



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-naïve 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-hemispheric and inter-hemispheric) ten major cortical regions in five frequency bands.

Recently diagnosed, drug-naïve patients showed an overall increase in alpha1 SL relative to controls. Cross-sectional analysis in all patients revealed that disease duration was positively associated with alpha2 and beta SL measures, while severity of parkinsonism was positively associated 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 inter-hemispheric 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 onward. With disease progression, neighboring frequency bands become increasingly involved. 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.

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Introduction

Synchronization of neuronal activity between distributed brain regions plays a key role in the integration of their activity (Varela et al., 2001). This phenomenon can be studied by measuring statistical interdependencies between physiological signals derived from different brain regions over a certain time interval (Pereda et al., 2005), a concept aptly named functional connectivity (Lee et al., 2003). 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 (Schnitzler and Gross 2005; Uhlhaas and Singer 2006), which might even be observed during a no-task, resting-state condition (Uhlhaas and Singer 2006). The resting state is a far more stable and active condition than previously assumed (Gusnard and Raichle 2001) and is characterized by activation within a series of functional-anatomic networks implicated in motor, sensory and cognitive functions (Damoiseaux et al., 2006). Each of these resting-state networks appears to have a specific electrophysiological signature that combines the involvement of different brain rhythms (Mantini et al., 2007). 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 (Stam et al., 2003; Pijnenburg et al., 2004; Koenig et al., 2005; Babiloni et al., 2006), Alzheimer's disease (Leuchter et al., 1992; Besthorn et al., 1994; Locatelli et al., 1998; Berendse et al., 2000; Stam et al., 2002; Pijnenburg et al., 2004; Koenig et al., 2005; Stam et al., 2006b, 2007), multiple sclerosis (Cover et al., 2006), brain tumor patients (Bartolomei et al., 2006a,b) and schizophrenia (Micheloyannis et al., 2006). In mild cognitive impairment and Alzheimer's disease, changes were correlated with cognitive deficits (Stam et al., 2003; Babiloni et al., 2006; Stam et al., 2006b).

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 (Silberstein et al., 2005). Both

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dopaminergic therapy and deep brain stimulation led to a reduction in coupling in parallel with 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 (Hammond et al., 2007).

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.

Materials and methods

Subjects

A total of 70 patients with idiopathic Parkinson's disease (disease duration 0–13 years) and 21 healthy controls were recruited and selected for analysis as described in a previous MEG study in the same subjects (Stoffers et al., 2007). Out of these 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. Dopaminomimetically treated patients were either using levodopa, a short half-life dopamine agonist (i.e. pramipexole, ropinirole or pergolide) or a combination of the two.

Subject characteristics

Level of education was determined using the International Standard Classification of Education (ISCED-1997; UNESCO, 2003). The pre-morbid level of intelligence was measured using the National Adult Reading Test (NART; Nelson and O'Connell 1978). Global cognitive function was examined using the CAMCOG scale (Hobson and Meara 1999). 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 first occurred. Unified Parkinson's disease Rating Scale motor scores (UPDRS-III; Fahn et al., 1987) and modified Hoehn and Yahr stages (Jankovic et al., 1990) were obtained in a so-called "practically defined OFF" state (see MEG data acquisition and pre-processing) by a trained physician prior to MEG registration. UPDRS tremor (items 20 and 21), rigidity (item 22), bradykinesia (items 23–26, 31) and axial involvement (items 27–30) subscores were computed by summing scores on items related to the motor domain in question. Items 18 and 19 (speech and facial expression) were not included in the subscore analysis. 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.

Neuropsychological evaluation

Cognitive functions were assessed using a set of neuropsychological tasks as described previously (Stoffers et al., 2007). 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 several hours after MEG registration. Dopaminomimetically treated PD patients were examined in an "ON" medication state.

