BRAIN AREAS WITH EPILEPTIC HIGH FREQUENCY OSCILLATIONS ARE FUNCTIONALLY ISOLATED IN MEG VIRTUAL ELECTRODE NETWORKS

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ABSTRACT

OBJECTIVE
Previous studies have associated network hubs and epileptiform activity, such as spikes and high frequency oscillations (HFOs), with the epileptogenic zone. The epileptogenic zone is approximated by the area that generates interictal epileptiform activity: the irritative zone. Our aim was to determine the relation between network hubs and the irritative zone.

METHODS
Interictal resting-state MEG recordings of 12 patients with refractory epilepsy were analysed. Beamformer-based virtual electrodes were calculated at 70 locations around the epileptic spikes (irritative zone) and in the contralateral hemisphere. Spikes and HFOs were marked in all virtual electrodes. A minimum spanning tree network was generated based on functional connectivity (phase lag index; PLI) between all virtual electrodes to calculate the betweenness centrality, an indicator of hub status of network nodes.

RESULTS
Betweenness centrality was low, and PLI was high, in virtual electrodes close to the centre of the irritative zone, and in virtual electrodes with many spikes and HFOs.

CONCLUSION
Node centrality increases with distance from brain areas with spikes and HFOs, consistent with the idea that the irritative zone is a functionally isolated part of the epileptic network during the interictal state.

SIGNIFICANCE
A new hypothesis about a pathological hub located remotely from the irritative zone and seizure onset zone opens new ways for surgery when epileptogenic areas and eloquent cortex coincide.
INTRODUCTION

Epilepsy surgery in patients with refractory epilepsy is successful in two thirds of the patients (Englot et al., 2015b; Jobst and Cascino, 2015; Wyllie et al., 1998). Presurgical evaluation is performed to outline the potential epileptogenic zone and eloquent cortex. Various non-invasive (e.g. electro- and magneto-encephalography (EEG/MEG), magnetic resonance imaging (MRI)) and invasive methods (e.g. intracranial grid EEG, depth electrodes) are used to record and find the source of epileptiform activity. MEG measures neural activity directly and can record ictal and interictal epileptiform activity, such as spikes and focal slowing, to localize the irritative zone. The irritative zone is defined as the area of cortex that generates interictal spikes (Lüders et al., 2006), and gives an indication of the location of the epileptogenic zone. The epileptogenic zone is based on post-surgical outcome and defined as the area that needs to be removed or disconnected to result in seizure freedom (Lüders et al., 2006). The source location found by MEG recordings is often used to guide depth electrode placement and to plan surgical intervention (Agirre-Arrizubieta et al., 2014; Stefan et al., 2011a).

High-frequency oscillations (HFOs) have been reported to be potential biomarkers of the epileptogenic zone (Bragin et al., 2010; Jacobs et al., 2012; Jirsch et al., 2006; Malinowska et al., 2014; Zijlmans et al., 2012). HFOs are characterised as at least four oscillations with an amplitude above baseline and a frequency of >80 Hz (Worrell et al., 2012). HFOs are more specific markers of the seizure onset zone than spikes (Jacobs et al., 2008; Melani et al., 2013) and they occur in smaller areas than spikes. The detection of HFOs is conventionally performed in intracranial EEG, but recent studies have reported HFOs in scalp EEG (Andrade-Valenca et al., 2011; Kobayashi et al., 2010) and MEG (Miao et al., 2014; van Klink et al., 2015; Xiang et al., 2009). The detection of HFOs requires a high sampling rate and low background noise. Van Klink et al. reported that HFOs were more readily identified in virtual electrodes than in physical MEG sensors (van Klink et al., 2015). Virtual electrodes refer to a spatial filtering approach (beamforming) that estimates the activity from a location within the brain on the basis of the extra-cranial MEG recordings (Hillebrand and Barnes, 2005; Hillebrand et al., 2005), resulting in an improved signal-to-noise-ratio.

