EPILEPSY
Epilepsy affects 4-10 per 1000 people worldwide (Banerjee et al., 2009; Forsgren et al., 2005; Sander, 2003). The diagnosis is based on the occurrence of at least two unprovoked seizures more than 24h apart (Fisher et al., 2014). The seizures by themselves can sometimes be disabling, but the largest impact follows from the unpredictability of their occurrence. There are three main types of seizures: seizures with a focal, generalized or unknown onset (Scheffer et al., 2017). Focal seizures start in a specific area of the brain; generalized seizures affect both hemispheres at the same time; and unknown onset seizures have an unknown beginning. The classification of seizure type is important as it indicates the choice of treatment, prognosis of the patient, and possible underlying causes. In addition, it provides a framework for communication between health care professionals. Epilepsy can be caused by many factors, even though the cause is often unknown (Berg et al., 2010). Some causes are congenital, such as genetic and developmental disorders. Other causes are acquired, such as brain trauma, infection, tumor, and stroke. The first group of causes is more prevalent in children, whereas the second group is more likely in adults. The mechanisms of seizure generation are not fully understood. It is generally assumed that a shift occurs from normal neuronal activity to excessive synchronization due to decreased inhibition (Lopes da Silva et al., 2003). After the onset in one area, the seizure can spread to other areas and sometimes to the entire brain. Recurring seizures damage the brain tissue (Sutula et al., 2003) and reinforce the epileptogenic network, which makes it important and progressively more difficult to control the seizures by treating them.

EPILEPSY SURGERY
Several treatment options exist for epilepsy. The first choice for treatment consists of anti-epileptic drugs. However, these drugs are only effective in two out of three people (Kwan et al., 2010; Sander, 2003). When at least two trials of appropriately chosen and tolerated anti-epileptic drugs have failed, the patient is considered to be drug-resistant (Kwan et al., 2010). For those patients, epilepsy surgery is an alternative treatment for focal-onset seizures. During the surgery, the brain regions involved in seizure generation are removed or disconnected. Epilepsy surgery has been increasingly applied since the late nineteenth century (Schijns et al., 2015) and in today’s clinical practice seizure freedom is achieved in up to two-thirds of the patients (Englot et al., 2015b; Jobst and Cascino, 2015; Spencer and Huh, 2008). The majority of the remaining patients experience a reduction in seizure frequency after surgery. Further improvement in epilepsy surgery will hopefully render more patients seizure-free. Therefore, the overall goal of this dissertation is to investigate new ways of improving epilepsy surgery. More specifically, new methods will be evaluated to localize the area to be removed during surgery: the epileptogenic zone.
The epileptogenic zone is defined as the brain area that needs to be removed or disconnected to render the patient seizure-free (Lüders et al., 2006; Rosenow and Lüders, 2001). By definition, this can only be confirmed after surgery, meaning that before surgery a hypothesis needs to be established about the location of the epileptogenic zone. This hypothesis is generated following the presurgical evaluation, which uses several imaging modalities, including magnetic resonance imaging (MRI), positron emission tomography (PET), electrocorticography (ECoG), stereotactic electroencephalography (SEEG), magnetoencephalography (MEG), and electroencephalography (EEG). MEG and standard (30 minutes) surface EEG record mainly interictal activity (between seizures), and therefore measure the irritative zone. The irritative zone is defined as the brain area that generates interictal spikes (Lüders et al., 2006) and is indicative of the location of the epileptogenic zone (Figure 1). Other presurgical evaluation modalities measure different cortical zones related to the seizures. For example, SEEG recordings indicate the seizure onset zone, which is defined as the brain area that initiates clinical seizures (Lüders et al., 2006). Seizure activity spreads from the seizure onset zone and subsequently invades the symptomatogenic zone, which is eloquent cortex and generates the first clinical seizure symptoms (Lüders et al., 2006). Another zone is indicated by high frequency oscillations (HFOs), usually measured invasively (Zijlmans et al., 2012). HFOs consist of at least four oscillations with an amplitude above baseline and a frequency above 80 Hz (Worrell et al., 2012). They have been shown to reliably indicate the location of the epileptogenic zone (Bragin et al., 2010; Jacobs et al., 2012; Jirsch et al., 2006; Malinowska et al., 2014; von Ellenrieder et al., 2016; Zijlmans et al., 2012) and often co-occur with interictal spikes (van Klink et al., 2015). Yet, HFOs appear in a smaller area and are a more specific marker of the epileptogenic zone than interictal spikes (Jacobs et al., 2008; Melani et al., 2013). Improving the hypothesis about the location of the epileptogenic zone from the presurgical evaluation will result in an improved surgery outcome. This dissertation focuses on two presurgical evaluation techniques, namely MEG recordings and SEEG recordings.