MEG data acquisition and pre-processing

MEG data acquisition and pre-processing were performed as described previously (Stoffers et al., 2007). In short, patients treated with levodopa were instructed to come to the hospital without taking their first morning dose of anti-Parkinson medication. This medication state is roughly equivalent to the "practically defined OFF" state

Table 1
Subject characteristics

	Controls ($N=21$)	Recently diagnosed Parkinson's disease patients ($N=18$)	Moderately advanced Parkinson's disease patients ($N=17$)	All Parkinson's disease patients ($N=70$)
Age (years, mean±SD)	59.4±7.3	59.4±7.9	64.2±5.8	62.1±6.8
Sex (♂/♀)	11/10	12/6	8/9	40/30
Education (ISCED 0/1/2/3/4/5/6)	0/0/6/4/2/8/1	0/0/5/5/0/8/0	0/1/7/1/2/6/0	1/0/27/16/4/21/1
Verbal IQ (Dutch NART)	111.7±9.4	109.2±11.2	109.2±12.9	107.7±13.1
Global cognition (CAMCOG)	98.9±4.2	98.2±4.7	97.2±5.1	97.0±5.1
Disease duration (years, mean±SD)	n.a.	0.9±0.5	13.3±1.3	5.5±3.7
Side of onset (left/right)	n.a.	4/14	10/7	33/37
H and Y modified "OFF" (1/1.5/2/2.5/3)	n.a.	9/1/7/1/0	0/0/8/7/2	14/1/29/18/8
UPDRS-III "OFF" (mean±SD)	0.6±1.4	13.1±6.1	19.6±5.4	17.1±6.9
LEDD (mg)	n.a.	n.a.	795±361	406±423

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 and 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; n.a.=not applicable.

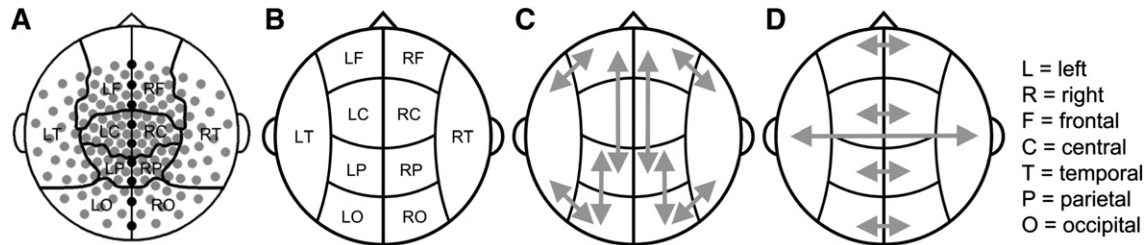


Fig. 1. (A) Clustering of MEG sensors above the major cortical areas. Midline sensors (depicted in black) were excluded from synchronization likelihood (SL) analysis. (B) Schematic representation of regions of interest (ROIs) used for within and between ROI SL calculations. (C) Intrahemispheric SL. (D) Interhemispheric SL. Arrows indicate between ROI SL measures used.

as was originally put forward by the CAPIT Committee (Langston et al., 1992). 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 s artifact-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 (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 (Figs. 1A and B). As ROIs were based on the extra-cranial position of the MEG sensors, underlying cortical areas are to be considered as indicative.

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 and van

Dijk, 2002). Parameter settings used for SL computation were explicitly based on the frequency content of the data (for lags and embedding dimensions used, see Montez et al., 2006). P_{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 (Fig. 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 (Fig. 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 (Fig. 1D). As local SL measures statistical interdependencies between sensors within a ROI, it is fundamentally different from local spectral power within a ROI, a measure that was used in a previous study in the same subjects (Stoffers et al., 2007). Local power within a ROI is an average of the local fields measured at each individual sensor within that ROI and reflects the synchronous activity of underlying populations of neurons. 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 artifacts 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

Table 2
Means \pm SDs of synchronization likelihood (SL) measures in five major frequency bands

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

Significant differences between patients and controls are indicated in bold.

