Disrupted brain network topology in Parkinson’s disease: a longitudinal magnetoencephalography study

Kim T. E. Olde Dubbelink,1 Arjan Hillebrand,2 Diederick Stoffers,1 Jan Berend Deijen,3 Jos W. R. Twisk,4 Cornelis J. Stam2 and Henk W. Berendse1

1 Department of Neurology, Neuroscience Campus Amsterdam, VU University Medical Centre, Amsterdam, The Netherlands
2 Department of Clinical Neurophysiology and Magnetoencephalography Centre, VU University Medical Centre, Amsterdam, The Netherlands
3 Department of Clinical Neuropsychology, VU University, Amsterdam, The Netherlands
4 Department of Clinical Epidemiology and Biostatistics, VU University Medical Centre, Amsterdam, The Netherlands

Correspondence to: Ms. Kim T.E. Olde Dubbelink, MD,
Department of Neurology,
VU University Medical Centre,
PO Box 7057,
1007 MB Amsterdam,
The Netherlands
E-mail: kte.oldedubbelink@vumc.nl

Although alterations in resting-state functional connectivity between brain regions have previously been reported in Parkinson’s disease, the spatial organization of these changes remains largely unknown. Here, we longitudinally studied brain network topology in Parkinson’s disease in relation to clinical measures of disease progression, using magnetoencephalography and concepts from graph theory. We characterized whole-brain functional networks by means of a standard graph analysis approach, measuring clustering coefficient and shortest path length, as well as the construction of a minimum spanning tree, a novel approach that allows a unique and unbiased characterization of brain networks. We observed that brain networks in early stage untreated patients displayed lower local clustering with preserved path length in the delta frequency band in comparison to controls. Longitudinal analysis over a 4-year period in a larger group of patients showed a progressive decrease in local clustering in multiple frequency bands together with a decrease in path length in the alpha2 frequency band. In addition, minimum spanning tree analysis revealed a decentralized and less integrated network configuration in early stage, untreated Parkinson’s disease that also progressed over time. Moreover, the longitudinal changes in network topology identified with both techniques were associated with deteriorating motor function and cognitive performance. Our results indicate that impaired local efficiency and network decentralization are very early features of Parkinson’s disease that continue to progress over time, together with reductions in global efficiency. As these network changes appear to reflect clinically relevant phenomena, they hold promise as markers of disease progression.

Keywords: Parkinson’s disease; resting-state functional brain networks; magnetoencephalography (MEG); longitudinal; graph theory

Abbreviations: CAMCOG = Cambridge Cognitive Examination; MEG = magnetoencephalography
Introduction

Normal brain function relies on the interaction of different brain regions that are integrated within a large-scale complex network (Schnitzler and Gross, 2005; Bullmore and Sporns, 2012). In recent years, the organization of the human brain network has been studied extensively using concepts from graph theory (Watts and Strogatz, 1998; for review see Reijneveld et al., 2007; Bullmore and Sporns, 2009). We have come to appreciate that brain networks in humans are organized according to a highly efficient topology that combines a high level of local integration (i.e. dense local clustering of connections) with a high level of global efficiency (i.e. critical long-distance connections); that is, a so-called small-world organization (Bassett and Bullmore, 2006; Stam and van Straaten, 2012). In addition, brain networks in healthy subjects contain a subset of relatively important highly connected regions (‘hubs’) (Achard et al., 2006), that are also mutually and densely interconnected, forming a connectivity backbone or ‘rich club’ crucial for efficient brain communication (van den Heuvel and Sporns, 2011).

Several studies have demonstrated that brain diseases disrupt the normal network organization. In Alzheimer’s disease, a deviation from the small-world network organization towards a more randomly organized network has been described in multiple studies. In addition to a loss of both global network efficiency and local integration (Supékar et al., 2008; Stam et al., 2009; Sanz-Arigita et al., 2010), a selective vulnerability of hub regions has been reported (Buckner et al., 2009; Stam et al., 2009). In many other brain diseases such as epilepsy, schizophrenia and brain tumours, the study of large-scale brain networks has equally contributed to a better understanding of the underlying pathophysiological mechanisms (Ponten et al., 2007; Rubinov et al., 2009; Douw et al., 2010).