MEG
Magneetoencephalography (MEG) is a neuroimaging technique that records magnetic fields due to neuronal activity of the brain. Active neurons transport ions (charged particles) across their membranes, which results in an electrical current (primary current). This changing current generates both an electric and magnetic field, which are perpendicular to each other. If a sufficient number of neurons (tens of thousands) are active simultaneously and in synchrony (Lopes da Silva, 2010), they generate a magnetic and electric field that are measurable extracranially by MEG and EEG, respectively. MEG has the advantage that it 1)
Figure 1: Illustration of the different cortical zones related to epilepsy. The epileptogenic zone (blue circle) is defined as the surgical excision in seizure-free patients. The irritative zone generates interictal epileptiform abnormalities, such as spikes. Ictal activity starts in the seizure onset zone, which is smaller than the irritative zone. Seizure activity spreads from the seizure onset zone to the symptomatogenic zone, which gives rise to the initial seizure symptomatology. The symptomatogenic zone constitutes eloquent cortex and should not be resected to avoid functional deficits. The area generating high-frequency oscillations is also smaller than the irritative zone and a more specific marker of the epileptogenic zone.

is non-invasive, 2) has a high temporal resolution and a moderate to high spatial resolution (Troebinger et al., 2014), 3) is a direct measure of brain activity, 4) does not need a reference, and 5) the measured magnetic field is unperturbed by the skull and brain tissue (Baillet, 2017). On the other hand, MEG is not portable, more expensive than a standard surface EEG, and does not achieve the high spatial resolution of an MRI (Boto et al., 2017). MEG is clinically applied in the presurgical evaluation for epilepsy surgery (De Tiege et al., 2017). Its usage has two purposes: 1) to determine eloquent cortex (areas whose functions should be preserved, such as speech, motor and sensory areas) and 2) to localize the irritative zone to generate a hypothesis about the location of the epileptogenic zone. Eloquent cortex and the irritative zone are localized with various MEG analysis methods.

**Source reconstruction**

The signals measured at the sensors are computed to sources in the brain during source reconstruction. This reconstruction requires that the so-called MEG inverse problem is solved, which is an ill-posed problem (von Helmholtz, 1853), since different source
distributions can give rise to the same external electromagnetic field. To solve the inverse problem, further assumptions or prior knowledge are needed, such as knowledge about the distribution and orientation of the sources. One commonly used source reconstruction method is beamforming (Hillebrand and Barnes, 2005; Hillebrand et al., 2005). Beamforming is a spatial filtering approach that passes brain activity from a specified location and attenuates activity from other locations. To achieve this, it assumes that no two sources are perfectly linearly correlated (Hillebrand and Barnes, 2005). However, the spatial resolution varies over brain regions; superficial sources are measured with a higher signal-to-noise ratio than deep sources (Hillebrand and Barnes, 2002).

