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Slowing of oscillatory brain activity is a stable characteristic of Parkinson's disease without dementia

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Extensive changes in resting-state oscillatory brain activity have recently been demonstrated using magnetoencephalography (MEG) in moderately advanced, non-demented Parkinson's disease patients relative to age-matched controls. The aim of the present study was to determine the onset and evolution of these changes over the disease course and their relationship with clinical parameters. In addition, we evaluated the effects of dopaminomimetics on resting-state oscillatory brain activity in levodopa-treated patients. MEG background oscillatory activity was studied in a group of 70 Parkinson's disease patients with varying disease duration and severity (including 18 *de novo* patients) as well as in 21 controls that were age-matched to the *de novo* patients. Whole head 151-channel MEG recordings were obtained in an eyes-closed resting-state condition. Levodopa-treated patients ($N = 37$) were examined both in a practically defined 'OFF' as well as in the 'ON' state. Relative spectral power was calculated for delta, theta, low alpha, high alpha, beta and gamma frequency bands and averaged for 10 cortical regions of interest (ROIs). Additionally, extensive clinical and neuropsychological testing was performed in all subjects. *De novo* Parkinson's disease patients showed widespread slowing of background MEG activity relative to controls. Changes included a widespread increase in theta and low alpha power, as well as a loss of beta power over all but the frontal ROIs and a loss of gamma power over all but the right occipital ROI. Neuropsychological assessment revealed abnormal perseveration in *de novo* patients, which was associated with increased low alpha power in centroparietal ROIs. In the whole group of Parkinson's disease patients, longer disease duration was associated with reduced low alpha power in the right temporal and right occipital ROI, but not with any other spectral power measure. No association was found between spectral power and disease stage, disease severity or dose of dopaminomimetics. In patients on levodopa therapy, a change from the 'OFF' to the 'ON' state was associated with decreases in right frontal theta, left occipital beta and left temporal gamma power and an increase in right parietal gamma power. Widespread slowing of oscillatory brain activity is a characteristic of non-demented Parkinson's disease patients from the earliest clinical stages onwards that is (largely) independent of disease duration, stage and severity and hardly influenced by dopaminomimetic treatment. Some early cognitive deficits in Parkinson's disease appear to be associated with increased low alpha power. We postulate a role for hypofunctional non-dopaminergic ascending neurotransmitter systems in spectral power changes in non-demented Parkinson's disease patients.

Keywords: Parkinson's disease; magnetoencephalography (MEG); resting-state; oscillations; spectral analysis

Abbreviations: EEG = Electroencephalography; MEG = Magnetoencephalography

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Introduction

In patients with Parkinson's disease with dementia, considerable diffuse slowing of resting-state oscillatory brain activity has consistently been reported in comparison to both non-demented Parkinson's disease patients as well as healthy controls. Electroencephalography (EEG) and magnetoencephalography (MEG) studies in demented

patients generally show increases in delta and theta activity and decreases in alpha and beta activity (Neufeld *et al.*, 1988; Soikkeli *et al.*, 1991; Neufeld *et al.*, 1994; Tanaka *et al.*, 2000; Sinanovic *et al.*, 2005; Bosboom *et al.*, 2006). Until very recently, the presence of slowing of resting-state oscillatory brain activity in non-demented Parkinson's disease patients was a more controversial issue. Generally, EEG studies in non-demented Parkinson's disease patients have not shown (pronounced) slowing of resting-state brain activity (Neufeld *et al.*, 1994; Gagnon *et al.*, 2004; Sinanovic *et al.*, 2005). Some older studies, however, have shown a slowing of the occipital background rhythm (Neufeld *et al.*, 1988; Soikkeli *et al.*, 1991), a decrease of beta power (Pezard *et al.*, 2001) and, remarkably, an increase in alpha power (Tanaka *et al.*, 2000) in non-demented Parkinson's disease patients relative to controls. In one MEG study, in which cognitive status was unfortunately not reported, a decreased frequency of the dominant alpha rhythm and an increase in low frequency power were found (Kotini *et al.*, 2005). In a very recent MEG study, we were the first to demonstrate extensive changes in resting-state oscillatory brain activity also in non-demented Parkinson's disease patients at moderately advanced stages of disease (mean subjective disease duration 9.7 ± 4.5 years) relative to age-matched controls (Bosboom *et al.*, 2006). The exact time of onset of slowing of resting-state oscillatory brain activity in Parkinson's disease and its evolution over the disease course, however, are still unknown.

Brain processes not only require the activation of specialized brain areas but also some mechanisms to integrate their activity. There is increasing evidence from neurophysiological studies that synchronized oscillatory neuronal activity plays a crucial role in integrating higher brain processes (Schnitzler and Gross, 2005). As adequate brain function requires the integration of specialized brain areas, clinical deficits in neurological diseases such as Alzheimer's disease and Parkinson's disease are expected to be associated with changes in oscillatory brain activity (Schnitzler and Gross, 2005). In Alzheimer's disease, which is also characterized by a diffuse slowing of resting-state oscillatory brain activity, measures of global cognition such as Folstein's Mini-Mental State Examination [MMSE (Folstein *et al.*, 1975)], as well as more specific neuropsychological test scores are associated with slowing of resting-state oscillatory brain activity (for a review of EEG dynamics in patients with Alzheimer's disease, see Jeong, 2004). With the noteworthy exception of studies using intra-cerebral recordings in Parkinson's disease patients undergoing functional neurosurgery, only a few studies have reported associations of (changes in) resting-state oscillatory brain dynamics with clinical (motor or cognitive) parameters in demented or non-demented Parkinson's disease patients. To our knowledge, only a single study has reported an association between disease (motor) severity and oscillatory brain dynamics; Neufeld and co-workers found the degree of motor disability to be associated with the frequency of slowing of occipital background activity in mentally intact patients (Neufeld *et al.*, 1988), which they interpreted as an indication that subcortical structures (involved in motor control) can influence occipital background activity. Two studies have reported associations between spectral power and cognitive parameters: Sinanovic and co-workers found slowing of background activity to be associated with lower MMSE scores in demented Parkinson's disease patients (Sinanovic *et al.*, 2005). In the MEG study by Bosboom and co-workers (Bosboom *et al.*, 2006), increased theta power in occipital and right temporal channels was associated with lower scores on a measure of global cognitive function [CAMCOG scale (Roth *et al.*, 1986; Hobson and Meara, 1999)] in non-demented Parkinson's disease patients. To our knowledge, no study has explored the relationship of more specific measures of cognitive performance with oscillatory brain activity in non-demented (or demented) Parkinson's disease.

An important neuropathological feature of Parkinson's disease is a dopamine (DA) deficit in the striatum, which results in abnormal basal ganglia outflow to the (pre)frontal cortex. In addition, degeneration of the mesocortical dopaminergic system in Parkinson's disease produces a loss of DA terminals in the (pre)frontal cortex itself. Either of these two pathological features of Parkinson's disease might constitute the mechanism behind changes in oscillatory brain activity and (frontal) cognitive dysfunction. The role of the nigrostriatal dopaminergic projection in cognitive function is supported by a functional magnetic resonance imaging (fMRI) study, in which a specific effect of dopaminergic manipulation on the effective connectivity between the caudate and the ventral midbrain was found in healthy subjects (Honey *et al.*, 2003). An EEG study showed Parkinson's disease to be associated with impaired cortical connectivity that could be normalized by levodopa treatment (Cassidy and Brown, 2001). Assuming that the dopaminergic deficit in Parkinson's disease is a key factor in causing alterations of oscillatory brain activity, restoring brain dopamine levels by drug treatment should at least partly restore normal patterns of oscillatory brain activity.

According to a newly introduced neuropathological staging system for Parkinson's disease (Braak *et al.*, 2003), degeneration of nuclei that give rise to ascending corticopetal neurotransmitter systems is one of the earliest pathological features of Parkinson's disease. This includes not only the dopaminergic neurons in the substantia nigra, but also noradrenergic neurons in the locus coeruleus and serotonergic neurons in the dorsal raphe nuclei. Interestingly, involvement of the corticopetal non-dopaminergic projection systems may even precede degeneration of dopaminergic substantia nigra neurons. In these very early pathological stages of Parkinson's disease, the forebrain cholinergic system arising from the basal nucleus of Meynert is affected to a much lesser extent. Degeneration of noradrenergic and serotonergic corticopetal projection systems may therefore at least contribute to any early stage alterations in cortical oscillatory brain activity.

