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Resting-state functional connectivity as a marker of disease progression in Parkinson's disease: A longitudinal MEG study☆

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ABSTRACT

The assessment of resting-state functional connectivity has become an important tool in studying brain disease mechanisms. Here we use magnetoencephalography to longitudinally evaluate functional connectivity changes in relation to clinical measures of disease progression in Parkinson's disease (PD).

Using a source-space based approach with detailed anatomical mapping, functional connectivity was assessed for temporal, prefrontal and high order sensory association areas known to show neuropathological changes in early clinical disease stages.

At baseline, early stage, untreated PD patients (n = 12) had lower parahippocampal and temporal delta band connectivity and higher temporal alpha1 band connectivity compared to controls. Longitudinal analyses over a 4-year period in a larger patient group (n = 43) revealed decreases in alpha1 and alpha2 band connectivity for multiple seed regions that were associated with motor or cognitive deterioration.

In the earliest clinical stages of PD, delta and alpha1 band resting-state functional connectivity is altered in temporal cortical regions. With disease progression, a reversal of the initial changes in alpha1 and additional decreases in alpha2 band connectivity evolving in a more widespread cortical pattern. These changes in functional connectivity appear to reflect clinically relevant phenomena and therefore hold promise as a marker of disease progression, with potential predictive value for clinical outcome.

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1. Introduction

Parkinson's disease (PD) is characterized by neuropathological changes that extend beyond the nigrostriatal system and involve cortical regions in a progressive manner from early disease stages onward (Braak et al., 2003; Lim et al., 2009). Clinically, the phenotype of PD is heterogeneous and can be classified into distinct subtypes that have a different progression of both motor and non-motor symptoms over time (Eggers et al., 2012; Foltynie et al., 2002; van Rooden et al., 2011). A better understanding of the pathophysiological mechanisms underlying these symptoms is essential both for prognostic purposes and the development of targeted treatment strategies.

Coordinated and integrated activity of different brain regions is required for a variety of cognitive and motor functions (Bullmore and Sporns, 2012; Schnitzler and Gross, 2005; Varela et al., 2001). The synchronization of activity between distributed brain regions is assumed to reflect functional interactions between brain regions and is referred to as functional connectivity (Aertsen et al., 1989; Friston, 2001). The assessment of functional connectivity has become an important tool in the study of pathophysiological mechanisms in a variety of brain disorders such as Alzheimer's disease (AD), epilepsy and brain tumors (Stam and van Straaten, 2012; Uhlhaas and Singer, 2006).

In PD, functional connectivity has been studied by means of electro- (EEG) and magnetoencephalography (MEG) in patients with advanced as well as early-stage disease. Excessive resting-state cortico-cortical coupling characterizes both early-stage and advanced-stage non-demented patients (Silberstein et al., 2005; Stoffers et al., 2008a). In contrast, Parkinson's disease dementia (PDD) is associated with decreases in functional connectivity (Bosboom et al., 2009; Ponsen et al., 2013). The results of these cross-sectional studies suggest

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that changes in functional connectivity evolve over the course of the disease in relation to clinical (both motor and non-motor) symptomatology. However, this has not yet been confirmed in a longitudinal study.

An important limitation in interpreting the results of most previous neurophysiological studies lies in the fact that functional connectivity was calculated at the sensor-level. Consequently, the spatial distribution of the connectivity patterns has to be interpreted with care, as volume conduction and field spread may hinder correct relation to anatomical substrates. To overcome these problems, we developed a new methodological approach using an atlas-based source space in combination with beamforming and a functional connectivity estimator, the Phase Lag Index (PLI) that is insensitive to the effects of field spread and volume conduction (Hillebrand et al., 2012). This allows for improved anatomical interpretability of MEG functional connectivity data from distinct cortical regions as well as a comparison with results obtained from other imaging modalities, notably functional magnetic resonance imaging (fMRI).

In the present study we used the newly developed source-space method to longitudinally investigate the resting-state functional connectivity patterns of eight cortical seed regions including temporal, prefrontal and high order sensory association areas. The selection of these seed regions was based on their progressive neuropathological involvement in the early clinical stages of PD (Alafuzoff et al., 2009; Braak and Del Tredici, 2009; Braak et al., 2003) and we hypothesized that these regions would display changes in overall functional connectivity with the rest of the brain over time that would be related to motor and/or cognitive measures of disease progression.

2. Materials and methods

2.1. Participants

Participants were selected from a longitudinal study cohort in which a total of 70 patients (disease duration 0–13 years, including 18 early stage drug-naïve (de novo) patients) with idiopathic PD and 21 healthy controls (age-matched to the de novo patients) were included at baseline (Stoffers et al., 2007). After an interval of 4.3 ± 0.8 (mean ± standard deviation) years, 59 patients and 16 controls completed follow-up measurements. Three patients had passed away and 13 participants (8 patients and 5 controls) were lost to follow-up. Ten PD patients and a single control had relatively severe artifacts during MEG registration and were therefore excluded from further analysis. Six patients had no MRI performed at the follow-up evaluation, and a single control had an MRI scan with extensive white matter lesions. The latter subjects were also excluded from the current source-space based analysis, leaving baseline and follow-up measurements in 43 (including 12 initially de novo) PD patients and 14 controls for further analyses.