Parkinson’s disease is a neurodegenerative disorder characterized by prominent motor features as well as a variety of nonmotor disturbances including cognitive impairment (Chaudhuri et al., 2006). Clinically, the phenotype of Parkinson’s disease is heterogeneous and can be classified into distinct subtypes that are characterized by different rates of progression of motor and nonmotor symptoms over time (Foltynie et al., 2002; van Roode et al., 2011; Eggers et al., 2012). Previous imaging studies in patients with Parkinson’s disease have shown changes in the strength of functional connectivity between distributed brain regions associated with clinical features (Stoffers et al., 2008a; Tessitore et al., 2012; Ponsen et al., 2013). To what extent the overall brain network topology is affected by these changes is not well known. So far, only a single study addressed overall functional network topology in Parkinson’s disease and, using functional MRI, uncovered a loss of both local and global efficiency in the patients’ brain networks when compared to healthy controls (Skidmore et al., 2011). However, these observations have not been confirmed by other techniques, nor have relative regional importance (high centrality or ‘hubness’) and the relationship with clinical features been addressed using a longitudinal design.

A critical point in graph theoretical studies is the difficulty in comparing networks across different groups and conditions. Analysis of graphs derived from brain networks requires a normalization step, commonly performed through thresholding and/or comparing the observed network to randomized networks. However, these approaches do not provide a unique or consistent solution (van Wijk et al., 2010; Langer et al., 2013). An alternative method that overcomes this problem is the construction of a so-called minimum spanning tree. With this approach a unique graph is constructed from a weighted network (Wang et al., 2008; van Steen, 2010). It connects all network elements in such a way that the connection cost (sum of all connection distances) is minimized without forming cycles. In this way, networks with the same number of nodes and connections are obtained, which facilitates the direct comparison of network topology across conditions and groups while circumventing potential biases that can be introduced by a normalization step.

So far, a minimum spanning tree has been constructed in only a few brain imaging studies. A study in epilepsy identified critical nodes in a temporal lobe epileptic network on the basis of the minimum spanning tree (Ortega et al., 2008), whereas a study in Alzheimer’s disease showed segregation differences between the default mode brain network of patients and control subjects (Ciftci, 2011). More recently, a minimum spanning tree analysis was successfully applied in a whole brain network study that investigated brain maturation in children by means of EEG (Boersma et al., 2013). In this study, minimum spanning trees captured developmental changes in network topology, supporting results derived from a conventional network analysis (Boersma et al., 2011).

The aim of the present study was to examine the large-scale structure of resting-state brain networks in Parkinson’s disease, using concepts from graph theory. We set out to assess (i) whether network topology is affected in early-stage, untreated Parkinson’s disease; and (ii) whether alterations in network topology develop with progression of disease in association with clinical deterioration. We used magnetoencephalographic (MEG) recordings from a longitudinal cohort study involving patients with Parkinson’s disease with varying disease duration (including 12 early-stage, untreated patients) and controls that had been analysed previously with respect to local brain activity patterns (Olde Dubbelink et al., 2013). Whole-brain functional networks were constructed using the commonly used approach that involves a normalization step, as well as by means of minimum spanning tree construction. We calculated several measures to assess local integration, global efficiency and relative node importance within the networks and hypothesized that brain networks of patients with Parkinson’s disease would display progressive decreases in both local integration and global efficiency over time that would be accompanied by a loss of centrality of individual brain regions within the network.

Materials and methods

Participants

Participants were selected from a longitudinal study cohort in which a total of 70 patients (disease duration 0–13 years, including 18 de novo
patients) with idiopathic Parkinson’s disease and 21 healthy control subjects (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 control subjects completed follow-up measurements. Three patients had passed away and 13 participants (eight patients and five control subjects) were lost to follow-up. Ten patients and a single control subject had relatively severe artefacts 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) 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 Centre. Ethics review criteria conformed to the Declaration of Helsinki.

Participant characteristics

Table 1 summarizes the clinical characteristics of all participants included in the current analysis. 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 (Fahn et al., 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). None of the patients fulfilled clinical diagnostic criteria for Parkinson’s disease-related dementia at baseline, whereas at the follow-up evaluation four patients were classified as having Parkinson’s disease-related dementia, 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 (UNESCO, 1997). The total dose of dopaminomimetics was converted to a levodopa equivalent daily dose using a previously described conversion rate (Olde Dubbelink et al., 2013). At the time of the follow-up evaluation, two patients were using rivastigmine.