In the present study, we set out to determine whether changes in resting-state oscillatory brain activity occur already in the earliest clinical stages of Parkinson's disease, before the start of dopaminergic treatment. In addition, we aimed to study the effects of disease duration, disease stage, disease severity and dopaminergic medication on the observed changes, as well as the relationship of any alterations in spectral power with changes in cognitive function.

Material and Methods

Subjects

A total of 84 patients with idiopathic Parkinson's disease diagnosed according to the United Kingdom Parkinson's Disease Society Brain Bank (UK-PDSBB) clinical diagnostic criteria (Hughes *et al.*, 1992) were recruited from the outpatient clinic for movement disorders at the VU University Medical Center (VUMC). Parkinson's disease patients were recruited pseudo-randomly as they were selected on the basis of their (subjective) disease duration and use of medication and concurrently classified into two groups; *de novo* Parkinson's disease patients (untreated, subjective disease duration <2 years) and early to moderately advanced Parkinson's disease (all other patients with a subjective disease duration <14 years). A group of 21 self-declared healthy controls, who were age-matched to the *de novo* Parkinson's disease patients, was composed of spouses of the patients as well as of other healthy volunteers. All subjects were screened for neurological disorders by a trained physician. Exclusion criteria for Parkinson's disease patients were stereotactic surgery in the past and the current use of psychoactive compounds such as antipsychotics, antidepressants, anticholinergics, cholinesterase inhibitors, benzodiazepines and stimulants. Eleven Parkinson's disease patients were excluded from further analysis due to irremediable artefacts during MEG registration, usually caused by dental implants. Two other Parkinson's disease patients were excluded on the basis of MRI findings: one patient with extensive, probably ischaemic cerebral white matter lesions in frontal and parietal areas and a patient with a tumour of the pituitary gland. Additionally, one patient exhibited cognitive deficits interfering with activities of daily living (ADL) and was as such suspected of (developing) dementia, leaving a total of 21 controls and 70 Parkinson's disease patients, including 18 *de novo* and 37 levodopa-treated Parkinson's disease patients, for further analysis.

Subject characteristics

Level of education was determined using a standard Dutch educational scale [SOI-2003 (Centraal Bureau voor Statistiek, 2003)] which was subsequently converted to the International Standard Classification of Education [ISCED-1997 (UNESCO, 1997)]. Verbal IQ was measured using the Dutch version of the National Adult Reading Test [NART (Nelson and O'Connell, 1978; Schmand *et al.*, 1991)]. The NART yields a good estimate of the pre-morbid level of intelligence of cerebrally damaged patients (Bright *et al.*, 2002). Global cognitive function and the presence of dementia were examined using the CAMCOG scale (Roth *et al.*, 1986; Hobson and Meara, 1999). None of the patients fulfilled DSM-IV-TR clinical diagnostic criteria for dementia (American Psychiatric Association, 2000), which was also evidenced by scores above 78 out of a total of 107 points on the CAMCOG scale. Disease duration was calculated on the basis of the patients' subjective estimate of the time of occurrence of the first motor symptoms, which has been shown to have high convergent reliability both with medical records as well as with an estimate derived from a structured face-to-face interview by a medical professional (Reider *et al.*, 2003). Side of onset was based on the body-half in which the first motor symptoms occurred. Unified Parkinson's Disease Rating Scale [UPDRS-III, (Fahn *et al.*, 1987)] motor scores and modified Hoehn and Yahr stages (Jankovic *et al.*, 1990) were obtained by a trained physician. Additionally, total tremor score was determined by summing scores on tremor-related items of the UPDRS-III (items 20 and 21). The total daily dose of dopaminomimetics was converted to a so-called levodopa equivalent daily dose (LEDD) using the following conversion rate: 100 mg levodopa equalling 125 mg levodopa sustained release, 1 mg pergolide, 1 mg pramipexol or 6 mg ropinirole. Additionally, 5% was added to the total levodopa dose for every 5 mg of selegiline, up to a maximum of 10%. Levodopa was always used in combination with a peripheral decarboxylase inhibitor. None of the patients had severe motor response fluctuations or exhibited serious dyskinesias during testing. All subjects gave written informed consent to the research protocol, which was approved by the local medical ethical committee of the VUMC. Ethics review criteria conformed to the Helsinki declaration. Subject characteristics are listed in Table 1.

MRI

MR imaging on a Siemens Magnetom Impact Expert 1.0 T system (Siemens AG, Erlangen, Germany), using an MP-RAGE and fast-FLAIR scanning sequence, was performed in all subjects in an attempt to exclude other potential causes of parkinsonism as well as major brain pathology such as tumours and relatively large cerebrovascular lesions [early confluent or confluent, Fazekas score higher than 1 (Fazekas *et al.*, 1987)]. Vascular lesions at the level of the basal ganglia on fast-FLAIR MR images were considered supportive of vascular parkinsonism. A hypointense putamen with a hyperintensive rim and/or hyperintensities in the brainstem on fast-FLAIR images were considered supportive of multiple system atrophy. All MR images were reviewed by a radiologist specialized in neuroradiology.

Specific neuropsychological evaluation

Specific cognitive, mainly executive (frontal) functions were assessed using a comprehensive set of neuropsychological tasks. Six tasks were taken from the Cambridge Neuropsychological Test Automated Battery (CANTAB Eclipse 2.0, Cambridge Cognition, Cambridge, UK), a series of extremely well standardized and validated computerized paradigms. This battery includes an introductory motor screening task which acts as a training procedure for the other tasks and yields measures of both speed and accuracy that provide an index of the subjects' motor skill. Other tasks included in the battery are spatial span (spatial short-term memory), spatial working memory (working memory, strategy), stockings of Cambridge (working memory, planning) and intra-dimensional/extra-dimensional shift (set-shifting). These tasks are known to be (selectively) sensitive to cognitive deficits in Parkinson's disease and Parkinson's disease-related dementia (for a review, see Robbins *et al.*, 2002). An additional two tasks were taken from the Vienna Test System version 6.0 (Dr G. Shuhfried

Table 1 Subject characteristics

	Controls ($N = 21$)	All Parkinson's disease patients ($N = 70$)	*De novo* Parkinson's disease patients ($N = 18$)	Levodopa-treated Parkinson's disease patients ($N = 37$)
Age (years, mean ± SD)	59.4 ± 7.3	62.1 ± 6.8	59.4 ± 7.9	63.5 ± 6.6
Sex (♂/♀)	11/10	40/30	12/6	18/19
Education (ISCED 0/1/2/3/4/5/6)	0/0/6/4/2/8/1	1/0/27/16/4/21/1	0/0/5/5/0/8/0	0/0/18/6/3/9/1
Verbal IQ (Dutch NART)	111.7 ± 9.4	107.7 ± 13.1	109.2 ± 11.2	106.6 ± 14.1
Global cognition (CAMCOG)	98.9 ± 4.2	97.0 ± 5.1	98.2 ± 4.7	96.5 ± 5.7
Disease duration (years, mean ± SD)	n.a.	5.5 ± 3.7	0.9 ± 0.5	8.0 ± 2.7
Side of onset (left/right)	n.a.	33/37	4/14	21/16
H&Y modified 'OFF' (1/1½/2/2½/3)	n.a.	14/1/29/18/8	9/1/7/1/0	1/0/16/12/8
UPDRS-III 'OFF' (mean ± SD)	0.6 ± 1.4	17.1 ± 6.9	13.1 ± 6.1	19.1 ± 6.8
UPDRS-III 'ON' (mean ± SD)	n.a.	n.a.	n.a.	12.6 ± 4.6
LEDD DA agonists (mg)	n.a.	171 ± 183	n.a.	285 ± 168
LEDD levodopa (mg)	n.a.	235 ± 288	n.a.	445 ± 252
LEDD total dose (mg)	n.a.	406 ± 423	n.a.	729 ± 329

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 modified = 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, DA = dopamine agonist; n.a. = not applicable.

GmbH, Mödling, Austria). To assess abstract reasoning, the Vienna version of Raven's standard progressive matrices was administered. This computerized version is particularly well suited for non-verbal assessment of the 'eductive' (analytical, reasoning) component of general intelligence (Raven *et al.*, 2000). To assess the amount of perseveration in the generation of random motor behaviour, the Vienna Perseveration Task was administered. This computerized version of the pointing task by Mittenecker (1958) is ideally suited for measuring the internal control of behaviour, since this task does not contain any external cues. Previous research in our department has shown the task to be sensitive to cognitive dysfunction in Parkinson's disease, even at the earliest clinical stage (Stoffers *et al.*, 2001). Verbal fluency was assessed using the 1 min categorical fluency test (animals) which is part of the CAMCOG scale (Roth *et al.*, 1986; Hobson and Meara, 1999).