**Localization of epileptiform activity**

The current standard method for clinical analysis of MEG recordings is equivalent current dipole (ECD) fitting (Bagic et al., 2011). First, epileptiform abnormalities (e.g. spikes, spike-wave complexes, spike trains) are visually marked by an expert. Second, each abnormality is fitted to a model of a current dipole. Third, this fit is compared to the measured magnetic field, and the model parameters (location, orientation, and strength of the current dipole) are adjusted until the model best represents the measured data. ECD assumes that the measured neuronal activity is focal and can be explained by a small number of current dipoles. However, not all patients show interictal epileptiform abnormalities on their recordings. Additionally, ECD localization is not always indicating the epileptogenic zone.

An alternative method to the standard ECD method is kurtosis beamforming (Kirsch et al., 2006; Robinson et al., 2004). With this approach, beamforming is used to first reconstruct the time series of neuronal activity for every location in the brain. Then locations where time series show excess kurtosis are indicated. Kurtosis is a higher-level statistic that is sensitive for sharp waveforms such as spikes. Those regions with time-series that contain spikes will show positive excess kurtosis. Whereas ECD analysis requires visual or semi-automatic spike detection, kurtosis beamforming automatically detects locations displaying spikes. The advantage of kurtosis beamforming is that 1) fewer assumptions are needed compared to ECD analysis (such as number of active sources and the dipole model), 2) no prior visual identification of spikes is needed, and 3) it may pick up epileptiform activity that may be missed with ECD analysis (e.g. sharp activity that was not identified as a spike by the expert). Both ECD analysis and kurtosis beamforming rely on the presence of epileptiform abnormalities (e.g. spikes) in the recording. However, not all recordings contain spikes and rapid spread of seizure activity poses a problem to the current methods; for those cases new localization methods are needed. A potent framework for developing a new, spike-independent localization is provided by a relatively new area of research: modern network theory.
NETWORK THEORY

Network theory is embedded in the mathematical field of graph theory (Newman, 2010; Stam and Van Straaten, 2012b), and it studies the topology of networks. A network consists of only two elements: nodes and links (the connection between the nodes) (Figure 2). The links can have a weight (weighted network) or be binary (unweighted network). They can also have a direction (directed network) or have no direction (undirected network). Usually, a threshold is applied to select only the strongest or most important links. However, the selection of a threshold is arbitrary, might result in unconnected nodes, and leads to different connection density when applied to different networks. This is problematic for network comparisons across groups or studies, as network topology measures are biased by the connection density of the network (van Wijk et al., 2010). One solution to this problem is to convert the network into a minimum spanning tree (MST) (Kruskal, 1956). Initially, the MST consists only of nodes with no links. Subsequently, the links with the minimum weight (representing the strongest links: often link weights have to be inverted) are added one by one, while links that form loops are discarded, until all nodes are connected. The MST has the same number of links for all networks with the same number of nodes, and is uniquely determined when all the link weights are unique. This allows for unbiased comparison of network topology between conditions, patients, or groups (Stam et al., 2014; Tewarie et al., 2015). On the other hand, most connections are excluded, but they might carry important information. Strong links might be discarded because they form loops, but the MST has been shown to robustly include the strongest links (Tewarie et al., 2015).

