Increased cortico-cortical functional connectivity in early-stage Parkinson’s disease: An MEG study


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 magnetoencephalography (MEG) 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...
Subject characteristics

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.

Table 1

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

ISCED=International Standard Classification of Education, NART=National Adult Reading Test, CAMCOG=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.

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 2
Means±SDs of synchronization likelihood (SL) measures in five major frequency bands

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

Significant differences between patients and controls are indicated in bold.
paradigm was designed such that subbands were only 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-naive Parkinson’s disease patients ($N=18$) and controls ($N=21$) in analysis A. Specific SL measures have been included if they showed a trend 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).

### Analysis A

Differences in SL between recently diagnosed, drug-naive Parkinson’s disease patients ($N=18$) and controls ($N=21$) were analyzed by means of three univariate GLM analyses per frequency band using each of the overall SL measures (local, 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 $P$ 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 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-naive patients to exclude confounding effects of medication on task performance (analysis D). Relations between cognitive performance and functional connectivity were only analyzed for parameters that showed differences between recently diagnosed Parkinson’s disease patients and controls, in this way diminishing the likelihood of type-I statistical errors.

All analyses were performed at a significance level of 5% (two-tailed) using the SPSS 15.0.1.1 software package (SPSS Inc., Chicago, IL, 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 ($\eta^2$) was calculated when performing GLM analyses and beta squared ($\beta^2$) 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 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 covariants in all initial analyses of SL. Since age and pre-morbid IQ are well-known modifiers of cognitive performance, they were initially added as covariants in all analyses of cognitive performance, as was sex.

Analysis A: effect of early-stage, untreated disease

In recently diagnosed Parkinson’s disease patients, local \(P=0.001, \eta^2=26.4\%\), 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 SDs 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 \(P=0.15\). 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 alpha1 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 broad-band 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. 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 (\(\eta^2\)) are indicated in the top left corner of each plot.
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.

Acknowledgments

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References


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 (η²) are indicated in the top left or right corner of each plot.