MEG data acquisition

MEG data were acquired using a 151-channel whole-head radial gradiometer MEG system (CTF Systems Inc., Port Coquitlam, BC, Canada). Average distance between sensors in this system is 3.1 cm. Patients were seated in a magnetically shielded room (Vacuum-schmelze GmbH, Hanau, Germany). The recording pass-band was 0.25–125 Hz with a sample rate of 312.5 Hz. A third-order software gradient (Vrba *et al.*, 1999) was applied. Patients treated with levodopa were instructed to come to the hospital without taking their first morning dose of anti-Parkinson medication, which ensured that this registration was carried out at least 9 h after the last dose of dopaminomimetics (practically defined 'OFF'). To assess the acute effect of dopaminomimetics on spectral power, a second registration was performed in patients treated with levodopa at least 1 h after the normal morning dose of anti-Parkinson medication had been taken ('ON' state). At the beginning of the measurement, head position relative to the coordinate system of the helmet was recorded by leading small alternating currents through three position coils situated at the left and right pre-auricular points and the nasion on the subject's head. MEG was recorded in an eyes-closed resting-state condition and during the performance of two executive tasks. Task-related data were not analysed in the current study.

MEG data analysis

Three 13.083 s epochs per registration (sample rate 312.5 Hz; 4096 samples per epoch), free of significant artefacts involving multiple adjacent channels, were selected for further analysis by two of the investigators blinded for the clinical diagnosis (D.S. and J.L.W.B.). Of the original 151 channels, one channel above the left occipital region was excluded in all patients because of technical problems during the recordings. For further off-line processing and spectral power analysis epochs were converted to ASCII-files and imported into the DIGEEGXP 2.0 software package (C.J. Stam, Amsterdam, The Netherlands). Subsequently, the MEG data were digitally filtered off-line with a band-pass of 0.5–48 Hz. Relative spectral power was determined in the following frequency bands: 0.5–4 Hz (delta), 4–8 Hz (theta), 8–10 Hz (low alpha), 10–13 Hz (high alpha), 13–30 Hz (beta) and 30–48 Hz (gamma). The MEG channels were grouped into regions of interest (ROIs) roughly corresponding to the major cortical areas (frontal, central, temporal, parietal and occipital) on the left and right side of the brain. As ROIs were based on the extra-cranial position of the MEG sensors, underlying cortical areas are to be considered as indicative. The nine midline channels were left out of this clustering, leaving a total of 141 channels divided over 10 ROIs for analysis (Fig. 1). In a small number of subjects in both the control ($N = 5$) and patient ($N = 8$) groups, four channels showed artefacts of a technical nature during visual inspection of the epochs (i.e. dysfunctional super-conducting quantum interference device or channel amplifier, yielding a high frequency, high-amplitude signal). These channels were located in the left frontal and left central ($N = 7$ subjects), left occipital ($N = 5$ subjects) and right temporal ($N = 1$ subjects) ROIs. Power values for the respective individual channels in these particular subjects, as well as for the earlier mentioned left occipital MEG channel in all subjects, were left out of the averaging, ensuring that the mean relative power in the ROI containing the bad channel

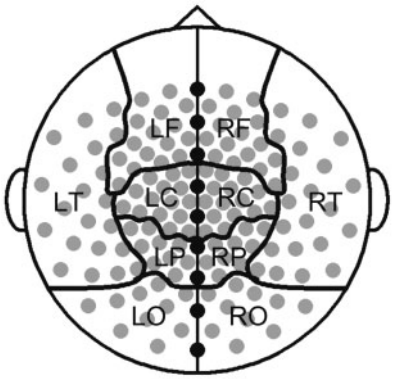

Fig. 1 Clustering of MEG sensors into regions of interest above the major cortical areas. Midline sensors (depicted in black) have been excluded from power spectral analysis. R = right, L = left, F = frontal, C = central, T = temporal, P = parietal, O = occipital.

was not distorted. Mean relative power for each ROI was used in the statistical analysis. Fast Fourier transformation was separately applied for every subject on the three epochs in the previously mentioned frequency bands. Results of the three epochs were averaged for each subject. Reported spectral power values are all relative.

Statistical analysis

Differences between Parkinson's disease patients and controls in the distribution of sex and education level were analysed by means of chi-square tests. All analyses with regard to group differences in age, pre-morbid IQ and CAMCOG scores were performed by means of univariate analysis of variance (ANOVA). To increase statistical power we attempted to normalize spectral power parameters by means of a transformation that is considered to be superior to other transformations when it comes to normalizing relative power in healthy controls (Gasser *et al.*, 1982); $\log[x/(1-x)]$. Unfortunately 10 out of 60 regional spectral power measures could not be sufficiently normalized in all groups by means of this transformation to pass Kolmogorov–Smirnov tests of normality. However, in the current study, we made use of ANCOVA testing with repeated measures. ANCOVA testing is usually not seriously confounded when the repeated measure (relative power in a ROI) does not follow the normal distribution in a limited number of levels (ROIs). More importantly, in our *post hoc* analyses of regional spectral power we made use of linear regression. This technique is rather robust when it comes to violations of the assumption of normality as long as there are (roughly) more than 10 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 18 observations per parameter and relative power usually does not result in extreme outliers we chose, in the interest of uniformity, to report parametric analyses of log-transformed power values only.

Since our initial exploratory analyses even showed abnormal spectral power in *de novo* Parkinson's disease patients, we chose to first compare spectral power in controls with *de novo* Parkinson's disease patients to show the effect of disease (experiment A) and, subsequently, to perform analyses of the relation of spectral power to disease duration, disease stage, disease severity and dose of dopaminomimetics within the whole group of Parkinson's disease patients (experiment B). The acute effects of levodopa administration (comparison of 'ON' and 'OFF' states) were analysed in levodopa-treated Parkinson's disease patients (experiment C). Principal component analysis (PCA) was used in all subjects to reduce the number of cognitive parameters to be used in the assessment of the relation of spectral power with cognitive performance (experiment D). Subsequent analyses were limited to *de novo* patients, to exclude confounding effects of medication on task performance. Relations between cognitive performance and spectral power were only analysed for cognitive components, frequency bands and ROIs that showed differences between *de novo* Parkinson's disease patients and controls. This approach further reduces the number of analyses, in this way reducing the likelihood of type-I statistical errors. All analyses were performed at a significance level of 0.05 (two-tailed) using the SPSS 14.0.2 software package (SPSS Inc., Chicago, IL, USA). Partial eta squared (η^2) was calculated when performing ANCOVA. Coefficients for relevant determinants when performing regression analysis were standardized and subsequently squared (β^2). Both η^2 as well as β^2 represent the proportion of the total variability in the dependent variable (relative power) that is accounted for by the relevant determinant, when controlling for all other covariants/determinants in the ANCOVA model or regression equation. Throughout the paper, η^2 and β^2 are expressed as a percentage of the total variance.

Experiment A: For each frequency band, ANCOVA with repeated measures was performed, using Greenhouse–Geiser corrected *P*-values for interaction effects. The repeated measures factor had 10 levels (relative power in the frontal, central, temporal, parietal and occipital ROI for the left and right side of the brain). The between-subjects factor had two levels (*de novo* Parkinson's disease patients and controls; effect of disease). Relevant confounders were used as covariates. In case of a significant main effect for group or an interaction effect of group with ROI, subsequent *post hoc* analyses with regard to regional differences in spectral power between *de novo* Parkinson's disease patients ($N = 18$) and controls ($N = 21$) were performed by means of linear regression using each of the regional spectral power measures as dependent and group membership (effect of disease) and any relevant confounders as determinants.

Experiment B: Analyses of the relation of spectral power with disease parameters in Parkinson's disease patients ($N = 70$) involved a separate ANCOVA with repeated measures for each frequency band, using Greenhouse–Geiser corrected *P*-values for interaction effects. The repeated measures factor was identical to experiment A. Relevant confounders as well as disease duration (effect of disease duration), modified Hoehn and Yahr stage in the 'OFF' medication phase (effect of disease stage), UPDRS motor score in the 'OFF' medication phase (effect of disease severity) or LEDD (effect of dose of dopaminomimetics) were used as covariates. In case of a significant main effect for a disease parameter or an interaction effect for the disease parameter with ROI, subsequent *post hoc* analyses of the relation of regional spectral power with disease parameters were performed by means of linear regression using each of the regional spectral power measures in the 'OFF' medication phase as dependent and either disease duration, modified Hoehn and Yahr stage in the 'OFF' medication phase, UPDRS motor score in the 'OFF' medication phase or LEDD as well as any relevant confounders as determinants. Relations of total

tremor score (UPDRS motor sub-score) with spectral power were explored using Spearman's non-parametric correlation analysis, as exploratory scatterplots showed severe non-normality with outliers in the *X*–*Y* data.