Data acquisition

MEG data were recorded in an eyes-closed resting-state condition for 5 min, as described previously (Olde Dubbelink et al., 2013). During acquisition, patients were in the ‘ON’ medication state, with exception of the de novo subgroup at baseline. Structural T1-weighted MRI was performed in all subjects (baseline: 1.0 T, Impact, Siemens; follow-up: 3.0 T, Signa, GE healthcare). Vitamin E capsules were placed at the same anatomical landmarks where head position coils had been placed during MEG-registration.

Data preprocessing

Both baseline and follow-up data sets were split up into epochs of 4096 samples (13.11 s). Channels and epochs containing artefacts were discarded after visual inspection by one of the investigators blinded for diagnosis and time registration point (K.O.D.). 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 onto an anatomical framework consisting of 78 cortical regions identified by means of automated anatomical labelling (Tzourio-Mazoyer et al., 2002; Gong et al., 2009). For this purpose, MRI and MEG data were co-registered 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 were then spatially normalized to a template MRI using a segmentation toolbox in Statistical Parametrical Mapping 8 (Ashburner and Friston, 2005; Weiskopf et al., 2011), after which anatomical labels were applied.

Time-series of neuronal activation were estimated for six 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 s (range 105–380 s) as input for the beamformer computations. This resulted in a total of six sets (one for each frequency band) of 78 time-series (one for each cortical region). For each subject, five artefact-free epochs per frequency band were selected for further analysis by one of the investigators still blinded for diagnosis and time registration point (K.O.D.). The stability of findings over different epoch selections was investigated in supplementary analyses (Supplementary Tables 1 and 2).

Functional connectivity analysis

Functional connectivity matrices were determined by computing the phase lag index between all pair-wise combinations of cortical regions (Stam et al., 2007). This measure (range between 0 and 1) reflects true interactions between two oscillatory signals through quantification

Table 1 Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Parkinson’s disease patients</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (n = 14)</td>
<td>Follow-up (n = 14)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>10/4</td>
<td>8/4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.0 ± 8.55</td>
<td>64.7 ± 9.81</td>
</tr>
<tr>
<td>Education level (0/1/2/3/4/5/6)</td>
<td>0/0/1/3/1/8/1</td>
<td>0/0/1/3/1/8/1</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>CAMCOG</td>
<td>99.2 ± 2.79</td>
<td>99.5 ± 1.56</td>
</tr>
<tr>
<td>Unified Parkinson’s Disease Rating</td>
<td>0.71 ± 1.59</td>
<td>2.62 ± 3.55</td>
</tr>
<tr>
<td>Scale motor score</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Levodopa equivalent daily dose (mg/day)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
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</table>

Values are expressed as mean ± standard deviation unless otherwise indicated. Note that the de novo group (n = 12) is a subgroup of the total patient group at baseline (n = 43).
of the (non-zero lag) phase coupling, thereby discarding the effects of volume conduction and field spread. Functional connectivity analyses and subsequent analyses of network topology were performed with BrainWave software (CS, version 0.9.72, available from http://home.kpn.nl/stam7883/brainwave.html).

**Analysis of network topology**

Figure 1 provides a schematic overview of the different analysis steps that resulted in the computation of network measures for both the commonly used weighted graph analysis approach (normalized mean clustering coefficient and normalized averaged shortest path length) and the minimum spanning tree (leaf number, tree hierarchy, eccentricity and betweenness centrality). Supplementary Fig. 1 provides an example of a minimum spanning tree with the associated measures.

**Weighted graph analysis**

In principle, networks can be represented by graphs, which are sets of vertices (nodes) and corresponding sets of edges (connections).
between those vertices. We constructed graphs of 78 vertices (i.e. the 78 cortical regions) and used all information from the functional connectivity (phase lag index) matrix. This resulted in fully connected, weighted, undirected networks, in which the connection strength between each pair of vertices (i.e. the weight) was defined as their connectivity value (Fig. 1).