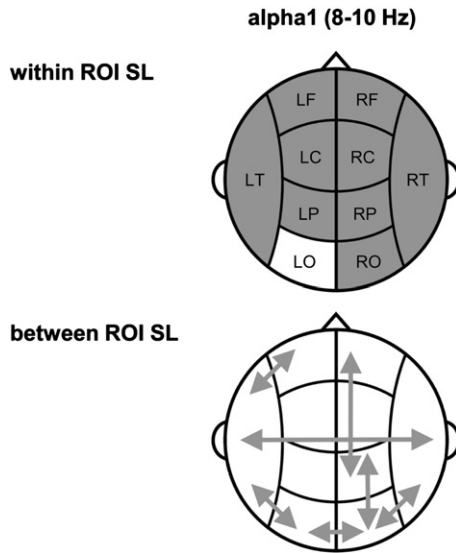


Fig. 2. Schematic overview of specific synchronization likelihood (SL) measures that have mainly contributed to the differences in local, intrahemispheric and interhemispheric SL measures found in univariate testing between recently diagnosed, drug-naïve Parkinson's disease patients ($N=18$) and controls ($N=21$) in analysis A. Specific SL measures have been included if they showed a trend toward 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.

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., 2007).

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, pre-morbid 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 artifacts, 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-naïve 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 additionally involve neighboring 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-naïve 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, USA). 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 analysis. Partial eta squared (η^2) was calculated when performing GLM analyses and beta squared (β^2) when performing regression analyses, which both represent the proportion of the total variability in the dependent variable that is accounted for by the relevant determinant, when controlling for all (other) determinants in the analysis.

Analysis A

Differences in SL between recently diagnosed, drug-naïve 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, intrahemispheric and interhemispheric SL) as dependent and group as well as any relevant confounders as determinants.

Analysis B

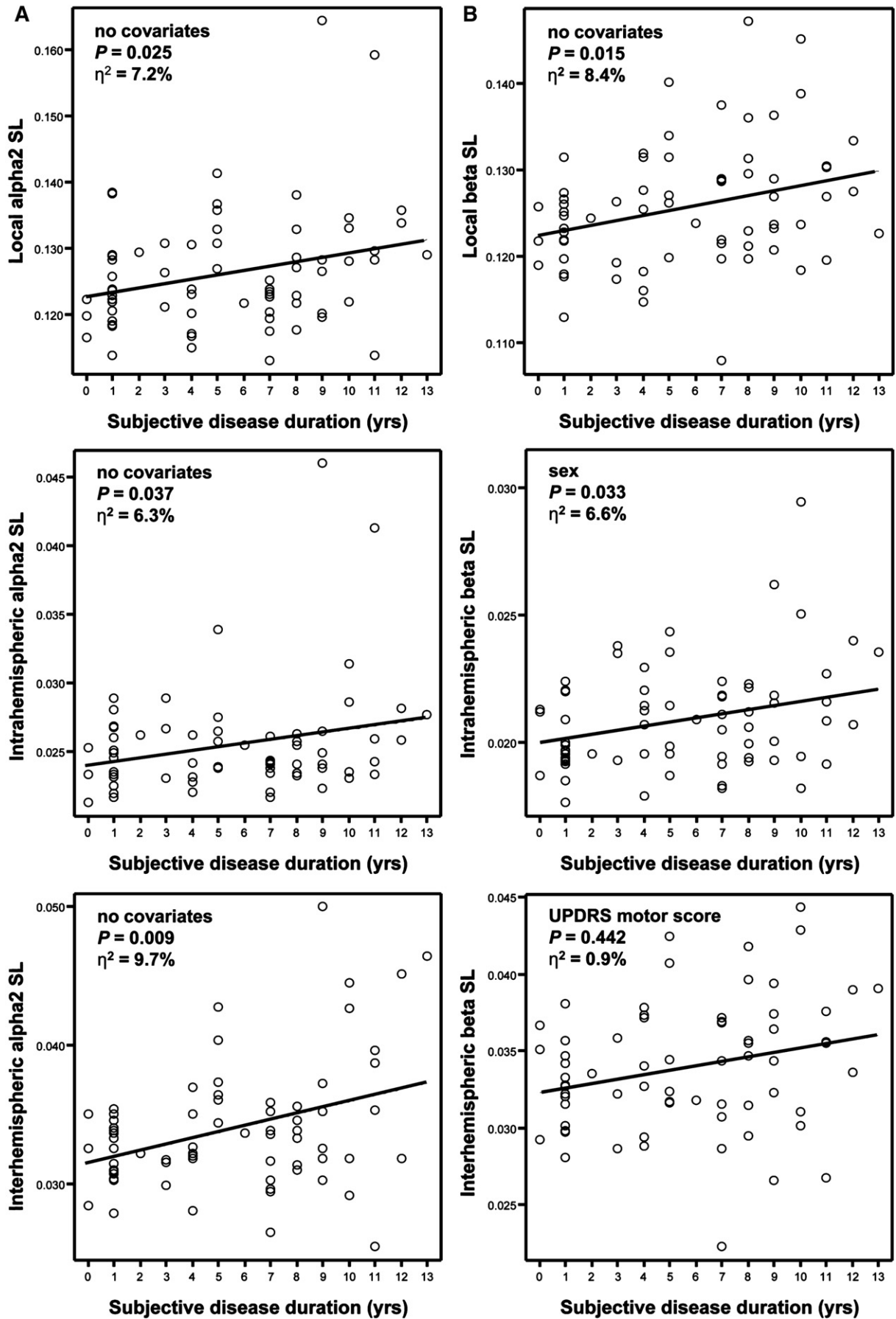
The relation of SL with disease parameters in Parkinson's disease patients ($N=70$) was analyzed by means of three univariate GLM analyses per selected frequency band using each of the SL measures as dependent and both disease duration and UPDRS motor score as well as any relevant confounders as determinants. A disease parameter was maintained in the final analysis if at a minimum a trend involving the disease parameter (P below 10%) was observed. The relation with UPDRS motor subscores was analyzed using stepwise linear regression analysis (0.05 probability of F for entry, 0.10 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. (2007). 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 recently diagnosed Parkinson's disease patients ($N=18$) was 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 covariates.