Experiment C: Analyses to determine the immediate modulatory effect of dopaminomimetic drugs on power values (effect of acute dopaminomimetic challenge) in levodopa-treated Parkinson's disease patients ($N = 37$) were performed by means of Student's paired *t*-tests.

Experiment D: Analyses to reduce the number of cognitive parameters were performed by means of PCA with varimax rotation and Kaiser normalization in all subjects ($N = 91$) on the following 11 variables: span length (spatial span), between search errors (spatial working memory), strategy (spatial working memory), mean initial thinking time on five move problems (stockings of Cambridge), mean subsequent thinking time on five moves (stockings of Cambridge), problems solved in five moves (stockings of Cambridge) stages completed (intra-dimensional/extra-dimensional shift), adjusted total errors (intra-dimensional/extra-dimensional shift), redundancy of the second order (Vienna perseveration), analytical IQ (Raven's standard progressive matrices) and category fluency (CAMCOG). All analyses with regard to differences in cognitive performance between *de novo* Parkinson's disease patients ($N = 18$) and controls ($N = 21$) were analysed by means of linear regression using each of the cognitive components from the PCA as dependent and group membership (effect of disease) and any relevant confounders as determinants. All analyses of the relation of spectral power with cognitive performance in Parkinson's disease patients involved a separate ANCOVA with repeated measures for each frequency band, using Greenhouse–Geiser corrected *P*-values for interaction effects. The repeated measures factor was identical to experiment A. Relevant confounders as well as one of the cognitive components from the PCA (relation with cognitive function) were used as covariates. In case of a significant main effect for a cognitive parameter or an interaction effect for a cognitive parameter with ROI, subsequent *post hoc* analyses of the relation of cognitive performance with regional spectral power involved linear regression using each of the regional spectral power measures in the 'OFF' medication phase as dependent and a cognitive component from the principal component analysis (relation with cognitive function) and any relevant confounders as determinants.

Results

Subject characteristics and confounders

There were no differences in the distribution of sex or education level over the various groups (controls, all Parkinson's disease patients, *de novo* Parkinson's disease patients, levodopa-treated Parkinson's disease patients). There were no significant differences in age, pre-morbid IQ (NART) or global cognitive function (CAMCOG) between groups. Since age is known to be a modifier of spectral power (Van Sweden *et al.*, 1999), it was nonetheless added as a covariate/determinant in all analyses of spectral power in experiments A, B and D, as was sex. Since age and pre-morbid IQ are known to be modifiers of cognitive performance, they were added as determinant in analyses of differences in cognitive performance between patients and controls in experiment D, as was sex.

Experiment A: effect of disease

ANCOVA testing showed *de novo* Parkinson's disease patients to have higher power than controls in the theta ($P = 0.004$, $\eta^2 = 21.7\%$) and low alpha ($P < 0.001$, $\eta^2 = 35.4\%$) frequency bands and lower power in the beta ($P < 0.001$, $\eta^2 = 32.4\%$) and gamma ($P < 0.001$, $\eta^2 = 29.8\%$) frequency bands. No differences in spectral power between controls and *de novo* Parkinson's disease patients were found in the delta and high alpha frequency bands. An interaction for group with ROI was observed in the low alpha ($P = .007$, $\eta^2 = 11.0\%$), high alpha ($P = 0.036$, $\eta^2 = 7.7\%$) and beta ($P = 0.014$, $\eta^2 = 10.7\%$) frequency bands. *Post hoc* regression analysis showed *de novo* Parkinson's disease patients to have higher power than controls in all 10 cortical areas in the theta and low alpha band. Beta power was significantly lower in *de novo* Parkinson's disease patients when compared to controls in all but the frontal ROIs. In the gamma band, lower power was found in all but the right occipital ROI, when comparing *de novo* Parkinson's disease patients to controls. No differences in regional spectral power between controls and *de novo* Parkinson's disease patients were found in the delta and high alpha frequency bands. For means and standard deviations of regional relative spectral power values and accompanying statistics see Table 2, for a graphical depiction see Fig. 2. The overall changes in relative power can be appreciated from a line chart showing global relative spectral power density for *de novo* Parkinson's disease patients, age-matched controls as well as the full group of Parkinson's disease patients (Fig. 3).

Experiment B: effect of disease duration, disease stage, disease severity and effect of dose of dopaminomimetics

ANCOVA testing revealed no main effects of disease duration in any frequency band. An interaction of disease duration with ROI was observed in the low alpha ($P = 0.023$, $\eta^2 = 4.4\%$) frequency band. Subsequent *post hoc* regression analyses showed an effect of disease duration on regional spectral power in two ROIs in the low alpha band: relative spectral power decreased with increasing disease duration in both the right temporal ($b = -0.020$, 95%CI $= -0.038$ to -0.003, $P = 0.026$, $\beta^2 = 7.12\%$) and right occipital ($b = -0.023$, 95% CI $= -0.041$ to -0.004, $P = 0.016$, $\beta^2 = 8.18\%$) ROIs (Fig. 4A and B). ANCOVA testing showed no main or interaction effects of disease stage, as assessed by the modified Hoehn and Yahr scale, or disease severity, quantified using the total score of the motor section of the UPDRS, on spectral power values in any frequency band. Furthermore, total tremor score

Table 2 Means ± SDs and accompanying statistics of relative spectral power values in the six frequency bands for controls and *de novo* Parkinson's disease patients in experiment A