Various measures can be used to characterize a weighted network (for a comprehensive overview, see Rubinov and Sporns, 2010). Two fundamental measures are the clustering coefficient and the average shortest path length. The unweighted clustering coefficient denotes the likelihood that neighbours of a vertex are also connected to each other, and characterizes the tendency of nodes to form local clusters. We used the weighted equivalent of this measure to characterize local clustering (Stam et al., 2009). The average weighted shortest path length is a measure for global integration of the network. It is defined as the harmonic mean of shortest paths between all possible vertex pairs in the network, where the shortest path between two vertices is defined as the path with the largest total weight (Stam et al., 2009).

Values of both the weighted clustering coefficient and the weighted path length depend on edge weights (connectivity values) and network structure, but also on network size (number of nodes). To enable comparison of results across studies, clustering coefficient and path length were normalized, that is, they were expressed as a function of measures generated from random networks. To this end, an ensemble of 1000 surrogate random networks was derived from the original networks by randomly reshuffling the edge weights. Subsequently, the ratio between the weighted and random clustering coefficient as well as the ratio between the weighted and random path length was computed. In this way, the normalized average weighted clustering coefficient as well as the ratio between the weighted and random path length depend on edge weights (connectivity values) and network structure, but also on network size (number of nodes). To enable comparison of results across studies, clustering coefficient and path length were normalized, that is, they were expressed as a function of measures generated from random networks. To this end, an ensemble of 1000 surrogate random networks was derived from the original networks by randomly reshuffling the edge weights. Subsequently, the ratio between the weighted and random clustering coefficient as well as the ratio between the weighted and random path length was computed. In this way, the normalized average weighted clustering coefficient and the normalized average weighted path length were obtained for each epoch, reflecting local integration and global efficiency, respectively. Results of five epochs were averaged per subject.

Minimum spanning tree
The minimum spanning tree of an undirected weighted graph is a unique subgraph that connects all the nodes in such a way that the total cost (the sum of all the edge distances) is minimized without forming cycles. In our case, the tree with the maximum connection strength (phase lag index) was determined for every connectivity matrix, equivalent to a minimum spanning tree as obtained by Kruskal’s algorithm (Kruskal, 1956). In short, first all connectivity values were ordered in an ascending way. Then the construction of the tree was started with the edge, with the highest edge phase lag index. Consecutive high phase lag index links were added until all nodes (n) were connected in a loopless subgraph consisting of n – 1 edges (Fig. 1). When adding an edge resulted in the formation of a cycle, this edge was skipped. In our study, trees of 78 nodes and 77 edges were constructed. Extreme topologies of a minimum spanning tree are a decentralized line-like tree with all nodes on a single line and, at the other end of the spectrum, a star-like or centralized configuration with one central node (Supplementary Fig. 1).

From these minimum spanning tree graphs several measures can be computed that give information about the topological properties of the tree. Leaves are nodes on the tree with degree = 1. Each tree has a lower bound of two and an upper bound of n – 1 leaves (with n being the number of vertices). For each tree, leaf number is defined as the number of leaves divided by the maximum number of leaves possible given the size of the tree. Eccentricity of a node is defined as the longest distance between that node and any other node of the tree.

The eccentricity of a node is low if this node is central in the tree. The betweenness centrality of a node i is the number of shortest paths between any pair of nodes h and j that are running through i, divided by the total number of paths between h and j. Betweenness centrality scales between 0 and 1. Eccentricity and betweenness centrality are different criteria for centrality or relative nodal importance and may point out the hubs (high centrality nodes) in a tree. For the initial comparison between groups and the changes over time with regard to these node-specific measures, eccentricity was averaged over nodes whereas for betweenness centrality, the maximum value was taken per tree. In additional analyses we subsequently studied node-specific values for all individual cortical regions.