All participants gave written informed consent to the research protocol, which was approved by the medical ethical committee of the VU University Medical Center. Ethics review criteria conformed to the Helsinki declaration.

2.2. Participant characteristics

Disease duration was calculated on the basis of the patients' subjective estimate of the time of occurrence of the first motor symptoms. Unified Parkinson's Disease Rating Scale motor ratings (UPDRS-III) (Fahn and Elthon, 1987) were obtained in the "ON" medication state by a trained physician. Global cognitive function was assessed using the Cambridge Cognitive Examination (CAMCOG) scale (Roth et al., 1986). The presence of dementia was evaluated according to the clinical criteria recommended by the Movement Disorder Society Task Force (Dubois et al., 2007). Education level was determined using the International Standard Classification of Education (ISCED) (UNESCO, 1997). The total dose of dopaminomimetics was converted to a so-called levodopa equivalent daily doses (LEDD) as described previously (Olde Dubbelink et al., 2013). Levodopa was always used in combination with a peripheral decarboxylase inhibitor. At the time of the follow-up evaluation, two patients were using rivastigmine.

2.3. Specific neuropsychological evaluation

Specific cognitive, mainly frontal and temporal, functions were assessed using a set of neuropsychological tasks. Three tasks were taken from the Cambridge Neuropsychological Test Automated Battery (CANTAB), a computerized test battery (CANTAB Eclipse 2.0, Cambridge Cognition, Cambridge, UK). Tasks included were spatial span (SSP; outcome measure: spatial span length, reflecting frontal cognitive function), spatial working memory (SWM; outcome measures: total errors and strategy, reflecting frontal cognitive function), and pattern recognition memory (PRM; outcome measure: correct responses, reflecting temporal cognitive function). Additionally, the Vienna perseveration task (VPT) was administered (Vienna Test System, Dr. G. Shuhfried GmbH, Mödling, Austria), measuring the amount of perseveration in the generation of random motor behavior (reflecting frontal cognitive function). Verbal fluency (reflecting temporal cognitive function) was assessed using the 1 minute semantic fluency test (animals), which is part of the CAMCOG examination.

2.4. Data acquisition

MEG data were recorded in an eyes-closed resting-state condition for 5 min with a sample rate of 312.5 (baseline) or 625 (follow-up) Hz, while subjects were in the "ON" medication state, as described previously (Olde Dubbelink et al., 2013). Structural T1-weighted MR imaging was performed in all subjects (baseline: 1.0 T, Impact, Siemens, Erlangen, Germany; follow-up: 3.0 T, Signa, GE healthcare, Waukesha, USA). Vitamin E capsules were placed at the same anatomical landmarks where head position coils had been placed during MEG-registration.

2.5. Data preprocessing

Follow-up MEG-data were downsampled to 312.5 Hz. Subsequently, both baseline and follow-up datasets were split up into epochs of 4096 samples (13.11 s). Channels and epochs containing artifacts were discarded after visual inspection (KOD). On average 2.4 (range: 2–7) channels and 3.1 (range: 0–13) epochs were discarded. An atlas-based beamformer approach (Hillebrand et al., 2012) was used to project MEG sensor signals to an anatomical framework consisting of 78 cortical regions identified by means of automated anatomical labeling (AAL) (Gong et al., 2009; Tzourio-Mazoyer et al., 2002) (Inline Supplementary Table S1). For this purpose, MRI and MEG data were co-registered for each subject through identification of the same anatomical landmarks (left and right pre-auricular points and nasion). Only data with an estimated co-registration error < 1.0 cm were accepted for further analysis. MRI-data was then spatially normalized to a template MRI using the SEG toolbox in SPM8 (Ashburner and Friston, 2005; Weiskopf et al., 2011), after which anatomical labels were applied.

Time-series of neuronal activation were estimated for 6 frequency bands (delta (0.5–4 Hz), theta (4–8 Hz), alpha1 (8–10 Hz), alpha2 (10–13 Hz), beta (13–30 Hz) and gamma (30–48 Hz)), using an average time-window of 236 (range 105–380) seconds as input for the beamformer computations. This resulted in a total of 6 sets (one for each frequency band) of 78 time-series (one for each AAL region). For each subject, five artifact free epochs per frequency band were selected for further analysis (KOD).

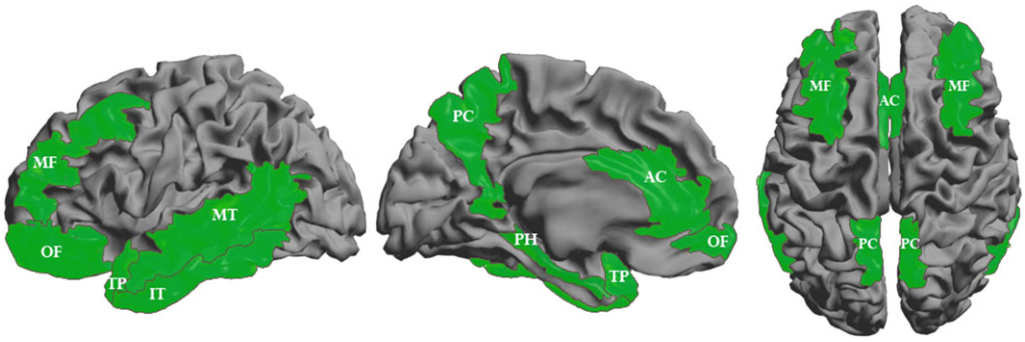

Fig. 1. Seed regions overlaid on a template brain. Left lateral, left medial and dorsal views are shown. PH, parahippocampal; TP, temporal pole; IT, inferior temporal; OF, orbitofrontal; MT, middle temporal; AC, anterior cingulate; PC, precuneus; MF, middle frontal.