Frequency band	Area	Controls ($N = 21$)	*De novo* Parkinson's disease patients ($N = 18$)	Statistic			
				b	95%CI	*P*	β^2
Delta (0.5–4 Hz)	L frontal	0.351 ± 0.101	0.314 ± 0.129	−0.061	−0.212 to 0.090	0.416	1.79
	R frontal	0.338 ± 0.099	0.314 ± 0.140	−0.036	−0.192 to 0.119	0.639	0.59
	L central	0.211 ± 0.073	0.198 ± 0.067	−0.024	−0.141 to 0.104	0.711	0.36
	R central	0.198 ± 0.066	0.190 ± 0.074	−0.014	−0.135 to 0.108	0.821	0.13
	L temporal	0.318 ± 0.076	0.271 ± 0.085	−0.104	−0.217 to 0.010	0.072	8.82
	R temporal	0.310 ± 0.089	0.283 ± 0.122	−0.064	−0.208 to 0.079	0.368	2.28
	L parietal	0.166 ± 0.067	0.147 ± 0.063	−0.066	−0.220 to 0.088	0.389	2.16
	R parietal	0.168 ± 0.070	0.143 ± 0.060	−0.086	−0.241 to 0.068	0.263	3.65
	L occipital	0.175 ± 0.076	0.185 ± 0.104	0.006	−0.168 to 0.179	0.949	0.01
	R occipital	0.178 ± 0.066	0.187 ± 0.100	−0.006	−0.167 to 0.156	0.941	0.02
Theta (4–8 Hz)	L frontal	**0.138 ± 0.029**	**0.187 ± 0.064**	**0.136**	**0.033 to 0.239**	**0.011**	**16.24**
	R frontal	**0.138 ± 0.032**	**0.186 ± 0.055**	**0.135**	**0.042 to 0.229**	**0.006**	**17.98**
	L central	**0.131 ± 0.033**	**0.170 ± 0.055**	**0.115**	**0.003 to 0.227**	**0.044**	**10.98**
	R central	**0.123 ± 0.031**	**0.161 ± 0.050**	**0.116**	**0.009 to 0.223**	**0.035**	**11.08**
	L temporal	**0.120 ± 0.026**	**0.181 ± 0.071**	**0.179**	**0.079 to 0.279**	**0.001**	**25.20**
	R temporal	**0.124 ± 0.032**	**0.168 ± 0.066**	**0.129**	**0.026 to 0.232**	**0.016**	**13.76**
	L parietal	**0.109 ± 0.032**	**0.156 ± 0.065**	**0.152**	**0.033 to 0.272**	**0.014**	**14.98**
	R parietal	**0.100 ± 0.025**	**0.152 ± 0.065**	**0.164**	**0.044 to 0.285**	**0.009**	**16.24**
	L occipital	**0.102 ± 0.034**	**0.167 ± 0.082**	**0.199**	**0.077 to 0.321**	**0.002**	**19.71**
	R occipital	**0.105 ± 0.033**	**0.159 ± 0.068**	**0.177**	**0.064 to 0.290**	**0.003**	**19.62**
Low alpha (8–10 Hz)	L frontal	**0.077 ± 0.041**	**0.116 ± 0.049**	**0.187**	**0.048 to 0.326**	**0.010**	**16.81**
	R frontal	**0.071 ± 0.020**	**0.111 ± 0.057**	**0.170**	**0.027 to 0.312**	**0.021**	**14.44**
	L central	**0.096 ± 0.059**	**0.159 ± 0.061**	**0.267**	**0.122 to 0.413**	**0.001**	**26.83**
	R central	**0.092 ± 0.039**	**0.171 ± 0.075**	**0.304**	**0.167 to 0.441**	**<0.001**	**34.57**
	L temporal	**0.112 ± 0.072**	**0.215 ± 0.078**	**0.365**	**0.209 to 0.521**	**<0.001**	**38.31**
	R temporal	**0.113 ± 0.060**	**0.223 ± 0.096**	**0.356**	**0.187 to 0.526**	**<0.001**	**33.53**
	L parietal	**0.141 ± 0.101**	**0.277 ± 0.118**	**0.400**	**0.214 to 0.585**	**<0.001**	**33.29**
	R parietal	**0.144 ± 0.098**	**0.297 ± 0.123**	**0.436**	**0.244 to 0.627**	**<0.001**	**35.40**
	L occipital	**0.158 ± 0.105**	**0.277 ± 0.118**	**0.347**	**0.154 to 0.539**	**0.001**	**26.73**
	R occipital	**0.159 ± 0.107**	**0.277 ± 0.123**	**0.337**	**0.140 to 0.533**	**0.001**	**24.80**
High alpha (10–13 Hz)	L frontal	0.077 ± 0.025	0.082 ± 0.029	0.022	−0.084 to 0.128	0.678	0.46
	R frontal	0.084 ± 0.026	0.080 ± 0.032	−0.052	−0.177 to 0.073	0.401	1.85
	L central	0.110 ± 0.035	0.116 ± 0.036	0.027	−0.078 to 0.131	0.610	0.76
	R central	0.122 ± 0.109	0.112 ± 0.038	−0.045	−0.154 to 0.063	0.403	1.98
	L temporal	0.117 ± 0.035	0.103 ± 0.101	−0.064	−0.172 to 0.043	0.230	3.96
	R temporal	0.125 ± 0.037	0.101 ± 0.037	−0.105	−0.214 to 0.003	0.057	9.55
	L parietal	0.173 ± 0.068	0.155 ± 0.075	−0.064	−0.209 to 0.080	0.373	2.25
	R parietal	0.177 ± 0.062	0.147 ± 0.062	−0.093	−0.225 to 0.038	0.159	5.34
	L occipital	0.178 ± 0.084	0.130 ± 0.057	−0.143	−0.287 to 0.000	0.050	9.86
	R occipital	0.171 ± 0.086	0.128 ± 0.055	−0.124	−0.265 to 0.018	0.086	7.34
Beta (13–30 Hz)	L frontal	0.283 ± 0.089	0.247 ± 0.074	−0.091	−0.218 to 0.035	0.152	5.76
	R frontal	0.294 ± 0.089	0.256 ± 0.084	−0.111	−0.263 to 0.040	0.146	5.76
	L central	**0.380 ± 0.094**	**0.310 ± 0.078**	**−0.133**	**−0.240 to −0.026**	**0.016**	**14.06**
	R central	**0.391 ± 0.098**	**0.317 ± 0.083**	**−0.141**	**−0.249 to −0.032**	**0.013**	**14.52**
	L temporal	**0.264 ± 0.058**	**0.188 ± 0.057**	**−0.191**	**−0.289 to −0.093**	**<0.001**	**29.70**
	R temporal	**0.268 ± 0.069**	**0.185 ± 0.061**	**−0.212**	**−0.328 to −0.097**	**0.001**	**27.87**
	L parietal	**0.358 ± 0.073**	**0.238 ± 0.078**	**−0.250**	**−0.356 to −0.144**	**<0.001**	**34.93**
	R parietal	**0.358 ± 0.078**	**0.232 ± 0.080**	**−0.269**	**−0.380 to −0.157**	**<0.001**	**40.96**
	L occipital	**0.300 ± 0.073**	**0.193 ± 0.057**	**−0.253**	**−0.361 to −0.144**	**<0.001**	**38.56**
	R occipital	**0.305 ± 0.071**	**0.193 ± 0.055**	**−0.262**	**−0.361 to −0.163**	**<0.001**	**44.49**
Gamma (30–48 Hz)	L frontal	**0.075 ± 0.028**	**0.053 ± 0.017**	**−0.149**	**−0.259 to −0.039**	**0.009**	**17.98**
	R frontal	**0.075 ± 0.028**	**0.053 ± 0.021**	**−0.181**	**−0.318 to −0.045**	**0.011**	**17.39**
	L central	**0.071 ± 0.026**	**0.046 ± 0.016**	**−0.186**	**−0.294 to −0.079**	**0.001**	**24.70**
	R central	**0.074 ± 0.024**	**0.047 ± 0.018**	**−0.202**	**−0.311 to −0.092**	**0.001**	**27.56**
	L temporal	**0.069 ± 0.032**	**0.050 ± 0.015**	**−0.219**	**−0.344 to −0.094**	**0.001**	**25.50**
	R temporal	**0.060 ± 0.026**	**0.040 ± 0.020**	**−0.206**	**−0.350 to −0.061**	**0.007**	**19.36**
	L parietal	**0.053 ± 0.021**	**0.029 ± 0.014**	**−0.283**	**−0.431 to −0.134**	**<0.001**	**27.98**
	R parietal	**0.054 ± 0.020**	**0.029 ± 0.015**	**−0.284**	**−0.425 to −0.143**	**<0.001**	**31.25**
	L occipital	**0.086 ± 0.065**	**0.049 ± 0.029**	**−0.242**	**−0.468 to −0.016**	**0.037**	**11.97**
	R occipital	0.083 ± 0.052	0.055 ± 0.034	−0.200	−0.408 to 0.007	0.057	10.11

Significant differences between *de novo* Parkinson's disease patients and controls are indicated in bold. L = left, R = right; *b* = regression coefficient, 95% CI = 95% confidence interval of *b*, *P* = probability of finding the present or a more extreme result, assuming the null hypothesis is true, β^2 = explained variance (%).

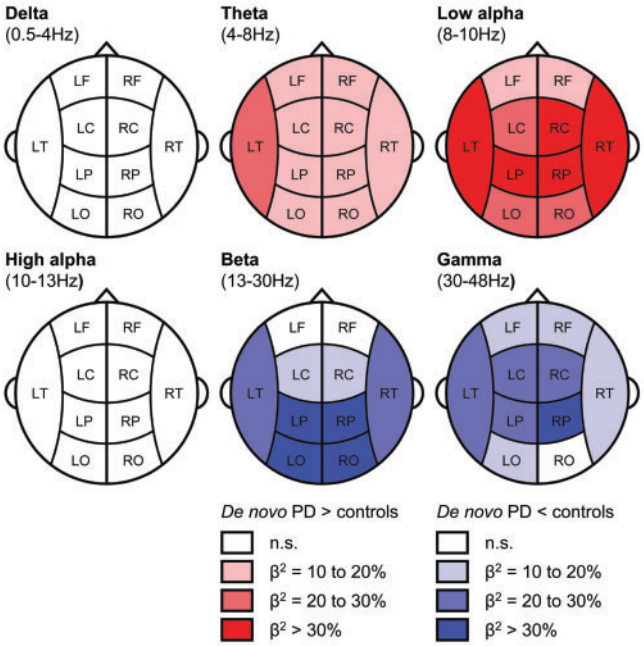

Fig. 2 Schematic view of differences in relative spectral power in experiment A in the various frequency bands and regions of interest between *de novo* Parkinson's disease patients ($N = 18$) and age-matched controls ($N = 21$). Colour indicates direction of effect, colour intensity signifies magnitude of effect (variance explained by group membership in the regression equation, which further included sex and age as determinants). R = right, L = left, F = frontal, C = central, T = temporal, P = parietal, O = occipital, n.s. = not significant, β^2 = explained variance (%).