Epilepsy is nowadays thought of as a network disorder, where the epileptogenic networks produce abnormal activity (ictal and interictal epileptiform activity, HFOs, and focal slowing), which may result in seizures (Kramer and Cash, 2012; Stam, 2014). The epileptogenic network consists of spatially distributed cortical and subcortical structures that are abnormally connected; it includes the seizure onset zone, the irritative zone, and the connections along which the seizures spread (Bartolomei et al., 2001; Bartolomei et al., 2004; Briellmann et al., 2004). Brain networks in epilepsy patients are disturbed and deviate...
from an optimal configuration (Douw et al., 2010b; Horstmann et al., 2010; Ponten et al., 2007). Hubs are regions that play a central role in the network, for example because they are well-connected, and/or because much of the communication over the network goes through these nodes (Bullmore and Sporns, 2012; van den Heuvel and Sporns, 2013). Regions that normally function as a hub in brain networks are more likely to be abnormal in brain disorders (Crossley et al., 2014; Stam, 2014). Therefore, hubs are of particular interest in epilepsy. They are thought to play a central role in seizure generation, namely by enabling the spread of the epileptiform activity that arises in the seizure onset zone (defined as the area that initiates clinical seizures (Lüders et al., 2006)) to the rest of the network (Bernhardt et al., 2011; Jin et al., 2015; Morgan and Soltesz, 2008). An indicator for hub status is betweenness centrality (Boccaletti et al., 2006). Invasive recordings (electrocorticography (ECoG) and depth electrodes) have shown that betweenness centrality is highest within brain areas that generate ictal and interictal epileptiform activity (Varotto et al., 2012), and that this correlates with the resection area in seizure-free patients (Wilke et al., 2011). In contrast, a study by van Diessen et al., using depth electrodes recordings during the interictal state, showed that contact points in the seizure onset zone had a decreased hub status compared to contact points outside the seizure onset zone (van Diessen et al., 2013b). The opposing results could be due to methodological differences, as the studies differed in modality, patient population, connectivity measure, recorded state, and hub measure. Non-invasive studies have also found that the hub status of some regions differ in epilepsy patients compared to controls (Bernhardt et al., 2011; Jin et al., 2015; Liao et al., 2010; van Dellen et al., 2014; Zhang et al., 2011b). However, there is no consensus (1) whether the hub status is higher or lower in patients compared to controls and (2) whether the hubs are located within or outside the epileptogenic zone. An fMRI study showed that interictal hub nodes differed between patients with idiopathic generalized epilepsy and controls, but both increased and decreased hub status in patients was reported (Zhang et al., 2011b). In temporal lobe epilepsy, functional and structural MRI studies found the majority of abnormal (interictal and structural) hubs outside the temporal lobe (Bernhardt et al., 2011; Liao et al., 2010). In addition, a recent MEG study found the interictal hub in the hippocampus in left mesial temporal lobe epilepsy (mTLE) patients, whereas this was not the case for right mTLE patients (Jin et al., 2015). An MEG study by van Dellen et al. reported post-surgical decreases in betweenness centrality in regions close to the resection area, but only for patients with lesional epilepsy who became seizure free (van Dellen et al., 2014).

Taken together, both invasive and non-invasive recordings of structural and functional networks have found that (pathological) hubs play an important role in the epileptogenic network. However, it is as yet unclear whether the area that generates epileptiform activity
itself functions as a hub or not. Does it function as a hub during the interictal state, from which activity can spread to the rest of the epileptogenic network, or is it functionally isolated to prevent spreading? The aim of our study was to determine the spatial relationship between network hubs and regions that generate interictal epileptiform activity. Do functional connectivity and betweenness centrality (as an indicator of hub status) increase or decrease with distance from the location of interictal epileptiform activity (i.e. spikes and HFOs)? A spatial correlation of hub status and location of interictal epileptiform activity would support the hypothesis that the irritative zone functions as a pathological hub from which epileptiform activity can spread. In this case, hub measures could be used as a non-invasive biomarker for the irritative zone. On the other hand, a spatial anticorrelation of hub status and the location of interictal epileptiform activity would be consistent with the idea that the irritative zone is kept functionally isolated, indicating that generation and spread of epileptiform activity happen by separate mechanisms.

**MATERIAL & METHODS**

*Patients*

Twelve patients with refractory epilepsy had a clinical MEG recording in 2013 as part of their preoperative evaluation at the VU University Medical Center, Amsterdam, The Netherlands. The dataset has previously been published in (van Klink et al., 2015). All patients had epileptiform activity in the MEG recording. Table 1 provides an overview of the patient characteristics, presurgical evaluation results, and surgery outcome. Written informed consent was obtained from patients or their caretakers prior to the MEG recording.