2.6. Functional connectivity analysis

For the current analysis we defined 8 seed regions including temporal, prefrontal and high order sensory association areas known to show neuropathological changes in mild to moderate clinical disease stages in PD (i.e. Braak stages 4, 5) (Alafuzoff et al., 2009; Braak and Del Tredici, 2009; Braak et al., 2003): parahippocampal, inferior and middle temporal, temporal pole, orbitofrontal, precuneus, anterior cingulate and middle frontal regions (Fig. 1). A seed region consisted of the bilateral homologous AAL regions, and for the temporal pole and orbitofrontal regions of multiple bilateral AAL regions (Inline Supplementary Table S1). Functional connectivity was computed between each seed region and all other AAL regions using the PLI (Stam et al., 2007). This measure (range between 0 and 1) reflects true interactions between two oscillatory signals through quantification of the (non-zero lag) phase coupling, thereby discarding the effects of volume conduction and field spread. Functional connectivity analyses were performed with BrainWave software (CS, version 0.9.58, available form http://home.kpn.nl/stam7883/brainwave.html). PLI values for AAL regions were subsequently averaged per seed region. This resulted in a single PLI value per seed region per frequency band, reflecting mean connectivity of that region with the rest of the brain. Results of five epochs were averaged per subject.

Inline Supplementary Table S1 can be found online at http://dx.doi.org/10.1016/j.nicl.2013.04.003.

2.7. Statistical analysis

2.7.1. Participant characteristics

Baseline differences between de novo PD patients (n = 12) and controls (n = 14) regarding sex distribution, age and education level were analyzed by means of chi-square and independent sample *t*-tests. Longitudinal changes in cognitive test performance in the full group of PD patients (n = 43) were analyzed with paired-sample *t*-tests.

2.7.2. Functional connectivity in early stage, untreated disease

To study functional connectivity in early-stage untreated disease, baseline PLI was assessed in de novo PD patients (n = 12) compared to age-matched controls (n = 14) by means of a single General Linear Model (GLM) analysis per seed region and per frequency band. PLI values were transformed with a natural logarithmic function to obtain a Gaussian distribution. Sex was added as a covariate.

2.7.3. Longitudinal changes in functional connectivity

Longitudinal changes in functional connectivity were analyzed within the full group of PD patients (n = 43) and in the control group (n = 14) by means of a single GLM analysis for repeated measures per seed region and frequency band with time. Sex, baseline age and mean difference in LEDD were added as covariates.

2.7.4. Relationship between functional connectivity and clinical measures of disease progression

Within the group of PD patients (n = 43) the relationship between the longitudinal course of functional connectivity and clinical measures of motor and cognitive function was investigated by means of Generalized Estimated Equations (GEE) with exchangeable working correlation matrix (Zeger et al., 1988) using either UPDRS-III (parameter of motor disease severity); CAMCOG (global cognitive function); PRM correct responses or semantic fluency (temporal cognitive function); SSP span length, SWM between errors, SWM strategy or VPT redundancy (frontal cognitive function) as dependent, and mean PLI per seed region as independent variable. When appropriate, dependent variables were transformed in order to comply with assumptions of normality. Sex, age and LEDD were

Table 1
Participant characteristics.

	Baseline analysis		Longitudinal analysis	
	CTRL (n = 14)	de novo PD (n = 12)	PD baseline (n = 43)	PD follow-up (n = 43)
Sex (M/F)	10/4	8/4	28/15	28/15
Age (years)	60.0 ± 8.55	58.0 ± 6.95	61.5 ± 6.45	65.8 ± 6.53
ISCED (0/1/2/3/4/5/6)	0/0/1/3/1/8/1	0/0/2/2/0/8/0	0/1/14/13/1/13/1	0/1/14/13/1/13/1
Disease duration (years)	n.a.	0.92 ± 0.52	5.19 ± 3.63	9.56 ± 4.13
UPDRS-III	0.71 ± 1.59	14.9 ± 1.27	14.1 ± 5.93	27.1 ± 9.32
LEDD total dose	n.a.	n.a.	352 ± 416	807 ± 506
CAMCOG	99.2 ± 2.79	97.1 ± 4.94	95.9 ± 4.51	93.1 ± 8.28

Values are expressed as mean ± standard deviation unless otherwise indicated. Please note that the de novo PD group (n = 12) is a subgroup of the full PD group at baseline (n = 43). M/F, male/female; ISCED, International Standard Classification of Education (0 no education, 1 primary education, 2 lower secondary education, 3 upper secondary education, 4 post-secondary non-tertiary education, 5 lower tertiary education, 6 upper tertiary education); UPDRS-III, Unified Parkinson's Disease Rating Scale motor ratings; LEDD, Levodopa Equivalent Daily Dosis; CAMCOG, Cambridge cognitive examination; n.a. non-applicable.