For optimal performance of the network, different tree topological criteria have to be met. First, efficient communication between all vertices is needed, which would require a star-like topology (i.e. maximum number of leaves; shortest possible average path length of two). However, in such a tree the central node might easily be overloaded since it has a betweenness centrality of one. Therefore, the second criterion would be prevention from overloading hubs by setting a maximal betweenness centrality for any of the tree nodes. The optimal tree should then reflect the best possible balance between both criteria. To aim at, a tree hierarchy measure TH was developed as an indicator of the balance between efficient communication paths and overload prevention (Boersma et al., 2013). It is defined as:

\[ TH = \frac{L}{2mBC_{\text{max}}} \]

where \( L \) is the leaf number; \( m \) the number of vertices – 1; and \( BC_{\text{max}} \) the maximum value of betweenness centrality. To assure tree hierarchy ranges between 0 and 1, the denominator is multiplied by two. If leaf number = 2 (i.e. a line-like topology), and \( m \) approaches infinity, tree hierarchy approaches 0. If leaf number = \( m \) (i.e. a star-like topology) tree hierarchy approaches 0.5. For leaf numbers between these two extreme situations, tree hierarchy can have higher values.

Statistical analysis
To study network topology in early-stage untreated disease, baseline network measures were assessed in de novo patients (\( n = 12 \)) compared with age-matched controls (\( n = 14 \)) by means of a single general linear model analysis per network measure and per frequency band. Sex was added as a potential confounder.

Longitudinal changes in network measures were analysed within the total group of patients (\( n = 43 \)) and in the control group (\( n = 14 \)) by means of a single general linear model analysis for repeated measures per network measure and per frequency band. Sex, baseline age and mean difference in levodopa equivalent daily dose were added as potential confounders.

Within the total group of patients (\( n = 43 \)) the relationship between the longitudinal course of network measures and clinical measures of motor and cognitive function was investigated by means of generalized estimated equations with exchangeable working correlation matrix (Zeger et al., 1988) using Cambridge Cognitive Examination CAMCOG (measure of cognitive function) and Unified Parkinson’s Disease Rating Scale motor scores (measure of motor function) as dependent variables. Sex, baseline age and the levodopa equivalent daily dose were added as covariates in all analyses, as was education level (dichotomized) in analyses involving cognition. To limit the number of comparisons, only network measures that displayed time effects with \( P < 0.10 \) in the longitudinal general linear model analysis were selected as independent variables.
All analyses were performed using the SPSS Statistics 20.0 software package (IBM Corporation). A significance level of 0.05 (two-tailed) was applied. Because of the explorative character of the study, corrections for multiple testing were not applied.

## Results

### Network topology in early-stage, untreated disease

We first studied differences in network topology between early-stage, untreated (de novo) patients ($n = 12$) and controls ($n = 14$). We observed that delta band normalized weighted clustering coefficient was smaller in de novo patients when compared with controls [$F(1,23) = 5.33$, $P = 0.030$], whereas normalized weighted path length did not differ between groups in any frequency band (Fig. 2A and Supplementary Table 3; weighted graph analysis). Additionally, minimum spanning trees of de novo patients revealed lower leaf number [$F(1,23) = 9.69$, $P = 0.005$] and lower tree hierarchy $T_h$ [$F(1,23) = 5.60$, $P = 0.027$] in the alpha2 frequency band when compared to controls (Fig. 2B and Supplementary Table 3). Centrality measures (i.e. eccentricity and betweenness centrality values) of minimum spanning tree-derived brain networks did not differ between groups in any frequency band.

Taken together, these results point towards a decrease in local integration with preserved global efficiency of the brain network in the early motor stage of Parkinson’s disease.

### Longitudinal changes in network topology

Longitudinal changes in network topology were assessed in the full group of patients ($n = 43$) and also in controls ($n = 14$), to account for possible effects of normal ageing.

Within the group of patients, we observed ongoing decreases in local integration of the brain network over time: normalized weighted clustering coefficient decreased in theta [$F(1,39) = 4.33$, $P = 0.044$], alpha1 [$F(1,39) = 4.76$, $P = 0.035$] and alpha2 [$F(1,39) = 4.39$, $P = 0.043$] frequency bands. Normalized weighted path length also decreased over time, but in the alpha2 frequency band only [$F(1,39) = 12.79$, $P = 0.001$; Fig. 3A and Supplementary Table 4; weighted graph analysis]. Minimum spanning tree analysis was also able to capture longitudinal changes in brain network topology in the patient group (Fig. 3B and Supplementary Table 4): leaf number decreased over time in the theta band [$F(1,39) = 8.86$, $P = 0.005$], whereas tree hierarchy decreased in the delta band [$F(1,39) = 4.88$, $P = 0.033$]. Moreover, eccentricity increased over time in the theta band [$F(1,39) = 5.54$, $P = 0.024$], indicating a reduction in overall node centrality (Supplementary Fig. 1, shift towards a more line-like tree configuration). Post hoc results with regard to regional distribution patterns revealed this effect to be present for multiple regions, most prominently in (orbito-)frontal and temporal regions (Fig. 4 and Supplementary Table 5). We performed an additional analysis of node centrality distribution in the weighted graph in the theta band in comparison with these minimum spanning tree results (Supplementary material and Supplementary Fig. 2).