*MEG acquisition*

MEG recordings were obtained using a whole-head MEG system (Elekta Neuromag Oy, Helsinki, Finland) with 306 channels consisting of 102 magnetometers and 204 gradiometers.

The patients were in supine position inside a magnetically shielded room (Vacuumschmelze GmbH, Hanau, Germany). Typically, three datasets of 15 minutes each containing eyes-closed resting-state recordings were acquired for the identification and localization of interictal epileptiform activity. Paradigms for the localization of eloquent cortex, such as voluntary movements and somatosensory stimulation (see (Hillebrand et al., 2013)) were also recorded but not analysed in this study. The data were sampled at 1250 Hz, and filtered with an anti-aliasing filter of 410 Hz and a high-pass filter of 0.1 Hz. To localize the head position relative to the MEG sensors the signals from four or five
Table 1: Overview of patient characteristics, findings of MEG (affected region and HFO location), MRI, interictal and ictal EEG, PET, SPECT, pathology, surgery location and surgery outcome. HFO location between brackets means only one HFO-time on this side. M: male, F: female, R: right, L: left, MTS: mesial temporal sclerosis, FCD: focal cortical dysplasia, SEGA: subependymal giant cell astrocytoma, NA: not available. Reprinted from Clinical Neurophysiology, in press, ‘Identification of epileptic high frequency oscillations in the time domain by using MEG beamformer-based virtual sensors’, Nicole van Klink, Arjan Hillebrand, Maeike Zijlmans, Copyright (2015), with permission from Elsevier.

<table>
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<th>#</th>
<th>Gender</th>
<th>MEG affected side/ location</th>
<th>HFO location</th>
<th>MRI</th>
<th>Interictal EEG abnormalities</th>
<th>Ictal EEG onset</th>
<th>PET</th>
<th>SPECT</th>
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<th>Dura(ion)</th>
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<td>R frontal-temporal</td>
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head-localization coils were recorded continuously. The positions of the head-localization coils and the outline of the scalp (roughly 500 points) were measured with a 3D digitizer (Fastrak, Polhemus, Colchester, VT, USA). The points on the scalp surface were used for co-registration with the anatomical MRI of the patient through surface-matching.

Artifacts were removed by spatially filtering the raw data offline using the temporal extension of Signal Space Separation (tSSS) (Taulu and Hari, 2009; Taulu and Simola, 2006) using Maxfilter software (Elekta Neuromag, Oy; version 2.1). For a detailed description and parameter settings see (Hillebrand et al., 2013).

**MEG analysis**

Epileptic spikes were marked manually by trained MEG/EEG technicians. A single equivalent current dipole model was fitted to each selected spike using the ascending part of the spike from half-way the flank until just past the top. The dipole parameters were optimized according to the least-square error criterion, which minimizes the difference between the measured field and the field computed from the dipole model. As volume conductor a spherical head model was used, which was based on the scalp surface obtained from the anatomical MRI. Dipoles exceeding a goodness-of-fit of typically 70% were included. The outcome was visualized on the co-registered MRI of the patient. The locations of the fitted dipoles were used as a marker for the location of the irritative zone, referred to here as the ‘affected region’ (van Klink et al., 2015). All clinical MEG recordings were discussed in multidisciplinary meetings involving MEG technicians, MEG physicists and clinical neurophysiologists, to evaluate the reliability and clinical significance of the source localization and to decide upon further analyses (if needed).

**Virtual electrodes**

Time-series of single voxels were reconstructed using a scalar beamforming technique (Elekta Neuromag Oy, beamformer; version 2.2.10). Beamformer weights were calculated for each voxel separately in order to maximally let through signals originating from the voxel of interest and to attenuate all other signals, i.e. the weights form a spatial filter. The beamformer weights are based on the lead fields (using again a spherical head model) and the data and noise covariances. The data covariance was estimated from a 200ms window around each marked epileptic spike, which was band pass filtered at 80-600 Hz. The noise covariance was obtained from the first 10s of the unfiltered data. The broadband data was projected through the beamformer weights in order to obtain time-series (virtual electrodes) at the voxels of interest (Hillebrand and Barnes, 2005; Hillebrand et al., 2005).