Table 2
Cognitive performance of PD patients (n = 43) over time.

	Baseline	Follow-up	*p*-Value
Global cognitive function			
CAMCOG	95.9 ± 4.51	93.1 ± 8.28	.008
Specific neuropsychological evaluation			
PRM correct responses	21.6 ± 1.96	20.8 ± 2.73	.034
Semantic fluency	24.2 ± 6.09	20.1 ± 6.18	<.001
SSP span length	5.41 ± 0.92	4.71 ± 1.03	. 001
SWM between errors	32.6 ± 19.3	40.4 ± 23.7	.011
SWM strategy	33.5 ± 5.50	33.3 ± 6.23	.84
VPT redundancy	23.9 ± 8.49	24.8 ± 10.8	.38

All values are expressed as mean ± standard deviation.
CAMCOG, Cambridge Cognitive Examination; PRM, Pattern Recognition Memory; SSP, Spatial Span; SWM, Spatial Working Memory; VPT, Vienna Perseveration Test.

added as covariates in all analyses, as was education level (ISCED, dichotomized) in all analyses involving cognitive measures.

All analyses were performed using the IBM SPSS Statistics 20.0 software package (IBM Corporation, New York, USA). A significance level of .05 (two-tailed) was applied, with false discovery rate (FDR) control for the number of seed regions (GLM and GEE analyses) and the number of outcome measures within a cognitive domain (GEE analyses) to reduce the likelihood of type-I statistical errors.

3. Results

3.1. Participant characteristics

Participant characteristics are summarized in Table 1. There were no significant differences in age or sex distribution or education level between de novo patients and controls at baseline. None of the patients fulfilled clinical diagnostic criteria for dementia at baseline. Longitudinal assessment of cognitive function revealed decreases in cognitive test performance in PD patients over time (Table 2). Moreover, four patients fulfilled clinical diagnostic criteria for PDD at the time of follow-up evaluation.

3.2. Functional connectivity in early stage, untreated disease

In de novo PD patients, delta band PLI for parahippocampal [$F(1,23) = 6.45$, $p = .018$], inferior temporal [$F(1,23) = 12.3$, $p = .002$], temporal polar [$F(1,23) = 10.3$, $p = .004$] and middle temporal [$F(1,23) = 10.5$, $p = .004$] regions was lower compared to controls. For the middle temporal cortex, alpha1 band PLI was higher in PD patients compared to controls [$F(1,23) = 7.91$, $p = .010$] (Table 3). There were no significant PLI differences between groups for middle frontal, precuneus, anterior cingulate or orbitofrontal region.

3.3. Longitudinal changes in functional connectivity

Within the full group of PD patients (n = 43) alpha1 band PLI for the middle temporal cortex decreased over time [$F(1,39) = 10.8$, $p = .002$]. In the alpha2 band, decreases in PLI were present for several seed regions: parahippocampal [$F(1,39) = 4.17$, $p = .048$], inferior temporal [$F(1,39) = 4.94$, $p = .032$], middle temporal [$F(1,39) = 10.2$, $p = .003$] as well as precuneus [$F(1,39) = 8.25$, $p = .007$] regions (Table 3).

Controls did not show significant changes in PLI over time for any of the seed regions in any of the frequency bands.

To assess the possible confounding effect of dopaminergic medication more thoroughly, we studied the longitudinal relation of alpha1 and alpha2 band functional connectivity (dependent variable) with LEDD (independent variable) in more detail. These analyses demonstrated no association between functional connectivity and LEDD for any of the seed regions in these frequency bands (Inline Supplementary Table S2).

Inline Supplementary Table S2 can be found online at http://dx.doi.org/10.1016/j.nicl.2013.04.003.

3.4. Relationship between functional connectivity and clinical measures of disease progression

3.4.1. Motor function

Within the full group of PD patients (n = 43) GEE analysis revealed a longitudinal association between PLI values and UPDRS-III scores in the delta, alpha1 and alpha2 frequency bands. Worsening motor function (i.e. higher UPDRS-III scores) was associated with higher delta PLI values for orbitofrontal ($\beta = .214$; $p = .006$) and anterior cingulate ($\beta = .184$; $p = .005$) seed regions (Fig. 2A). In the alpha1 and alpha2 frequency bands, higher UPDRS-III scores were associated with lower PLI values for parahippocampal (alpha1 $\beta = -.364$; $p < .001$; alpha2 $\beta = -.221$; $p = .015$), inferior temporal (alpha2 $\beta = -.348$; $p < .001$), temporal pole (alpha1 $\beta = -.345$; $p < .001$; alpha2 $\beta = -.294$; $p = .019$), orbitofrontal (alpha1 $\beta = -.244$; $p = .009$; alpha2 $\beta = -.291$; $p = .009$), middle temporal (alpha2 $\beta = -.246$; $p = .017$) and precuneus (alpha2 $\beta = -.307$; $p = .003$) seed regions (Fig. 2B,C).