Brain networks

Network theory is independent of the nature of the underlying elements and can therefore be applied to, and characterize, a variety of systems such as those formed by the brain, internet, gene regulations, social relationships, transportation, and economics. In brain networks, nodes represent brain areas and links represent estimated connections (Bassett and Sporns, 2017; Bullmore and Sporns, 2009; Rubinov and Sporns, 2010). Brain areas are usually predefined in a commonly used atlas, such as the automated anatomical labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002). The choice of atlas depends on the purpose of the study, and also on the spatial resolution of the brain imaging method (Barnes et al., 2004; Farahibozorg et al., 2017). The network links are determined from a connectivity measure, which can measure structural (anatomical) or functional (activity-dependent) connectivity. Functional connectivity assesses the statistical dependencies between activity time series of nodes (Friston et al., 1993). Many different measures exist that quantify this dependency, but only some are insensitive to volume conduction and field spread (Bastos
and Schoffelen, 2015; Kida et al., 2015; Pereda et al., 2005; van Mierlo et al., 2014). The problem is that due to volume conduction and field spread, neighboring sensors may measure activity from the same neuronal source. Those neighboring sensors will appear to be functionally connected because their measurements are correlated, which is a redundancy of measurement instead of a true connection due to brain interaction. Volume conduction occurs when the current arising from the source is transmitted through the brain and skull tissue to the sensor, i.e. it needs a medium. MEG is sensitive to volume conduction, as it measures the magnetic field arising both from the source current as from the transmitted currents. Field spread on the other hand is independent of a medium and refers to the propagation of the electromagnetic field (often visualized by field lines) from the source outwards. Both effects give rise to spurious detection of connections that display zero time lag between nodes and are a major problem in sensor space analysis (each sensor represents a node), but it is also not eliminated when converting data from sensor to source space (each brain region represents a node) (Schoffelen and Gross, 2009). One connectivity measure that is insensitive to zero-lag connections is the phase lag index (PLI) (Stam et al., 2007), which is an undirected connectivity measure. Directed connectivity measures, such as those based on the concepts of Granger causality or transfer entropy, are useful for indicating the seizure origin (Coito et al., 2015; Epstein et al., 2014; van Mierlo et al., 2013; Wilke et al., 2009), as the seizure spread occurs in a direction away from it.

Network measures

Once the brain network has been reconstructed, network measures can be utilized to characterize the topology of the network (Figure 2). Simple network measures, such as path length and clustering coefficient characterize the integration and segregation of a network (Watts and Strogatz, 1998). The path length is the average number of steps required to go from one node to another via the shortest path. A low path length indicates a well-integrated network, that allows efficient communication between the nodes. The clustering coefficient quantifies the number of times two nodes that both are connected to a third node are also connected to each other (to form a triangle of three connected nodes), compared to the number of possible triangular connections. A high clustering coefficient denotes a well-connected network locally, which is required for a segregated network. Together, a low path length and a high clustering coefficient are characteristic of a small-world network (Watts and Strogatz, 1998), which represents an optimal balance between integration and segregation, in terms of e.g. wiring cost and information transport (Bullmore and Sporns, 2012). Other network measures indicate nodes that in some aspect are central to the network. These so-called hubs can be characterized by, for example, a high degree (Barabasi and Albert, 1999), betweenness centrality (Freeman, 1977), or
eigenvector centrality (Bonacich, 1972). All these measures are nodal measures, i.e. they constitute one value per node. The degree is defined as the number of links a node possesses (unweighted network) or the total link weight of a node (weighted network); betweenness centrality is the fraction of the shortest paths that pass through a node; and eigenvector centrality takes into account not only a node’s centrality, but also the centrality of its neighbors (Newman, 2008; Stam and Van Straaten, 2012b). Modules are subnetworks whose nodes show higher connectivity to nodes of the same module than to nodes in other modules (Meunier et al., 2010). Hubs can be situated within a module (provincial hubs) or between modules (connector hubs) (Sporns et al., 2007). Hubs play an important role in brain networks and have been shown to be affected in a range of brain disorders (Buckner et al., 2009; Crossley et al., 2014; de Haan et al., 2012; Stam, 2014; van den Heuvel and Sporns, 2013).
Epilepsy as a network disease

Epilepsy is increasingly seen as a network disorder (Kramer and Cash, 2012; Lehnertz et al., 2009; Smith and Schevon, 2016; Stam, 2014; van Diessen et al., 2013a; Yaffe et al., 2012). Brain networks in epilepsy patients are disturbed compared to healthy controls (Horstmann et al., 2010; Pedersen et al., 2015; Ponten et al., 2007) and deviate from an optimal network configuration (Douw et al., 2010b). Among the various network measures, especially hubs play an important role in epilepsy as they may facilitate the spread of seizure activity to the rest of the brain (Figure 3) (Bernhardt et al., 2011; Jin et al., 2015; Morgan and Soltesz, 2008). Several studies have shown that network hubs are present in, or near, the affected brain area (Bernhardt et al., 2011; Jin et al., 2015; Liao et al., 2010; Storti et al., 2016; Varotto et al., 2012; Wilke et al., 2011) making measures indicating hubs potential measures to localize the area that should be removed during epilepsy surgery (i.e. the epileptogenic zone).