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, pre-morbid IQ (NART) or global cognitive function (CAMCOG). Since age and sex could be modifiers of functional connectivity, they were nonetheless added as covariates in all initial analyses of SL. Since age and pre-morbid IQ are well-known modifiers of cognitive performance, they were initially added as covariates 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 ($P=0.001$, $\eta^2=26.4\%$), intrahemispheric ($P=0.013$, $\eta^2=15.9\%$) 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, and a detailed illustration of changes in within ROI and between ROI SL measures can be found in Fig. 2.

Analysis B: relation with disease duration and motor function

In the full group of Parkinson's disease patients, we found positive associations of disease duration with local, intrahemispheric and interhemispheric alpha2 SL (Fig. 3A) and with local beta SL (Fig. 3B), as well as positive associations of UPDRS motor score with local, intrahemispheric and interhemispheric theta SL (Fig. 4A) and with interhemispheric beta SL (Fig. 4B). Intrahemispheric beta SL was positively associated with both disease duration (Fig. 3B) and UPDRS motor score (Fig. 4B), 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 ($P\approx 0.15$). Analyses of UPDRS motor subscores showed positive associations of the tremor subscore with local and interhemispheric theta SL (Fig. 5A) and positive associations of the bradykinesia subscore with intrahemispheric theta (Fig. 5A) as well as local, intrahemispheric and interhemispheric beta SL (Fig. 5B). No associations of SL with rigidity or axial involvement UPDRS subscores were found.

Analysis C: effect of moderately advanced disease

In moderately advanced Parkinson's disease patients, increases were found relative to controls in local ($P=0.002$, $\eta^2=23.2\%$),

intrahemispheric ($P=0.022$, $\eta^2=14.2\%$) and interhemispheric ($P=0.025$, $\eta^2=13.3\%$) theta SL; local ($P=0.003$, $\eta^2=22.5\%$) and intrahemispheric ($P=0.017$, $\eta^2=15.3\%$) alpha1 SL; local ($P=0.025$, $\eta^2=13.2\%$) and interhemispheric ($P=0.045$, $\eta^2=10.7\%$) alpha2 SL; and local ($P=0.044$, $\eta^2=11.1\%$) beta SL. For each frequency band, means and SDs of the SL measures are listed in Table 2.