3.4.2. Cognitive function

3.4.2.1. Global cognitive function. A longitudinal association between PLI and CAMCOG scores was found for multiple seed regions in the alpha1 frequency band: a worsening in test performance was associated with PLI decreases for parahippocampal ($\beta = .237$; $p = .007$), inferior temporal ($\beta = .197$; $p = .022$), temporal pole ($\beta = .286$; $p < .001$), orbitofrontal ($\beta = .205$; $p = .009$), anterior cingulate ($\beta = .210$; $p = .003$) and precuneus ($\beta = .162$; $p = .017$) seed regions (Fig. 2D).

As alpha1 PLI for parahippocampal, temporal pole and orbitofrontal regions was associated with both motor (UPDRS-III) and global cognitive (CAMCOG) function, we assessed relative importance by performing supplementary analyses in which we included CAMCOG and UPDRS-III scores, respectively, as a covariate. Worsening CAMCOG test performance remained associated with parahippocampal ($\beta = .147$; $p = .027$), temporal pole ($\beta = .164$; $p = .028$) and orbitofrontal (CAMCOG $\beta = .146$; $p = .024$) PLI when controlling for UPDRS-III scores. Likewise, UPDRS-III also remained associated with parahippocampal ($\beta = -.307$; $p < .001$) and temporal pole PLI ($\beta = -.262$; $p = .011$) when controlling for CAMCOG performance. The relation between UPDRS-III and orbitofrontal PLI was attenuated ($\beta = -.174$; $p = .073$) when controlling for CAMCOG performance.

3.4.2.2. Specific neuropsychological functions. Impaired semantic fluency was associated with higher delta band PLI of the precuneus seed region ($\beta = -.262$; $p = .002$) as well as with higher beta band PLI of the temporal pole seed region ($\beta = -.338$; $p < .001$).

For the SWM task, impaired strategy use was associated with higher delta band PLI of the temporal pole ($\beta = -.213$; $p = .002$) and middle frontal ($\beta = -.227$; $p < .001$) seed regions. No longitudinal association was found between performance on the PRM-, SSP- or VPT-task and PLI.

4. Discussion

The present study demonstrates decreased delta band and increased alpha1 band functional connectivity of temporal seed regions with the rest of the brain in the earliest clinical stages of PD. Longitudinal assessment over a 4-year period revealed a reversal of the initial changes in alpha1 band functional connectivity and

Table 3
Phase Lag Index measures at baseline and follow-up evaluation. Only frequency bands that yielded significant results in GLM analyses are displayed.

Frequency band	Seed region	Baseline		Longitudinal	
		controls (n = 14)	PD de novo (n = 12)	PD baseline (n = 43)	PD follow-up (n = 43)
Delta (0.5–4Hz)	Parahippocampal	**0.116 (0.108–0.123)**	**0.107 (0.103–0.113)**	0.109 (0.105–0.119)	0.110 (0.102–0.118)
	Inferior temporal	**0.122 (0.109–0.131)**	**0.110 (0.104–0.113)**	0.112 (0.106–0.116)	0.113 (0.105–0.119)
	Temporal pole	**0.116 (0.107–0.127)**	**0.105 (0.101–0.109)**	0.108 (0.104–0.115)	0.112 (0.107–0.116)
	Orbitofrontal	0.114 (0.106–0.124)	0.107 (0.101–0.115)	0.111 (0.106–0.117)	0.113 (0.104–0.118)
	Middle temporal	**0.114 (0.107–0.125)**	**0.106 (0.104–0.109)**	0.106 (0.102–0.114)	0.109 (0.104–0.115)
	Anterior cingulate	0.114 (0.105–0.122)	0.105 (0.099–0.116)	0.107 (0.103–0.115)	0.107 (0.103–0.115)
	Precuneus	0.108 (0.102–0.121)	0.105 (0.099–0.118)	0.108 (0.101–0.112)	0.108 (0.101–0.119)
	Middle frontal	0.108 (0.105–0.115)	0.101 (0.095–0.111)	0.107 (0.102–0.113)	0.108 (0.104–0.112)
Alpha1 (8–10Hz)	Parahippocampal	0.140 (0.129–0.149)	0.148 (0.138–0.157)	0.147 (0.140–0.156)	0.141 (0.137–0.150)
	Inferior temporal	0.142 (0.124–0.159)	0.110 (0.104–0.113)	0.146 (0.137–0.156)	0.142 (0.136–0.150)
	Temporal pole	0.136 (0.129–0.151)	0.142 (0.136–0.146)	0.142 (0.134–0.157)	0.140 (0.131–0.145)
	Orbitofrontal	0.134 (0.129–0.140)	0.139 (0.132–0.149)	0.139 (0.132–0.149)	0.138 (0.130–0.146)
	Middle temporal	**0.138 (0.134–0.142)**	**0.150 (0.140–0.166)**	**0.151 (0.139–0.166)**	**0.143 (0.134–0.152)**
	Anterior cingulate	0.138 (0.126–0.144)	0.134 (0.129–0.137)	0.140 (0.133–0.153)	0.138 (0.129–0.144)
	Precuneus	0.140 (0.132–0.160)	0.148 (0.136–0.167)	0.147 (0.138–0.162)	0.152 (0.133–0.156)
	Middle frontal	0.131 (0.127–0.148)	0.137 (0.134–0.144)	0.140 (0.132–0.148)	0.138 (0.132–0.156)
Alpha2 (10–13Hz)	Parahippocampal	0.109 (0.102–0.117)	0.109 (0.105–0.114)	**0.112 (0.107–0.118)**	**0.109 (0.103–0.116)**
	Inferior temporal	0.115 (0.101–0.117)	0.117 (0.102–0.124)	**0.113 (0.105–0.120)**	**0.108 (0.104–0.113)**
	Temporal pole	0.109 (0.105–0.116)	0.113 (0.110–0.119)	0.111 (0.107–0.116)	0.109 (0.105–0.114)
	Orbitofrontal	0.111 (0.105–0.115)	0.109 (0.105–0.113)	0.110 (0.106–0.113)	0.108 (0.104–0.112)
	Middle temporal	0.118 (0.112–0.120)	0.118 (0.114–0.123)	**0.115 (0.109–0.122)**	**0.110 (0.106–0.118)**
	Anterior cingulate	0.113 (0.102–0.121)	0.112 (0.107–0.119)	0.111 (0.106–0.117)	0.108 (0.103–0.114)
	Precuneus	0.118 (0.112–0.131)	0.117 (0.113–0.125)	**0.117 (0.111–0.129)**	**0.111 (0.105–0.116)**
	Middle frontal	0.111 (0.107–0.117)	0.108(0.105–0.115)	0.110 (0.105–0.115)	0.109 (0.104–0.114)