A set of 35 virtual electrodes were placed in the affected region. The virtual electrodes were positioned in a three-dimensional star-shaped configuration and were placed 1 cm
apart along the axes of the coordinate system (Figure 1). The entire star-shaped configuration was 6 cm in diameter. The centre of the star is referred to as the centre of the affected region as found by dipole fitting. Another set of 35 virtual electrodes was placed as a control in the homologous region in the contralateral hemisphere.

**Time-series analysis**

Spikes were marked in each virtual electrode by visually inspecting the signal filtered with a fourth order 100Hz low pass infinite impulse response filter. HFOs were marked in the signal filtered with a 80Hz high pass finite impulse response filter, first by visually inspecting the entire time-series and second by closer inspection of the time points at which spikes occurred. For details see (van Klink et al., 2015).

The time-series of the 70 virtual electrodes were visually analysed for epileptiform activity and artefacts. 20 interictal and artefact-free epochs of 4096 samples (3.28s) without spikes were selected for each patient, except for patient 8 who only had 8 epochs without artefacts and spikes (due to the presence of many interictal spikes in the recordings). The epochs were further analyzed in Brainwave (version 0.9.151.5 available from http://home.kpn.nl/stam7883/brainwave.html) in eight frequency bands: delta (0.5-4Hz), theta (4-8Hz), lower alpha (8-10 Hz), upper alpha (10-13Hz), beta (13-30Hz), gamma (30-48Hz), broadband (0.5-48Hz), and a high frequency band (80-250Hz).

**Network measures**

A functional network was generated using the 70 virtual electrodes as nodes. Functional connectivity values were estimated using the phase lag index (PLI) (Stam et al., 2007) and used as edges. The PLI quantifies phase synchronization between two (virtual electrode) time-series by measuring the asymmetry in the distribution of instantaneous phase differences between the time-series. The PLI ranges between 0 (no functional connectivity, which is obtained when the distribution is flat (noise), or when the phase difference is zero modulus pi (volume conduction, or functional connectivity at zero-lag)) and 1 (full synchronization), and is robust against the effects of volume conduction and field spread (Porz et al., 2014).

Average PLI was computed for each virtual electrode, i.e. the average of the connectivity of that electrode with all other electrodes. From the functional network we extracted the minimum spanning tree (MST) (Kruskal, 1956): First, the PLI was inverted so that strong connections (large PLI) have a small weight. Then edges are added to the nodes by first adding the edge with the smallest weight (i.e. the strongest connection), and consecutively the following smallest edges until the network is fully connected, but skipping edges that would result in loops in the network. Across groups, conditions or
studies, the MST has the same number of edges (number of nodes minus 1) when the number of nodes is kept constant, enabling direct comparison between networks (Stam et al., 2014; Tewarie et al., 2015) without relying on arbitrary thresholds (van Wijk et al., 2010). Betweenness centrality (Boccaletti et al., 2006) was calculated for the MST, which is one of several measures that can identify hubs, i.e. nodes that play an important role in the network (Sporns et al., 2007). The betweenness centrality of a node is based on the number of shortest paths passing through that node in comparison to the number of all shortest paths of the network (Freeman, 1977). PLI and MSTs were calculated for each frequency band separately.

**Statistical analysis**

The affected region was compared to the contralateral region regarding average betweenness centrality and PLI using a paired t-test (i.e. averaged over the 35 virtual electrodes per region) per patient. Each virtual electrode was related to epileptiform
activity through three parameters: (1) distance to the centre of the affected region, (2) number of spikes, and (3) number of HFOs. These three parameters were correlated to the PLI and the MST betweenness centrality for each electrode. Correlations were estimated using linear regression, from which the coefficient of determination ($R^2$) and F-statistic were calculated. The correlations were corrected for multiple comparisons across patients using false discovery rate (Benjamini and Hochberg, 1995). The three parameters were also correlated to each other, and binomial tests were used to test whether positive or negative correlations occurred more often, over all patients, than chance. A significance level of $p < 0.05$ was used for all tests. The statistical analyses were performed in MATLAB (MATLAB and Statistics Toolbox Release 2012a, The MathWorks Inc., Natick MA, United States) and IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, USA).