(UPDRS motor sub-score) and spectral power were unrelated, as determined using Spearman's non-parametric correlation analysis. Lastly, no main or interaction effects were found between LEDD and spectral power.

Experiment C: effect of an acute dopaminomimetic challenge

When comparing the 'ON' state with the 'OFF' state in levodopa-treated patients, we found only a slight modulatory effect of levodopa administration on spectral power. Changes were limited to decreases of relative power in right frontal theta ($P = 0.048$), left occipital beta ($P = 0.015$) and left temporal gamma ($P = 0.003$), and an increase in right parietal gamma power ($P = 0.048$) after intake of dopaminomimetics.

Experiment D: relation with cognitive function

PCA with varimax rotation in the complete group of subjects ($N = 91$) yielded four components. Based on the factor loadings of the various tasks on the individual components, these could in our opinion best be attributed to four cognitive (executive) functions: strategy/analysis, set-shifting, planning/spatial memory and perseveration (Table 3). Subsequent linear regression analyses showed *de novo* Parkinson's disease patients to have a lower capacity for planning/spatial memory ($b = 0.404$, 95% CI = 0.038 to 0.770, $P = 0.031$, $\beta^2 = 11.76\%$) and higher perseveration ($b = -0.705$, 95% CI = −1.335 to −0.075, $P = 0.029$, $\beta^2 = 11.76\%$) compared to controls. No significant differences in performance between *de novo* Parkinson's disease patients and controls were found with regard to strategy/analysis or set-shifting. In the group of *de novo* Parkinson's disease patients, ANCOVA testing revealed a main effect of perseveration on spectral power ($P = 0.049$, $\eta^2 = 24.8\%$). Subsequent *post hoc* regression analyses showed increased perseveration in *de novo* Parkinson's disease patients to be associated with higher low alpha power in left central ($b = -0.086$, 95% CI = −0.162 to −0.010, $P = 0.030$, $\beta^2 = 27.56\%$), right central ($b = -0.102$, 95% CI = −0.193 to −0.010, $P = 0.031$ $\beta^2 = 27.66\%$), left parietal ($b = -0.108$, 95% CI = −0.209 to −0.007, $P = 0.038$, $\beta^2 = 24.70\%$) and right parietal ($b = -0.124$, 95% CI = −0.230 to −0.018, $P = 0.025$, $\beta^2 = 27.77\%$) ROIs (Fig. 5). ANCOVA testing did not show any effects of planning/working memory on spectral power.

Discussion

In a previous MEG study using similar methodology to the current, we compared resting-state oscillatory brain activity in groups of demented ($N = 13$) and non-demented ($N = 13$) Parkinson's disease patients matched with regard to age, sex and disease duration, as well as in 13 age- and sex-matched controls (Bosboom *et al.*, 2006). This study showed extensive changes in oscillatory brain activity in non-demented Parkinson's disease patients at moderately advanced stages of disease relative to controls, which included an increase in theta power over all but the frontal ROIs as well as decreases in mainly posterior beta and centroparietal gamma power. When comparing demented to non-demented Parkinson's disease patients, the most conspicuous changes were a diffuse increase in delta power and a diffuse decrease in alpha power in the demented patients. Furthermore, in the demented Parkinson's disease patients, we observed an additional increase in theta power restricted to the left frontal, bilateral central and right parietal ROIs as well as further widespread decreases in beta power and a decrease in frontal gamma power. Since our previous study involved only moderately advanced, non-demented Parkinson's disease patients who had already been on dopaminomimetic treatment for many years, it was at that point unclear whether slowing of resting-state oscillatory brain activity is an early stage characteristic of Parkinson's disease or a phenomenon that develops over the course of the disease, as a result of disease progression and/or (prolonged) exposure to dopaminergic agents.

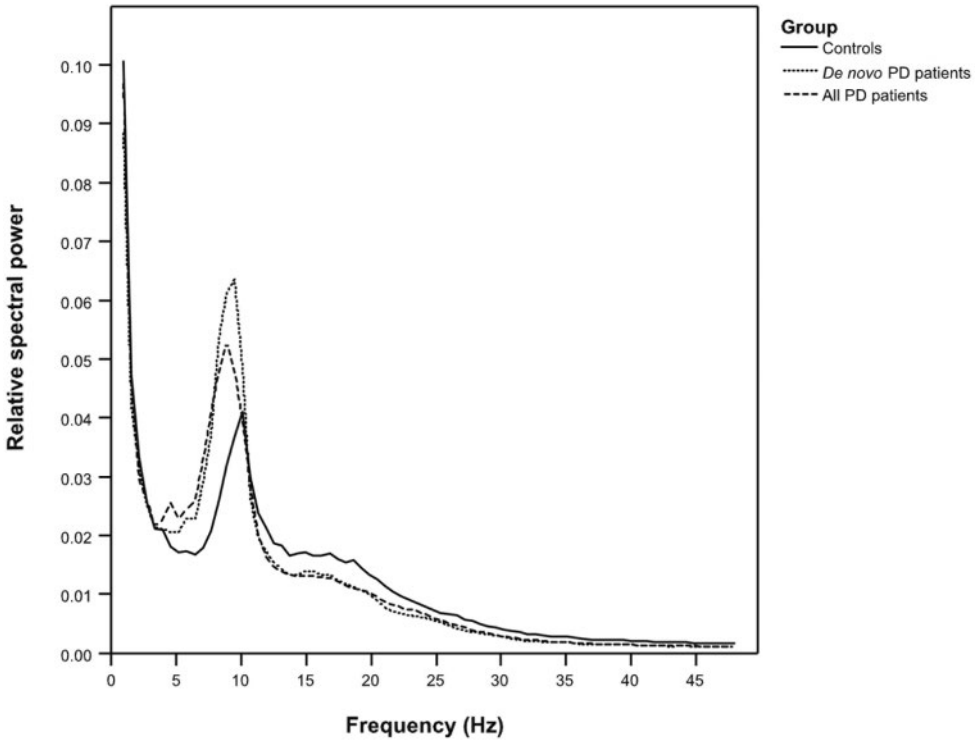

Fig. 3 Line chart showing relative spectral power averaged over all MEG sensors for *de novo* Parkinson's disease patients ($N = 18$), controls ($N = 21$) and the full group of Parkinson's disease patients ($N = 70$) in the 0.5 to 48 Hz frequency range. Note the differences in frequency distribution between controls and *de novo* Parkinson's disease patients as well as the similarities between *de novo* Parkinson's disease patients and the full group of Parkinson's disease patients.

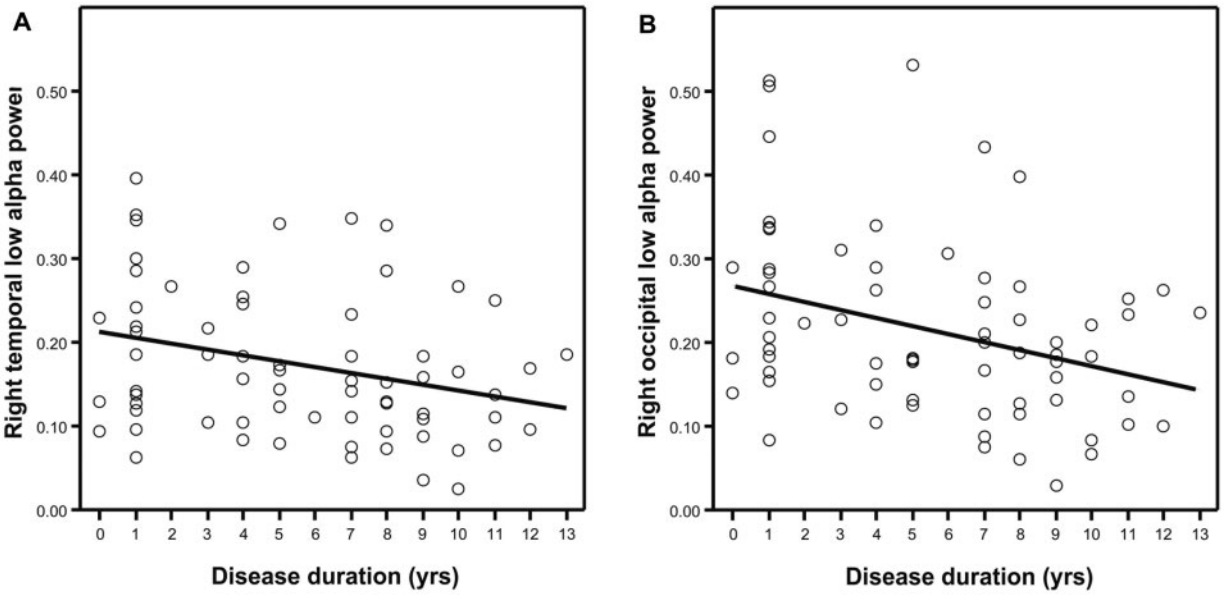

Fig. 4 Scatterplots showing relative spectral power in the low alpha band in the right temporal (**A**) and right occipital (**B**) regions of interest set out against disease duration in all Parkinson's disease patients ($N = 70$). In the *post hoc* regression analyses, which further included age and sex as determinants, disease duration explained 7.12% of the variance of spectral power in the right temporal region of interest and 8.18% of the variance in the right occipital region of interest.