Within the group of controls, we observed that normalized weighted clustering coefficient decreased over time in alpha1 [$F(1,11) = 5.68$, $P = 0.036$] and alpha2 [$F(1,11) = 5.36$, $P = 0.041$] frequency bands, whereas normalized weighted path length did not show any effect of normal ageing in any frequency band (Fig. 3A and Supplementary Table 2). With respect to the minimum spanning tree analysis, leaf number decreased over time in alpha1 [$F(1,11) = 6.54$, $P = 0.027$], alpha2 [$F(1,11) = 28.6$, $P < 0.001$] and gamma [$F(1,11) = 6.11$, $P = 0.031$] frequency bands, whereas $T_h$ decreased over time in the beta band only [$F(1,11) = 5.77$, $P = 0.035$] (Fig. 3B and Supplementary Table 2).
There were no changes in centrality measures (i.e. eccentricity or betweenness centrality values) over time in the control group.

In summary, we find a progressive impairment in local integration with an additional loss of global efficiency in Parkinson’s disease brain network topology with progression of disease. Minimum spanning tree results confirm the loss of global efficiency, while providing additional information about the efficiency of individual brain regions. With regard to local integration, effects of normal ageing have to be taken into account as well, in particular when studying the alpha frequency domain.

**Relationship between network topology and clinical features**

To evaluate the relationship between network topology and clinical measures of disease progression, we assessed the longitudinal relationship between network measures and CAMCOG (measure of cognitive function) as well as Unified Parkinson’s Disease Rating Scale motor scores (measure of motor function) in the full group of patients (n = 43). We observed a longitudinal association between worsening CAMCOG performance and lower theta band normalized weighted clustering coefficient (β = 0.173; P = 0.013) and path length (β = 0.201; P = 0.017). Higher Unified Parkinson’s Disease Rating Scale motor scores, reflecting deteriorating motor function, were associated with lower alpha2 band normalized weighted path length (β = −0.125; P = 0.028) over time. With respect to the minimum spanning tree analysis, a longitudinal association was found between worsening CAMCOG performance and higher alpha1 band eccentricity (β = −0.175; P = 0.043). Higher Unified Parkinson’s Disease Rating Scale motor scores were associated with lower delta band leaf number (β = −0.120; P = 0.048) and tree hierarchy...
Additional minimum spanning tree analysis, which is free of normalization biases, revealed a shift towards a decentralized network configuration as marked by decreases in leaf number and tree hierarchy, even in early-stage, untreated patients. With disease progression, the changes in network configuration accumulate, including further decreases in leaf number and tree hierarchy, and an additional increase in eccentricity in particular in (orbito)frontal and temporal regions. Taken together, the minimum spanning tree results confirm the loss of global efficiency, while providing additional information about the efficiency of individual brain regions.

The present results obtained using MEG confirm the overall findings of a recent functional MRI study in which graph theoretical analysis was applied for the first time to study the functional brain network organization in Parkinson’s disease (Skidmore et al., 2011). In this cross-sectional study in advanced patients, nodal and global efficiency of brain networks were smaller when compared with control subjects. The results of our MEG study extend the findings of the aforementioned functional MRI study by demonstrating that the alterations in Parkinson’s disease network topology are already present in the earliest clinical motor stages of the disease. Moreover, by using a longitudinal approach, we were able to demonstrate that the changes in brain network topology further evolve with disease progression, in close association with clinical measures of motor and cognitive function.