RESULTS

Affected versus contralateral region

Betweenness centrality was higher in the affected region ($M = 0.08, SD = 0.01$) compared to the contralateral region ($M = 0.07, SD = 0.01$) in the delta band ($t(11) = 3.24, p = 0.01$) (Figure S1). The PLI was also higher in the affected region ($M = 0.21, SD = 0.01$) compared to the contralateral region ($M = 0.20, SD = 0.01$) in the delta band ($t(11) = 2.37, p = 0.04$) (Figure S1). Those findings did, however, not remain significant after correction for multiple testing (across frequency bands) using the false discovery rate (Benjamini and Hochberg, 1995). No significant differences were found in other frequency bands (Table S1).

Betweenness centrality

Betweenness centrality across frequency bands was lower in virtual electrodes (1) that were close to the centre of the affected region (Figure 2 and 3), (2) with many spikes (Figure 4), and (3) with many HFOs (Figure 5).

Figure 2A shows the coefficients of determination ($R^2$) per patient and frequency band for the significant correlations of betweenness centrality with the distance of the virtual electrode to the centre of the affected region. All but one significant correlations had a positive slope, meaning that betweenness centrality increased with distance to the centre of the affected region. A representative example for the broadband is shown in Figure 2B.

Topological maps (Figure 3) display the spatial distribution of betweenness centrality in the broadband for two representative patients (patient 6 and 9). Betweenness centrality was low at the centre of the affected region, and increased with distance from the centre in most patients. The pattern was more or less symmetrical, with low values at or close to the centre of the affected region, which generally increased towards the edges in all directions.
Figure 4A shows the coefficients of determination (R²) per patient and frequency band for the significant correlations of betweenness centrality with number of spikes. All significant correlations had a negative slope, meaning that virtual electrodes with many spikes had a lower betweenness centrality. A representative example of such a correlation between number of spikes and the broadband betweenness centrality is shown in Figure 4B.

Figure 5A shows the coefficients of determination (R²) per patient and frequency band for the significant correlations of betweenness centrality with the number of HFOs. Only seven patients had HFOs in the affected region (white cells; grey cells indicate patients without HFOs). All significant correlations had a negative slope, meaning that virtual electrodes with many HFOs had a low betweenness centrality. A representative example for the theta band is shown in Figure 5B.

**PLI**

Functional connectivity was high for virtual electrodes (1) that were close to the centre of the affected region (Figure S2), (2) with many spikes (Figure S3), and (3) with many HFOs (Figure S4).

The PLI decreased with distance to the centre of the affected region in most patients (12 out of 14 significant correlations, Figure S2). It was mainly positively correlated to the number of spikes (12 out of 17 significant correlations, Figure S3), and it increased with number of HFOs in most patients (6 out of 9 significant correlations, Figure S4).

**Correlations between distance to the affected region, number of spikes, and number of HFOs**

Virtual electrodes in the centre of the affected region had significantly more spikes than the virtual electrodes further from the centre in all patients but patient 10 (Figure 6A) (11/12 patients, binomial test, p = 0.01). Similarly, virtual electrodes in the centre had significantly more HFOs than the virtual electrodes further away in all patients (Figure 6B) (7/7, binomial test, p = 0.02). Virtual electrodes with more spikes did not show significantly more HFOs, even though all patients but patient 4 showed a positive correlation (Figure 6C) (6/7, binomial test, p = 0.13).