Table 3 Factor loadings of the individual tests on the four components extracted in the principal component analysis with varimax rotation in all subjects ($N = 91$) in experiment C

Cognitive test variable	Component			
	1	2	3	4
SSP span length	**0.705**	0.020	−0.218	0.005
SWM between errors	**−0.880**	−0.072	−0.062	−0.044
SWM strategy	**−0.826**	−0.110	0.019	0.031
SOC mean initial thinking time (five moves)	0.066	0.160	**0.863**	0.011
SOC mean subsequent thinking time (five moves)	−0.289	−0.388	**0.619**	−0.065
SOC problems solved in minimum moves	0.481	0.069	−0.160	**0.572**
IED total errors (adjusted)	−0.120	**−0.955**	−0.023	−0.123
IED stages completed	0.105	**0.965**	−0.023	0.047
Vienna Raven SPM	**0.519**	0.321	0.236	0.225
Vienna perseveration	0.109	−0.070	−0.078	**−0.886**
Category fluency	**0.423**	0.178	−0.097	0.343

The highest factor loading per test has been indicated in bold. Component 1: strategy/analysis; 2: set-shifting; 3: planning/spatial memory; 4: perseveration. SSP = spatial span, SWM = spatial working memory, SOC = stockings of Cambridge, IED = intra/extra-dimensional shift, SPM = standard progressive matrices.

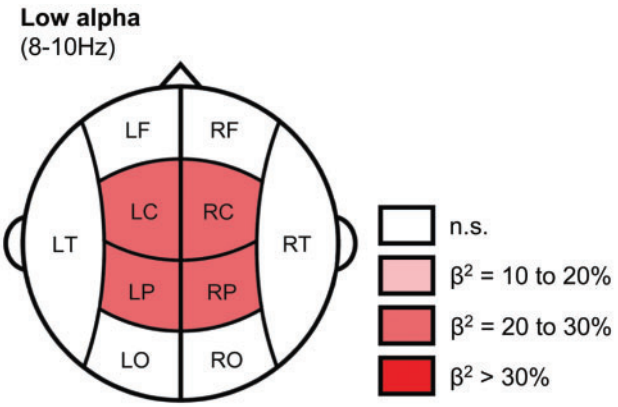

Fig. 5 Schematic view showing the location in MEG sensor space and magnitude of associations between relative spectral power in the low alpha band and the degree of cognitive perseveration in *de novo* Parkinson's disease patients ($N = 18$). Colour intensity signifies magnitude of effect (variance explained by cognitive perseveration in the regression equation, which further included sex and age as determinants). R = right, L = left, F = frontal, C = central, T = temporal, P = parietal, O = occipital, n.s. = not significant, β^2 = explained variance (%).

In the present whole-head MEG study, *de novo*, untreated Parkinson's disease patients showed considerable slowing of resting-state oscillatory brain activity compared to healthy controls, consisting of a widespread increase in theta and low alpha power, a slight change in the cortical distribution of high alpha power, as well as a loss of beta power over all but the frontal ROIs and a loss of gamma power over all but one ROI. Based on the results of the analysis in our whole group of Parkinson's disease patients, these changes in relative power values appear to be (largely) unrelated to disease duration, disease stage, disease severity and LEDD. Interestingly, power values in levodopa-treated patients were hardly modulated by an acute dopaminergic challenge. In addition, we found indications that an aspect of cognitive dysfunction in *de novo* Parkinson's disease patients is associated with increased low alpha power in central and parietal ROIs.

Contrary to our own results, most studies have been unable to show evidence of (pronounced) slowing of resting-state brain activity in non-demented Parkinson's disease patients. Several factors might account for this discrepancy. First, we used MEG instead of EEG in the current study. EEG measures the electrical derivative of underlying brain activity, which is based on the difference in potentials between an EEG electrode and a reference electrode. This signal is severely influenced by the electric conductivity of intermediate structures, especially the skull. MEG measures absolute magnetic flux, the magnetic derivative of electrical brain activity. Contrary to electricity, the magnetic fields measured with MEG pass through intermediate structures almost undisturbed. In addition, MEG is a reference-free technique that employs a higher sensor density than conventional EEG; our study uses 141 sensors as opposed to a maximum of 20 EEG electrodes in previous EEG studies. As a consequence of the resulting superior spatial resolution and signal-to-noise ratio of MEG compared to EEG, it might very well pick up changes of oscillatory brain activity not evident in the EEG signal. Another factor is that we used log-transformed relative power combined with advanced statistical techniques in the current study, which can significantly increase statistical power, especially in MEG studies.

The question may arise whether the eyes-closed resting state is the most effective condition for demonstrating changes in oscillatory brain activity in Parkinson's disease and, especially, their relation with indices of cognitive and motor function. For instance, the results of an EEG study using coherence and a visuomotor tracking task suggest that task-induced EEG changes might increase the discrimination between controls and Parkinson's disease

patients (Cassidy *et al.*, 2001). A number of recent fMRI studies, however, have shown that the resting state is a far more stable and active condition than has often been assumed (Gusnard and Raichle, 2001). The resting state is characterized by the activation of a 'default' network, which consists of frontal, posterior cingulate, parietal and medial temporal areas (Laufs *et al.*, 2003). Using MEG, abnormalities of resting-state network activity have been demonstrated in demented and non-demented Parkinson's disease patients (Bosboom *et al.*, 2006), multiple sclerosis (Cover *et al.*, 2006) and Alzheimer's disease (Stam *et al.*, 2006). The results of the present study further strengthen the idea that a simple resting-state condition is sufficient to demonstrate widespread changes in oscillatory brain dynamics in diseases of the brain. Obviously, this does not imply that the use of specific motor and cognitive tasks, aimed at activating brain areas assumed to be involved in Parkinson's disease, is not useful at all. However, brain pathology may be associated with either abnormally high or abnormally low task-related activation, thus seriously complicating the interpretation of the results of task-related data.

Parkinsonian tremor generally has a frequency in the theta range (4–7 Hz). Timmermann and co-workers reported coherence between tremor, measured with EMG, and several MEG oscillatory rhythms in the contralateral motor cortex (Timmermann *et al.*, 2003). In theory, these area-specific coherent oscillations could influence spectral power values. Since the observed theta power changes in the present study 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 through movement artefacts influencing power in the theta band. However, in our patients, UPDRS tremor scores did not correlate with MEG activity in any frequency band, most notably not in the theta band. Lastly, epochs were carefully selected for the absence of visible tremor. Taken together, we believe that it is highly unlikely that tremor could account for the differences between patients and controls observed in the theta band.

The use of dopaminergic medication is theoretically a confounding factor when performing analyses in controls and untreated Parkinson's disease patients on the one hand and the whole group of Parkinson's disease patients, including those dopaminomimetically treated, on the other. To minimize the potential effects of antiparkinsonian medication on the results of this study, MEG recordings in dopaminomimetically treated Parkinson's disease patients were obtained in a practically defined 'OFF' state, i.e. at least 9 h after the last dose of medication. In spite of this, effects of dopaminergic treatment may not have been fully abolished in this way, in particular in patients on dopamine agonists with a long half-life. Although these effects for obvious reasons have not influenced our finding of substantial power differences between controls and untreated *de novo* patients, they may have masked a stronger or more widespread correlation between disease duration and spectral power changes. On the other hand, the lack of major differences between 'ON' and 'OFF' registrations would argue against a significant confounding influence of dopaminergic treatment.