Previous MEG and EEG studies in Parkinson’s disease have focused on the strength of functional connectivity between distinctive brain regions, demonstrating increases and decreases in coupling strength in distinctive spatial patterns and at different clinical disease stages (Silberstein et al., 2005; Stoffers et al., 2008a; Tessitore et al., 2012). Although functional connectivity disturbances clearly represent an important pathophysiological mechanism in Parkinson’s disease, the interpretation of their significance is not straightforward. For example, although excessive synchronization has often been put forward as a compensatory mechanism (Appel-Cresswell et al., 2010; Ponsen et al., 2013), it may also be explained by pathological disinhibition following neurodegenerative damage (de Haan et al., 2012). The application of a network theory framework examines functional connectivity changes at a higher, integrative or network organization level, independent of coupling strength (Stam and van Straaten, 2012). In the present study, we demonstrated that brain network organization in Parkinson’s disease moves toward a more random structure. Therefore, it would seem that information processing in the parkinsonian brain is not only quantitatively altered, but also less efficiently organized.

The progressive changes in network properties we observed in this study were associated with clinical measures of disease severity. Over time, decreased clustering and shortened paths in the theta band were associated with worsening CAMCOG performance, independent of age or dopaminergic medication. In contrast, decreased path length in the alpha2 band correlated with worsening motor function. The differential association of network properties in these two frequency bands with motor and cognitive function, respectively, suggests that motor and cognitive impairments may have (partly) different underlying pathophysiological mechanisms. From these observations, it is tempting to speculate
that decreases in regional and global efficiency in the theta band functional brain network hold promise as (surrogate) markers of cognitive decline in Parkinson’s disease, and possibly involve non-dopaminergic pathophysiological pathways that include the cholinergic system (Ray and Strafella, 2012). Moreover, disruption of the normal functional brain network organization may have predictive value for the development of Parkinson’s disease-related dementia. We should, however, note that a potential effect of dopaminergic treatment cannot be completely 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 the levodopa equivalent daily dose as a covariate in all of our longitudinal analyses and studied the direct longitudinal relation between network measures and the levodopa equivalent daily dose in more detail, a modulatory role of dopaminomimetic treatment could therefore still be present in addition or even opposed to the pathophysiological effects of disease progression in Parkinson’s disease. This notion is supported by both electrophysiological and functional MRI studies that have shown effects of an acute dopaminergic challenge on measures of functional connectivity in Parkinson’s disease (Silberstein et al., 2005; Stoffers et al., 2008b; Helmich et al., 2010; Esposito et al., 2013), but this has not been investigated in the assessment of network topology so far.

In the present study we found a reduction in nodal efficiency in Parkinson’s disease that predominantly involved orbitofrontal and temporal regions, as indicated by increasing minimum spanning tree eccentricity values for these brain regions over time. As another measure of centrality, betweenness centrality, remained unchanged over time, the two measures may capture different aspects of node centrality within a network. Nevertheless, the observed region-specific increase in eccentricity implies that certain brain regions are selectively vulnerable to the progressive network injury that results from Parkinson’s disease-related pathology. Our observations are in line with structural imaging studies showing atrophy of frontal and temporal cortices in Parkinson’s disease (Burton et al., 2004; Biundo et al., 2011), and also with neuropathological observations that Lewy body pathology first invades the neocortex through these same regions (Braak et al., 2003; Alafuzoff et al., 2009; Braak and Del Tredici, 2009). Interestingly, in Alzheimer’s disease, a substantial spatial overlap between cortical hubs and regions showing large amounts of Alzheimer’s disease-related pathology (amyloid-β deposition) has been demonstrated (Buckner et al., 2009), implying that hubs are selectively vulnerable in Alzheimer’s disease. Although preliminary, we suggest that the approach taken in our study could similarly offer the future prospect of being able to relate Parkinson’s disease-specific pathology to changes in brain network structure, thus bridging the gap between neuropathological changes and clinical symptomatology that still exists in neurodegenerative diseases (Pievani et al., 2011).