**DISCUSSION**

In our interictal networks of virtual electrodes, betweenness centrality was low in the irritative zone, as measured by distance to the centre of the affected region, number of spikes, and number of HFOs. Thus, the region that contains interictal epileptiform activity does not function as a hub, but is functionally isolated.
Figure 2: Betweenness centrality increased with distance to the centre of the affected region. (A, B) Correlations between betweenness centrality and distance to the centre of the affected region are shown. (A) The coefficient of determination $R^2$ (the square of the correlation coefficient $r$) for each correlation per patient and frequency band (only significant correlations are shown). Green cells show positive correlations ($r > 0$), red cells show negative correlations ($r < 0$). (B) A representative example, showing the correlation for patient 4 in the broadband.
Figure 3: Betweenness centrality in the broadband was low in the centre of the affected region. Coronal cross-section (above) and axial cross-section (below) are shown for patient 6 (A) and patient 9 (B). Left column: The distribution of the broadband betweenness centrality according to virtual electrode placement is shown for a cross section of the affected region (interpolated between virtual electrodes). The locations of the virtual electrodes are indicated by the green circles. Right column: the cross-section of the MRI is shown with virtual electrode placement. The affected region was on the left side for patient 6 (A) and on the right side for patient 9 (B).
Figure 4: Betweenness centrality decreased with number of spikes. Correlations between betweenness centrality and number of spikes are shown. (A) The coefficient of determination $R^2$ (the square of the correlation coefficient $r$) for each correlation per patient and frequency band (only significant correlations are shown). Green cells show positive correlations ($r > 0$), red cells show negative correlations ($r < 0$). (B) A representative example, showing the correlation for patient 3 in the broadband.
Figure 5: Betweenness centrality decreased with number of HFOs. Correlations between betweenness centrality and number of HFOs are shown. (A) The coefficient of determination $R^2$ (the square of the correlation coefficient $r$) for each correlation per patient and frequency band (only significant correlations are shown). Green cells show positive correlations ($r > 0$), red cells show negative correlations ($r < 0$). (B) A representative example, showing the correlation for patient 6 in the theta band.
Figure 6: Correlation between distance of the virtual electrode to the centre of the affected region, number of spikes per virtual electrode, and number of HFOs per virtual electrode are shown per patient. (A) The number of spikes (as a percentage of the total number of spikes) decreased with distance from the affected region in most patients (11/12, binomial test, $p = 0.01$). (B) The number of HFOs decreased with distance from the affected region in all patients (7/7, binomial test, $p = 0.02$). (C) The number of HFOs and spikes per virtual electrode correlated positively in most patients (6/7, binomial test, $p = 0.13$).
A few previous studies have assessed the hub status of the affected region in epilepsy patients. Some studies noted a decreased hub status within the seizure onset zone (van Diessen et al., 2013b) and close to the resection area (van Dellen et al., 2014), corroborating our results. Van Diessen et al. used depth electrodes inserted in the hippocampus and amygdala of patients with suspected temporal lobe epilepsy. The hub status in the theta band, as measured by eigenvector centrality (Newman, 2008), was low in channels located in the seizure onset zone. Similar to our study, the number of HFOs correlated negatively with the eigenvector centrality. However, the authors found no correlation between number of spikes and hub status.

Other studies have found an association between the epileptogenic zone and hubs in the ictal (Varotto et al., 2012; Wilke et al., 2011) as well as in the interictal state (Jin et al., 2015; Varotto et al., 2012; Wilke et al., 2011). Jin et al (Jin et al., 2015) evaluated MEG functional connectivity based on mutual information in patients with mTLE and hippocampal sclerosis. The authors reported that hubs were outside the temporal lobe in controls but mainly inside the temporal lobe in patients. However, this was only the case for patients with left temporal lobe epilepsy and not for patients with right temporal lobe epilepsy. One of the detected hubs was in the hippocampus, but two other hubs were in the temporal gyrus, suggesting that hubs can be located close to, but outside, the affected region. In the studies by Wilke et al. and Varotto et al. (Varotto et al., 2012; Wilke et al., 2011), the epileptogenic zone showed an increased betweenness centrality. We also saw an overall increase of the betweenness centrality averaged over the affected region, however, a more detailed view using virtual electrodes revealed a lower betweenness centrality in the centre of the affected region. Interestingly, Wilke et al. noted that nodes with a high betweenness centrality during the interictal state were located further away from the seizure onset zone than nodes with a high betweenness centrality during the ictal state, even though those nodes were in proximity of the seizure onset zone in both states. This particular finding together with our results suggests that the epileptogenic zone might not function as a hub during the interictal state, but that there is a pathological hub nearby that plays an important role during the ictal state. Hubs seem to be located in areas that are distinct (and more or less distant) from the areas that generate interictal epileptiform activity, and are probably involved in seizure spread rather than seizure generation.