All values are expressed as median (interquartile range). Significant effects are indicated in bold ($p < 0.05$; FDR adjusted). Please note that the de novo PD group (n = 12) is a subgroup of the full PD group at baseline (n = 43).

additional decreases in alpha2 band functional connectivity evolving in a more widespread cortical pattern that also included the precuneus. Moreover, the longitudinal changes in functional connectivity were closely associated with clinical (both motor and cognitive) deterioration.

In a previous cross-sectional analysis involving the same patient group at baseline, we studied functional connectivity in signal-space and reported increases in alpha1 functional connectivity in early-stage disease (Stoffers et al., 2008a). The present source-space analysis enabled us to study the functional connectivity of specific anatomical brain areas in more detail. The fact that we found connectivity disturbances for temporal brain regions in the early motor stage of PD is in line with neuropathological observations that Lewy body pathology invades the cortex through these same regions in Braak stage 4 (Alafuzoff et al., 2009; Braak and Del Tredici, 2009; Braak et al., 2003). Furthermore, our findings imply that local cortical pathology within a brain region appears to affect the functional interactions of that region with the rest of the brain. The specific involvement of resting-state delta band synchrony has not been reported in early-stage PD before. Delta connectivity is related to memory performance in healthy subjects and disruptions have been reported in AD in relation to reduced cognitive performance. The observation that delta and alpha1 connectivity changes evolve simultaneously from the middle temporal cortex suggests the involvement of different or partly neuronal subpopulations within this seed region or may be the consequence of a frequency shift within a single population.

Our longitudinal GLM analysis revealed a reversal of the initial changes in alpha1 band functional connectivity and additional decreases in alpha2 band functional connectivity for temporal and precuneus seed regions over time. This suggests some degree of remapping of cortical connectivity with disease progression. In line with studies on mild cognitive impairment preceding AD, excessive synchronization at early disease stages might serve as a compensatory mechanism to maintain adequate information processing (Liang et al., 2011). Alternatively, the excessive synchronization may be explained by pathological disinhibition (de Haan et al., 2012). We should note that a potential effect of dopaminergic treatment cannot be ruled out, as in addition to motor disease progression, an increase in dopaminergic medication levels in individual patients over time is inevitable. Although we used LEDD as a covariate in all of our longitudinal analyses and studied the direct longitudinal relation between functional connectivity and LEDD in more detail, a modulatory role of dopaminomimetic treatment could still be present in addition or even opposed to the pathophysiological effects of disease progression in PD. This notion is supported by both electrophysiological and fMRI studies that have shown effects of an acute dopaminergic challenge on measures of functional connectivity (Esposito et al., 2013; Helmich et al., 2010; Silberstein et al., 2005; Stoffers et al., 2008b).

Another finding of our longitudinal analysis was an association between functional connectivity disturbances and clinical measures of disease progression over time for delta, alpha1, and alpha2 frequency bands. Worsening motor performance correlated with lower alpha1 and alpha2 functional connectivity, and with higher delta connectivity in a widespread cortical pattern that involved almost all seed regions. In our study limbic cortical regions were the first to manifest connectivity disturbance, whereas neocortical regions showed altered connectivity only with further progression of disease. Thus, even among the limited number of seed regions assessed in our study, the sequence of development of changes in functional connectivity fits well with the proposed topographical pattern of involvement of cortical brain regions based upon neuropathological studies, in which the transition from Braak stages 4 to 5 is characterized by the involvement of neocortical regions (Alafuzoff et al., 2009; Braak and Del Tredici, 2009; Braak et al., 2003).