Normal ageing is accompanied by alterations in brain network topology that include reduced local efficiency and changes in modular organization (Achard and Bullmore, 2007; Meunier et al., 2009). To account for the potential effects of normal ageing, we also studied brain network topology changes in control subjects and included age as a covariate in all our analyses. Our longitudinal analysis in controls revealed changes in local clustering, leaf number and tree hierarchy, but not in path length or eccentricity. In particular the observed decrease in alpha1 band local clustering in our patient sample could therefore have been confounded by an effect of normal ageing and therefore needs to be interpreted with caution. However, as the controls did not have any changes in network measures in the delta and theta frequency bands, the changes observed in these frequency bands in patients most likely represent a true disease effect. This assumption is further supported by the fact that most changes in network properties in our patient sample were associated with clinical measures of disease progression.

In the present study we constructed minimum spanning trees of brain networks in addition to a more conventional application of graph analysis techniques, as a possible solution for the threshold and normalization problems encountered with conventional approaches. An important caveat when using a minimum spanning tree is that not all, but only the ‘core’ connections are taken into account; some relevant information about the networks may therefore be lost. For instance, local clustering is a feature that cannot be examined in minimum spanning tree-derived brain networks. On the other hand, the construction of a minimum spanning tree creates a unique structure that reflects the most important routes of information flow through a network. In the present study, we have shown that a minimum spanning tree captures (clinically relevant) changes in network topology that are in agreement with results derived from more commonly used techniques, but without the need of a normalization step to correct for network size, connectivity strength and density. This is of particular importance, as there is currently no normalization step that allows for a reliable and fully unbiased characterization of network topology (van Wijk et al., 2010). When using conventional network measures, differences in connectivity strength, graph size and connectivity density within or between subjects may therefore yield spurious findings or mask true effects. Extraction and analysis of the minimum spanning tree seems to offer an elegant solution for these pitfalls, one that has not received much attention in the neuroscience literature so far.

A methodological limitation of our study is that several subjects were lost to follow-up or had to be excluded because of partial missing data. However, patients that had incomplete follow-up or that withdrew from the study reported a rather high level of subjective disease impairment. Their withdrawal or exclusion can therefore only have led to an underestimation of true effects. Furthermore, as our study is one of the first exploratory studies on network topology in Parkinson’s disease, we did not apply corrections for multiple testing. We focused on interpreting general patterns of the findings instead, but we report exact P-values for all individual statistical comparisons performed for the reader’s interpretation as well. Moreover, individual statistical calculations displayed robustness against a data resampling approach (Supplementary material). Nevertheless, future studies are necessary to confirm our results, using an independent study sample and in a hypothesis-driven study design.

Lastly, the possible influence of parkinsonian tremor, which generally has a frequency in the theta range (4–8 Hz), needs to be considered. Although epochs were carefully selected for the...
absence of visible tremor, some studies have reported linear synchrony (coherence) between tremor, as measured with EMG, and MEG oscillatory rhythms at tremor frequencies in the contra-lateral motor cortex (Volkmann et al., 1996; Timmermann et al., 2003). Although the influence of tremor on measures of brain network topology has never been studied, these area-specific coherent oscillations could theoretically have influenced the computation of network properties in our study via the intermediate computation of functional connectivity strength. However, as functional connectivity strength was corrected for in both our conventional graph and minimum spanning tree analyses, this is highly unlikely.

A major strength of our study is the application of a minimum spanning tree as a reliable and fully unbiased graph theoretical approach. It offers an elegant solution to the threshold and normalization problems experienced in most graph theoretical imaging studies. Furthermore, the performance of MEG data analysis in source-space with the use of a standard anatomical atlas offers the perspective of multimodal imaging, in which the relation between functional and structural network characteristics can be explored across imaging modalities, in particular MEG and functional MRI. Another strength of our study is its longitudinal design, which is superior over a cross-sectional design when studying the association between changes in functional brain network organization and clinical disease progression. Further (clinical) follow-up of our study cohort will enable us to evaluate MEG-derived neurophysiological measures as potential predictive markers for clinical outcome, in particular the development of Parkinson’s disease-related dementia.

In conclusion, in this first-ever longitudinal study of changes in functional brain network topology in Parkinson’s disease, we used both the construction of conventional graphs and the minimum spanning tree to demonstrate that impaired local efficiency and network decentralization are very early features of Parkinson’s disease: diagnosis and management. Lancet Neurol 2006; 5: 235–45.

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Supplementary material

Supplementary material is available at Brain online.

References