We showed that the connectivity (based on PLI) within the affected region was higher, both compared to the contralateral region and compared to the virtual electrodes located further away from the centre of the affected region. Other studies have also reported increased functional connectivity within the epileptogenic zone (Bettus et al., 2008; Liao et al., 2010; Maccotta et al., 2013; Ortega et al., 2008a). Together with the results for betweenness centrality, this suggests that the epileptogenic zone is well connected within
itself, but that it is isolated from the rest of the brain network during the interictal state (Ibrahim et al., 2013; Khambhati et al., 2015; Warren et al., 2010). Networks of inhibitory interneurons may provide a possible mechanism for such isolation. Interestingly, it has also been shown that such networks of inhibitory interneurons are important for the generation of spikes (de Curtis and Avanzini, 2001) and HFOs (Jefferys et al., 2012; Ylinen et al., 1995). The isolation possibly breaks down at the start of the ictal state (Ibrahim et al., 2013; Khambhati et al., 2015; Kramer et al., 2008), allowing seizure activity to spread to the rest of the epileptogenic network. This spread of seizure activity might be mediated by a nearby pathological hub (van Dellen et al., 2014), which would explain the overall increase of betweenness centrality in the affected region compared to the contralateral region. An interesting approach for future research is to follow the hub areas in networks that evolve from the interictal to the pre-ictal, ictal, and post-ictal state.

A possible scenario for an epileptogenic network consists of a pathological hub close by – or even at some distance, but in connection with the seizure onset zone – through which seizure activity can spread to the rest of the epileptogenic network. There are three components involved: 1) the irritative zone and seizure onset zone; 2) a surrounding zone of inhibition; 3) a pathological hub. When the surrounding zone of inhibition breaks down, the seizure onset zone connects to the hub, and the hub rapidly spreads seizure activity to the rest of the brain. According to this scenario, surgical intervention may aim to relieve seizures, either by i) removal of the irritative zone and seizure onset zone, or ii) removal of the pathological hub, or iii) disconnection of the irritative zone or seizure onset zone and the pathological hub (Ntsambi-Eba et al., 2013). These alternative strategies have practical consequences, in particular for patients in whom the irritative zone or seizure onset zone are in or near eloquent cortex.

Our results indicate that the hub is not located within the centre of epileptiform activity, but it might still be nearby. To prove that a pathological hub is not located within, but close to, the centre of epileptiform activity, the betweenness centrality should increase when moving away from the centre of epileptiform activity and peak at the area that contains the hub. The setup of our study was not suitable to test this thoroughly, but our results provide some support for this idea – especially the finding that betweenness centrality was low in the centre and high at one or two sides at the edge of the affected region (Figure 3). A strong prediction follows from the above working hypothesis: patients in whom the pathological hub is removed, yet in whom the area that generates epileptiform activity is left intact, should still be seizure free.

**Limitations**

We characterised the affected region by means of three variables: (1) distance to the centre
of the affected region, (2) number of spikes, and (3) number of HFOs, assuming that all three variables point to the centre of the irritative zone. Furthermore, we assumed that the centre of the virtual electrode configuration formed the centre of the epileptiform activity, which might not necessarily be the case. Additionally, the clinical spike locations might not be placed exactly at the origin of the source of epileptiform activity. Therefore, to be more certain about the centre of the irritative zone, we also correlated the three variables with each other. Both the number of spikes and the number of HFOs decreased with increasing distance to the centre of the affected region in significantly more patients than would have been expected at random. But the number of HFOs did not increase with the number of spikes in significantly more patients than expected at random, even though six out of seven patients showed a positive correlation (small sample size being a limited factor here). The observed correlations of the three variables assured us that the centre of the virtual electrode configuration was located at the centre of the irritative zone.