Analysis D: relation with cognitive dysfunction in early-stage, untreated disease

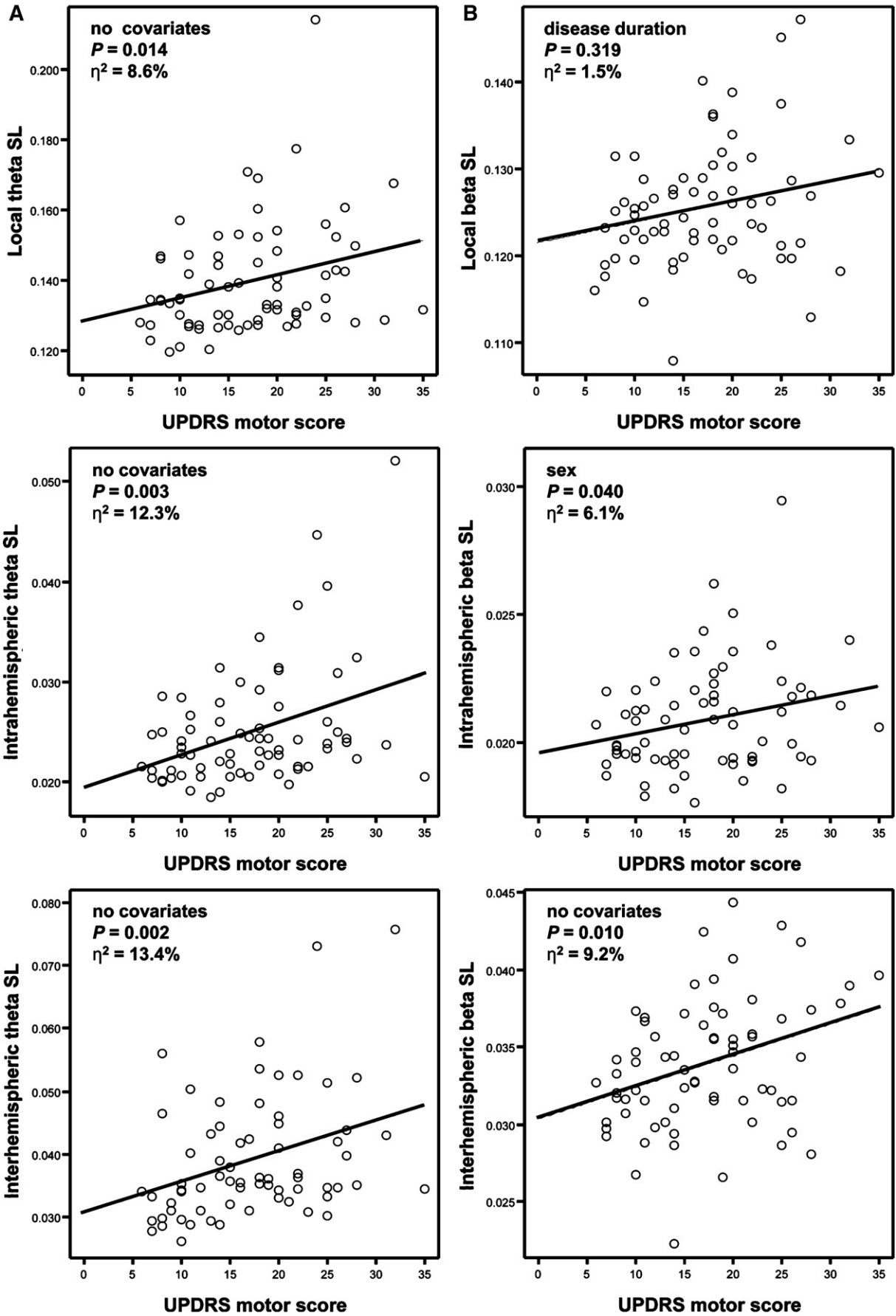
Recently diagnosed Parkinson's disease patients had a lower capacity for planning/spatial memory ($P=0.031$, $\eta^2=13.3\%$) and an increased tendency for cognitive perseveration ($P=0.029$, $\eta^2=13.6\%$) relative to controls. No significant differences in performance were found with regard to strategy/analysis or set-shifting. Within the group of recently diagnosed patients, analyses showed a positive association of the level of perseveration with interhemispheric alpha1 SL ($P=0.007$, $\eta^2=39.9\%$).