We chose to use relative power in the present study. As opposed to absolute power, relative power is not influenced by the distance between the MEG sensor and underlying neural populations. This distance is highly variable over individual subjects due to factors such as skull thickness and head position within the MEG helmet. Using relative power rather than absolute power results in a lower variance of power values within subject groups, which will substantially increase statistical power when making group comparisons. A drawback of using relative power is that it can complicate interpretation when making group comparisons. Relative power in a specific frequency band is influenced by the power in other bands; therefore group differences in relative power in a specific frequency band might not result from changes in absolute power in that frequency band *per se*, but rather from changes in absolute power in other frequency bands. Differences in relative power between groups within a specific frequency band should therefore always be interpreted with that proviso in mind.

In the present study, we adopted a pragmatic approach with regard to our spatial locations of interest, restricting the analyses to the actually recorded signal of the MEG sensors ('sensor space') and clustering the MEG sensors into 10 cortical ROIs based on their approximate position relative to the major cortical brain regions. An alternative method is the use of source modelling techniques such as synthetic aperture magnetometry, enabling investigation of the distribution of reconstructed sources over the various cortical regions ('source space'). However, no unique way exists to reconstruct the sources, and results from different techniques can show significant discrepancies. As a consequence of our approach, changes observed in regions of sensor space cannot be directly interpreted as reflecting physiological changes in the brain regions underlying the sensors. Even so, differences between controls and *de novo* Parkinson's disease patients in the present study were diffusely distributed over the 10 ROIs. Therefore, it is unlikely that our results were significantly influenced by using sensor space rather than source space.

The present study is the first to report widespread slowing of resting-state brain activity in *de novo*, untreated Parkinson's disease patients. Considering the fact that we were able to detect these changes in the earliest clinical stages, it is tempting to speculate that the onset of these alterations actually lies in the preclinical stages of Parkinson's disease, which precede the onset of the classical motor signs. In the preclinical stages (I and II) and the earliest clinical stage (III) of the Braak neuropathological staging system (Braak *et al.*, 2003),

Parkinson's disease-specific neuropathological changes are most prevalent in the brainstem, including dopaminergic, serotonergic and noradrenergic structures. To a lesser extent, also the forebrain cholinergic system is affected. The alterations of oscillatory cortical activity demonstrated in the present study may therefore find their origin in degeneration of ascending corticopetal projection systems originating in the brainstem.

The neuropathological hallmark of clinically non-demented Parkinson's disease is a progressive deterioration of the nigrostriatal dopaminergic system. In the present study, changes in resting-state oscillatory brain dynamics in *de novo* Parkinson's disease patients remained relatively constant with progression of subjective disease duration from 0 up to 13 years, modified Hoehn and Yahr stage from I up to III and UPDRS motor score from 6 up to 35. These findings strongly argue against a (major) involvement of the dopaminergic system in the observed changes in oscillatory brain dynamics in Parkinson's disease. Also, some of the other findings in our study argue against the dopaminergic system as a major player in early stage alterations of oscillatory brain activity in Parkinson's disease. Resting-state oscillatory activity was hardly modulated by dopaminomimetic treatment, as acute administration had only minimal effects on spectral power, whereas LEDD was not associated with spectral power values. Literature data on the effect of changes in dopaminergic neurotransmission on resting-state oscillatory brain activity in Parkinson's disease patients are scarce. As of yet, we are aware of a single EEG study utilizing frequency analysis, in which an increase in spectral power after treatment with levodopa was reported. The increase was localized to the left occipital lobe and was present over all frequency bands (Yaar and Shapiro, 1983). In this study, 25 parkinsonian patients (of which disease duration, stage and severity as well as use of concomitant maintenance medication is not reported) were examined prior to and 14 to 30 days after the initiation of levodopa therapy. Maintenance levels of levodopa (without a decarboxylase inhibitor) therapy reached 3 to 5 g/day. This is equivalent to roughly 750 to 1250 mg/day of levodopa with a peripheral decarboxylase inhibitor. Clearly, this is a much higher dose than the average 445 mg in our sample, which suggests that patients were at a more advanced stage of disease and therefore hard to compare with the present population of Parkinson's disease patients. It might be argued that with further increasing dopaminergic losses, effects of exogenous levodopa become stronger. This assumption should be addressed in future studies in more advanced Parkinson's disease patients.

As argued earlier, a role for the dopaminergic system in early changes in resting-state oscillatory brain dynamics in Parkinson's disease seems rather unlikely. A more likely alternative candidate to explain these changes in spectral power in Parkinson's disease is the noradrenergic system, arising in the brainstem locus coeruleus. In animal studies, stimulation of the locus coeruleus induces a shift in the electroencephalogram towards higher frequencies (Berridge and Foote, 1991) and conversely, suppression of noradrenergic neurotransmission induces slow wave activity (Berridge *et al.*, 1993). Another option is the serotonergic system originating in the dorsal raphe nuclei. 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 (Vanderwolf and Baker, 1986). On the other hand, lesions of this brainstem nucleus and therefore loss of the ascending serotonergic cortical projections have been found to result in a reduction or inhibition of this cortical activation (Vanderwolf *et al.*, 1990; Peck and Vanderwolf, 1991).

The cholinergic system also has a modulatory influence on cortical activity as evidenced by several animal as well as human studies (Détári and Vanderwolf, 1987; Buzsaki *et al.*, 1988). After cholinergic stimulation, a decrease of slow, mainly delta activity and an increase of fast EEG background activity has been found. In 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 (Riekkinen *et al.*, 1991; Ray and Jackson, 1991; Soininen *et al.*, 1992; Ebert and Kirch, 1998; Dringenberg *et al.*, 2000; Osipova *et al.*, 2003; Ricceri *et al.*, 2004). As lesions in the cholinergic system primarily result in an increase in delta activity, an observation not found in the present study in non-demented Parkinson's disease patients, and the cholinergic nucleus basalis appears only to be mildly affected in the earliest clinical stages of Parkinson's disease (Braak *et al.*, 2003), we think this system is not so much involved in altered oscillatory brain dynamics in non-demented Parkinson's disease patients. Instead, cholinergic deficits appear to come into play in demented Parkinson's disease patients as evidenced by the increases in delta power in these patients (Soikkeli *et al.*, 1991; Neufeld *et al.*, 1994; Tanaka *et al.*, 2000; Bosboom *et al.*, 2006).

In our untreated, *de novo* Parkinson's disease patients, worse performance on perseveration-related tasks was associated with increased low alpha in central and parietal ROIs. Alpha oscillations are thought to play an important role in attentional processes. Oscillatory alpha activity probably indicates that attention is actively suppressing cortical activity related to distracters as a part of the process of focusing attention on important targets (Ward, 2003). For example, alpha power increases with memory load in the Sternberg memory-scanning task, reflecting an increase in the need to suppress distraction (Jensen *et al.*, 2002). Moreover, when attention is directed internally towards mental imagery, alpha power at attention-relevant scalp sites is greater than during

externally directed, information-intake tasks, reflecting suppression of external input during the imagery task (Cooper *et al.*, 2003). Also in this study, alpha power increased with increasing external task load, reflecting the need to suppress competing information sources. Especially lower alpha synchronization (in the range of about 6–10 Hz) is thought to reflect general task demands and attentional processes, and is topographically widespread over the entire scalp (Klimesch, 1999). Although highly speculative, it may be that increased low alpha oscillations in central and parietal regions in our *de novo* Parkinson's disease patients are a sign of a pathologically high level of attention. This might cause an inability to switch behavioural programmes resulting in increased perseveration. This notion is further supported by the fact that the increase in low alpha power in the present study also involves the parietal lobe that has a well-established role in attention, especially with regard to attentional-shifting (Posner and Petersen, 1990). However, we cannot exclude the possibility that the associations found are produced by a single alpha source, which synchronizes activity in all the four ROIs and at the same time is associated with cognitive perseveration. Obviously, the current results do not warrant a definitive judgement in this matter.

In conclusion, our results demonstrate that a widespread slowing of resting-state oscillatory brain activity is a very early and probably preclinical feature of Parkinson's disease, largely unrelated to disease stage, duration and severity, and hardly modulated by dopaminomimetic treatment. We propose that these early changes in resting-state brain activity in Parkinson's disease mainly result from a degeneration of noradrenergic and/or serotonergic corticopetal projections arising in the brainstem. Longitudinal studies of resting-state brain activity in Parkinson's disease, including patients at more advanced disease stages, are necessary to confirm that slowing of resting-state oscillatory activity is indeed a stable feature in Parkinson's disease. Such studies may also clarify what changes in the pattern of oscillatory brain activity mark or even predict the onset of Parkinson's disease-related dementia.

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