendse, ECh Wolters**

The Neurophysiology of Dementia in Parkinson’s Disease: Does Connectivity Count?

In: Mental Disfunction in Parkinson’s Disease III, ECh Wolters, HW Berendse, CJ Stam.


**JLW Bosboom, ECh Wolters**


**JLW Bosboom, D Stoffers, ECh Wolters**

Cognitive dysfunction and dementia in Parkinson's disease.


**JLW Bosboom, ECh Wolters**

Psychotic symptoms in Parkinson’s disease: pathophysiology and management.


**JLW Bosboom, ECh Wolters**


**JLW Bosboom, D Stoffers, ECh Wolters**

The role of acetylcholine and dopamine in dementia and psychosis in Parkinson’s disease.


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