Discussion

This is the first study to demonstrate widespread increases in alpha band functional connectivity in early-stage Parkinson's disease. With increasing disease duration and severity of parkinsonism, there appear to be rising 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 for 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 stereotaxic implantation of macroelectrodes in the subthalamic nucleus (STN) for deep brain stimulation (Silberstein et al., 2005). 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 broadband increases in functional connectivity demonstrated in our most advanced patient group is in line with results from the study of Silberstein et al. (2005). Interestingly, a recent study in healthy controls, which combined 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 (Mantini et al., 2007). From our data, it would seem that increased functional connectivity over a broad frequency range

Fig. 3. Scatterplots showing SL 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. Maintained covariates, significance level (P) and percentage of explained variance (η^2) are indicated in the top left corner of each plot.



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.

Interestingly, worsening of bradykinesia has recently been reported following 5 Hz (Eusebio et al., 2008), 10 Hz (Timmermann et al., 2004; Eusebio et al., 2008) as well as 20 Hz (Fogelson et al., 2005; Chen et al., 2007; Eusebio et al., 2008) low-frequency stimulation of the STN in Parkinson's disease patients. Clearly, these observations further support the role of increased synchronization over a broad frequency range in Parkinson's disease motor deficits.

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 (Stam et al., 2006a). 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 (Braak et al., 2003), 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 not unlikely that there will be several distinct patterns of resting-state functional connectivity that are most typical for specific disease stages.