In addition to increasing motor impairment, cognitive decline also correlated with functional connectivity changes. In particular impaired global cognitive function was associated with lower alpha1 connectivity for parahippocampal, temporal, orbitofrontal and anterior cingulate regions. The fact that the reduction in functional connectivity of these regions was associated with increases in both

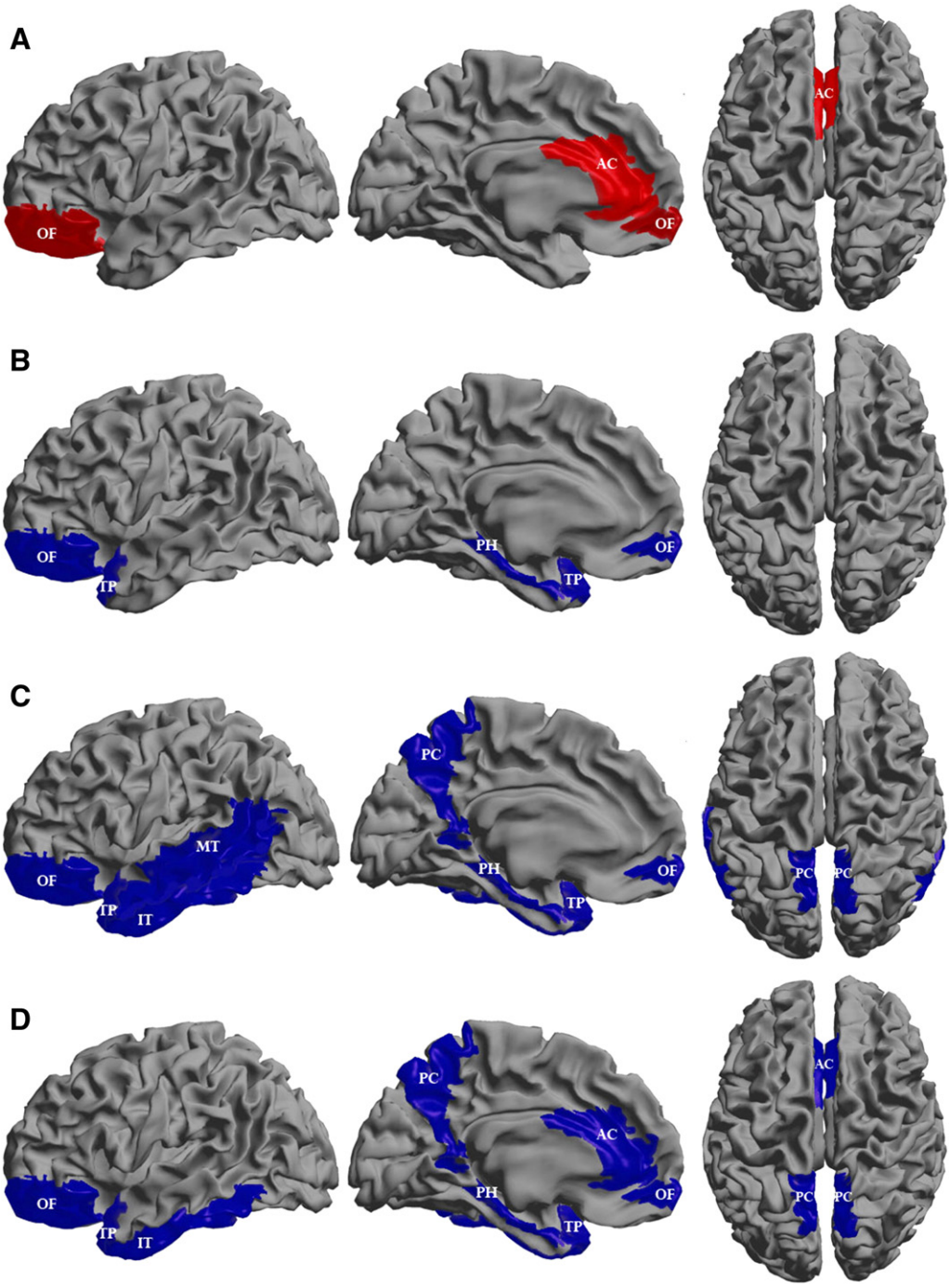

Fig. 2. Mean functional connectivity per seed region in longitudinal relation to UPDRS-III scores (A, delta band; B, alpha1 band; C, alpha2 band) and CAMCOG test performance (D, alpha1 band) in PD patients (n = 43). Left lateral, left medial and dorsal views are shown. Increases and decreases in functional connectivity are depicted in red and blue respectively ($p < 0.05$; FDR adjusted). PH, parahippocampal; TP, temporal pole; IT, inferior temporal; OF, orbitofrontal; MT, middle temporal; AC, anterior cingulate; PC, precuneus.

motor and cognitive impairments suggests a common underlying pathophysiological mechanism. However, even when controlling for motor performance we were still able to show significant associations between functional connectivity and cognitive performance, and vice versa. Therefore, motor and cognitive impairments must also have partly different pathophysiological mechanisms in spite of a common correlation with disease progression. For example, different pathophysiological profiles might underlie distinct clinical subtypes of PD as identified in previous studies (Lewis et al., 2005; Reijnders et al., 2009; van Rooden et al., 2011). The division of our patient group into different clinical variants would be of interest with regard to this "subtyping" hypothesis. However, this was not feasible in our relatively small study sample.