The affected region was not confirmed by surgery outcome, but instead was solely based on dipole fitting of interictal spikes in the MEG recording, i.e. the irritative zone (Lüders et al., 2006). The epileptogenic zone can only be confirmed post-operatively by seizure freedom (Lüders et al., 2006). Six of our 12 patients underwent surgery after the MEG recording, of which surgery outcome (measured by Engel classification (Engel Jr et al., 1993)) was known 3-20 month after surgery (van Klink et al., 2015). Of these six patients, three patients had concordant MEG localization and resection area with a good surgery outcome (Engel class I). Two patients had discordant MEG localization and resection area, but also a good surgery outcome (Engel class I). One patient had discordant MEG localization and resection area and a poor surgery outcome (Engel class IV). This means that the MEG localization was confirmed to be the epileptic zone in three cases, remains to be confirmed in seven cases, and was not confirmed in two cases. In the latter two cases, the irritative zone found by MEG did not overlap with the epileptogenic zone. The irritative zone is usually more extensive than the epileptogenic zone and is not necessarily positioned in the same location or even in proximity (Lüders et al., 2006). Our results were obtained for the irritative zone and cannot be generalized to the epileptogenic zone or seizure onset zone, although concordance and seizure freedom in three of six patients indicates that our results might also apply to the epileptogenic zone in some cases. Even though our patient group was heterogeneous, it was too small to perform analyses on subgroups. Future studies using MEG localization confirmed by surgery outcome are needed to extend our findings to the epileptogenic zone and seizure onset zone.
CONCLUSION
The irritative zone (i.e. the area that generates interictal epileptiform activity) does not function as a hub but is functionally isolated. The isolation might prevent seizures in the interictal state and possibly breaks down in the ictal state. A new working hypothesis is that pathological hubs are not necessarily located within the irritative zone or seizure onset zone, but at a more or less distant location, retaining a connection to these zones. This opens new ways for surgery in patients in whom the irritative zone or seizure onset zone are difficult to localize or are located within, or close to, eloquent cortex. If it is possible to remove a pathological hub, or the connections to it, instead of the area generating epileptiform activity, more patients will become eligible for surgery, operated upon, and rendered seizure free. To prove this hypothesis, future studies should aim at locating and characterising pathological hubs in epilepsy patients who had a good surgery outcome, but in whom the area generating spikes and HFOs were not or only partially removed. Ultimately, a prospective study would be necessary, where hubs are removed but not the area generating spikes and HFOs.

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Figure S1: Betweenness centrality (A) and PLI (B) differed significantly between the affected region and the mirror region in the delta band (paired t-test, betweenness centrality: $t(11) = 3.24, p = 0.01$, PLI: $t(11) = 2.37, p = 0.04$). The values for 35 virtual electrodes were averaged for the two regions and displayed for the 12 patients (one line per patient).
Figure S2: Correlation between PLI and distance to the centre of the affected region. (A) The coefficient of determination $R^2$ (the square of the correlation coefficient $r$) for each correlation per patient and frequency band (only significant correlations are shown). Green cells show positive correlations ($r > 0$), red cells show negative correlations ($r < 0$). (B) A representative example, showing the correlation for patient 12 in the lower alpha band.
Figure S3: Correlation between PLI and number of spikes. (A) The coefficient of determination $R^2$ (the square of the correlation coefficient $r$) for each correlation per patient and frequency band (only significant correlations are shown). Green cells show positive correlations ($r > 0$), red cells show negative correlations ($r < 0$). (B) A representative example, showing the correlation for patient 8 in the gamma band.
Figure S4: Correlation between PLI and number of HFOs. (A) The coefficient of determination $R^2$ (the square of the correlation coefficient $r$) for each correlation per patient and frequency band (only significant correlations are shown). Green cells show positive correlations ($r > 0$), red cells show negative correlations ($r < 0$). (B) A representative example, showing the correlation for patient 6 in the beta band.
Table S1: Paired t-test for the betweenness centrality and PLI between affected region and contralateral region per frequency band. The mean and standard deviation (SD) were over all patients. The findings marked with * were significant with \( p < 0.05 \), but did not remain significant after correction for multiple testing (across frequency bands) using the false discovery rate (Benjamini and Hochberg1995).

### Betweenness centrality

<table>
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<tr>
<th>Frequency band</th>
<th>Affected region Mean</th>
<th>SD</th>
<th>Contralateral region Mean</th>
<th>SD</th>
<th>t-value (df=11)</th>
<th>p-value</th>
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<tr>
<td>Delta</td>
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<td>0.006</td>
<td>0.067</td>
<td>0.005</td>
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<td>0.008*</td>
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<tr>
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<td>0.007</td>
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<td>0.007</td>
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<td>0.072</td>
<td>0.007</td>
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<td>0.599</td>
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<td>0.357</td>
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<tr>
<td>High frequency</td>
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<td>0.064</td>
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<td>0.376</td>
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### PLI

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<th>SD</th>
<th>t-value (df=11)</th>
<th>p-value</th>
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