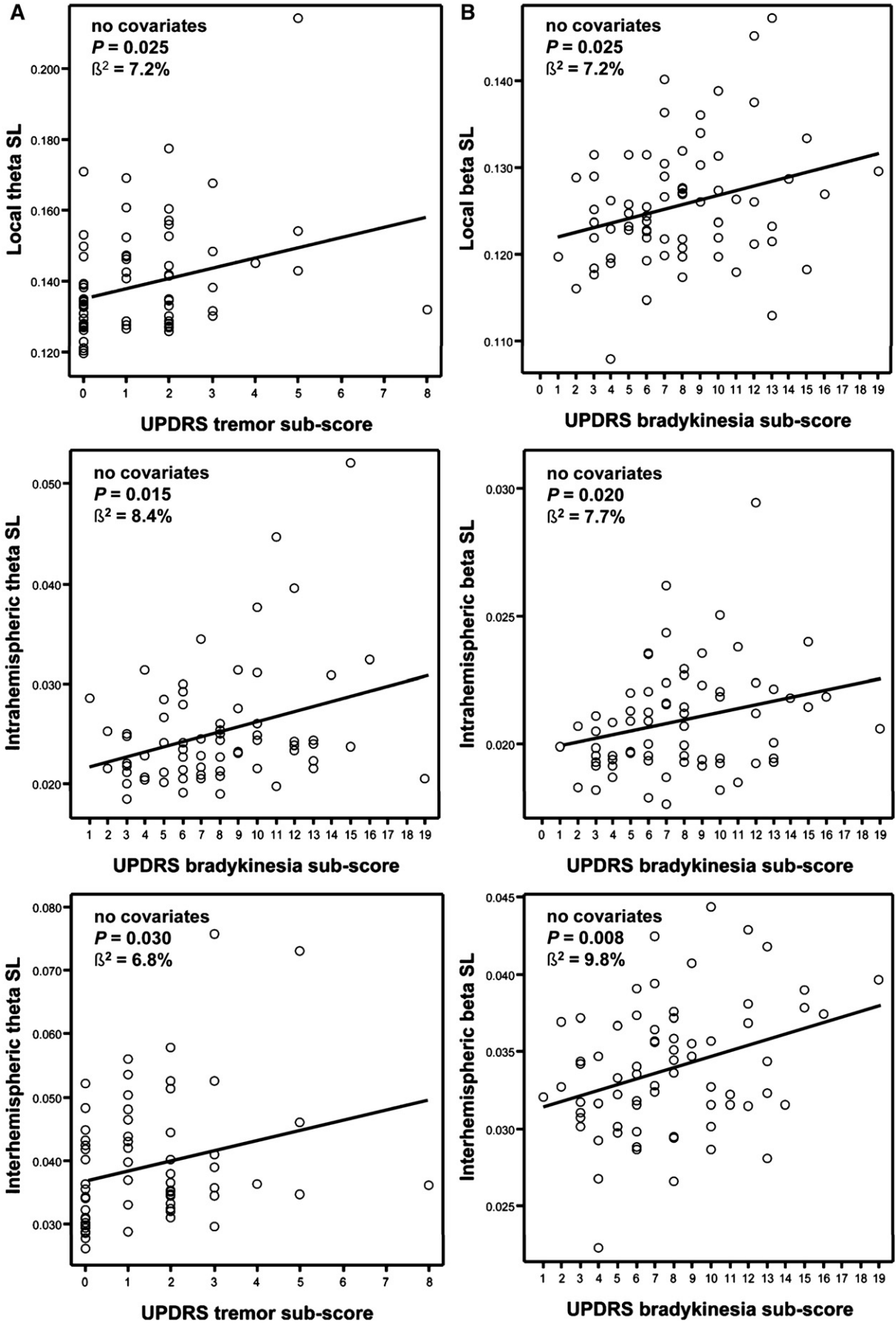
In the present study, increased local and interhemispheric theta band functional connectivity was associated with both total UPDRS motor scores and tremor subscores. Parkinsonian tremor generally has a frequency in the theta range (4–7 Hz). Consequently, tremor could directly influence measured theta band functional connectivity through movement artifacts. 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 (Stoffers et al., 2007). Alternatively, tremor might be related to SL in a more indirect way. Timmermann et al. (2003) have reported coherence between tremor, measured with electromyography, and MEG oscillatory rhythms at tremor (and double tremor) frequencies in the contralateral motor cortex. 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. On the other hand, the association of intrahemispheric theta band functional connectivity with the bradykinesia subscore (and total UPDRS motor score) suggests a role of this frequency band also in the pathophysiology of Parkinson's disease motor symptomatology other than tremor. This notion is further supported by the previously mentioned study by Eusebio et al. (2008), which demonstrated that 5 Hz stimulation of the STN can exacerbate bradykinesia in Parkinson's disease patients. 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 the mechanical or electromyography recording of tremor.

In a previous study using power spectral analysis on the same resting-state MEG data sets 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 (Stoffers et al., 2007). Although one of the major advantages of SL computation is that it is not influenced by spectral power (Stam and de Bruin, 2004), estimates of statistical interdependencies between channels can in theory still be confounded 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. Secondly, 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. Ebersbach et al., 1994; Robertson et al., 1996), which can be observed at the earliest clinical stages of disease (Stoffers et al., 2001). 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, untreated Parkinson's disease patients and showed them to be positively correlated with increased interhemispheric alpha1 coupling. In our previous study in the same subjects, increased alpha1 spectral power in central and parietal ROIs was associated with cognitive perseveration (Stoffers

Fig. 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. Maintained covariates, significance level (P) and percentage of explained variance (η^2) are indicated in the top left corner of each plot.



et al., 2007). 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 (Mantini et al., 2007). As effective cognition probably requires the constant changing of synchronous neural cell assemblies, enabling the rapid formation and decay of functional networks (Friston, 2000), 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 or 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 with disease progression, increased functional connectivity progressively involved a broader 4–30 Hz range. We were able to confirm the association between beta band coupling and severity of parkinsonism, in particular bradykinesia, and found some evidence for a similar association between functional connectivity in the theta band and motor symptoms, in particular tremor. Cognitive perseveration in early-stage Parkinson's disease was positively associated 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.

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Fig. 5. Scatterplots showing SL measures in the theta (A) and beta (B) frequency band set out against the UPDRS subscore 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 (η^2) are indicated in the top left or right corner of each plot.

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