The only change in functional connectivity that was strongly related to a decline in global cognitive function but not to worsening motor function was a decrease in anterior cingulate cortex functional connectivity in the alpha1 frequency band. This suggests a specific involvement of the anterior cingulate cortex in cognitive decline, an observation that fits well with the results of a recent fMRI study in PD in which anterior cingulate connectivity differentiated between patients with and without mild cognitive impairment (Ekman et al., 2012). Moreover, Lewy body pathology in the anterior cingulate

cortex in post-mortem studies correlates strongly with cognitive decline (Kovari et al., 2003).

Although performance on most specific neuropsychological tasks diminished over time in our PD group, only semantic fluency and spatial working memory task performance were associated with alterations in functional connectivity of the seed regions. This may seem counterintuitive, but could be related to the fact that we made a hypothesis-driven selection of cortical seed regions based upon neuropathological data. As a consequence, some brain regions that are known to be involved in specific cognitive tasks were not assessed, such as the superior temporal, inferior parietal and angular cortex, which are associated with semantic fluency (Wende et al., 2012). Moreover, one has to keep in mind that we studied the overall connectivity patterns of seed regions. Thus all direct and indirect connectivity loops of a seed region with the rest of the brain were assessed together, rather than only the selective direct connectivity between two distinct brain regions.

Changes in resting-state functional connectivity measured at the cortical level could find their origin in a common subcortical source (Sakkalis, 2011). In-depth subcortical neurophysiological recordings indeed show evidence of altered temporoparietal-brainstem network activity in the alpha frequency range in PD, but these recordings have only been performed in advanced stage patients receiving deep brain stimulation (Hirschmann et al., 2011; Litvak et al., 2011). Interestingly, these studies also found a frontal/sensorimotor-brainstem network in the beta domain. The absence of such changes in our study is most likely related to the fact that connectivity within this network is known to attenuate after the administration of levodopa (Hirschmann et al., 2013) and the fact that we studied early to moderate instead of advanced stage patients. MR-based functional connectivity studies could provide additional information, but so far focused on the assessment of cortico-striatal pathways in relation to motor symptomatology (Helmich et al., 2010; Wu et al., 2011). Moreover, such studies lack the temporal resolution to study subtle differences in phase relationships, or to examine frequency-specific connectivity patterns at frequencies above ~0.1 Hz.

A potential limitation of the present study is the fact that our control subjects were age-matched to the de novo PD patients, but not to the more advanced (i.e. older) PD patients, which precluded inclusion of the controls and patients in a single longitudinal GLM analysis. Instead, we chose to perform separate longitudinal GLM analyses for both patients and controls. As a consequence, the time effects observed in the PD patients are not controlled for potential effects of normal aging. However, the separate GLM analysis in our control group did not show any connectivity changes over time. Moreover, the longitudinal connectivity changes in our PD sample were associated with clinical measures of disease progression. Therefore, it is unlikely that an effect of normal aging has severely confounded our results. A second limitation of our study is that several subjects were lost to follow-up, which was partly due to mortality, but also due to withdrawal from the study. Additionally, some patient data had to be excluded from analysis due to partial missing data (MR scans at follow-up). However, since those patients that had incomplete follow-up or that withdrew from the study reported rather high subjective disease impairment (this being the main reason not to participate anymore), their exclusion can only have led to an underestimation of true effects. A last methodological consideration regards the choice of frequency bands in MEG/EEG studies. In our study, low and high alpha oscillations were analyzed separately, as these are considered to reflect different cognitive processes (Klimesch, 1997). Although alternative choices are possible (Foffani and Priori, 2006; Priori et al., 2004), we chose to assess the beta spectrum as a single band in line with our previous studies. Upper gamma band activity (>48 Hz) was excluded from all analyses, in order to avoid the 50 Hz electromagnetic radiation artifact from the environment.

A major strength of the present study is its longitudinal design, which is superior over a cross-sectional design, especially when searching for disease progression markers associated with clinical features. Further (clinical) follow-up of our study cohort will enable us to evaluate MEG-derived neurophysiological parameters that are associated with clinical measures of disease progression as potential predictive markers for clinical outcome, in particular for the development of dementia and psychosis. Both local oscillatory activity (Olde Dubbelink et al., 2013) as well as functional connectivity markers might serve this purpose. Another strength of the present study is that the MEG data analysis was performed in source-space, which offers the perspective of multimodal imaging, in which the relation between functional and structural connectivity can be explored in more detail. The method used also facilitates future direct comparisons between fMRI and MEG in the assessment of brain network topology (Bullmore and Sporns, 2009).

In conclusion, we found changes in resting-state functional connectivity for temporal seed regions with the rest of the brain in the earliest clinical stages of PD. With disease progression changes in functional connectivity evolved to include more widespread brain regions in close relation to clinical (both motor and cognitive) deterioration. Altered cortico-cortical resting-state functional connectivity in PD thus appears to reflect clinically relevant phenomena and holds promise as a marker of disease progression. Further longitudinal follow-up of our PD subjects will allow us to assess whether changes in functional connectivity can serve as a predictor of cognitive decline, dementia and/or psychosis.

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