The importance of generating an accurate hypothesis about the location of the epileptogenic zone has spurred research into the use of various measures: focal slow activity (Baayen et al., 2003; Englot et al., 2016b), functional connectivity (Bettus et al., 2008; Liao et al., 2010; Stufflebeam et al., 2011; Tarapore et al., 2012), directed functional connectivity (Li et al., 2016; Park and Madsen, 2017; van Mierlo et al., 2013; Varotto et al., 2015; Vlachos et al., 2016), and changes over time (dynamics) (Dickten et al., 2016; Khambhati et al., 2017; Steimer et al., 2017; Tomlinson et al., 2017). In this dissertation, the aim is to use network theory to identify the epileptogenic zone.

VIRTUAL ELECTRODES

The construction of a brain network from MEG measurements can be accomplished by beamforming, which reconstructs the neuronal sources and their time series – these are so-called virtual electrodes (Hillebrand et al., 2012). Virtual electrodes can be placed in regions of interest based on an atlas or in user-specified locations in the entire brain. Activity is reconstructed selectively at those specified virtual electrode locations, thereby achieving a higher signal-to-noise ratio (van Klink et al., 2015), which enables reliable detection of signals from deep structures (Mills et al., 2012). These signals can then be analyzed further using connectivity measures to reconstruct the brain network.

A long-standing discussion is whether MEG can measure neuronal signals arising from deep structures such as the hippocampus. The hippocampus is an important structure in epilepsy, as it is often involved in seizure generation in temporal lobe epilepsy (TLE), which is the most common form of drug-resistant focal epilepsy (Semah et al., 1998). Therefore, it is important to be able to differentiate between spikes arising from the hippocampus or from the neighboring neocortex. Whether the hippocampus is involved in
the seizure generation drastically changes the surgical procedure and long-term outcome. The virtual electrodes can be placed in the hippocampus to determine whether epileptiform activity can be detected in such a deep structure. Several virtual electrodes can also be placed in one region of interest (e.g. the epileptogenic zone), to virtually zoom in on this region.

**STEREOTACTIC EEG**

Virtual electrodes are reconstructions of the neuronal activity on the basis of extracranially recorded signals, and they rely on assumptions that may, or may not, be valid for a particular situation. Stereotactic EEG (SEEG) measure brain activity directly at the source. Epilepsy patients with inconclusive or conflicting findings from the standard presurgical evaluation modalities, are considered for invasive EEG recordings using SEEG (Bancaud and Talairach, 1973). SEEG is considered the gold standard among the presurgical evaluation modalities (Blount et al., 2008) and measures brain activity at subsequent
contact points situated approximately 1cm apart on an electrode that is inserted into the cortex. The placement of the electrodes occurs at pre-defined locations that are based on the findings from other presurgical evaluation modalities such as MEG (Knowlton et al., 2009; Stefan et al., 2011a). SEEG has both a high temporal and spatial resolution, although with limited spatial coverage, as the number of electrodes that can be used is limited (Jayakar et al., 2016). The contact points measure directly from the surrounding tissue with the signals being unattenuated by other brain tissue or the skull. The implantation is performed during a surgical procedure, which makes SEEG highly invasive and bears additional risks (Mullin et al., 2016; Vakharia et al., 2017). The procedure typically involves a two-week stay at an epilepsy monitoring unit, which is demanding for the patient, costly, and time-consuming. Not every patient is mentally or physically able to undergo this invasive procedure and it is only infrequently applied in pediatric patients. A replacement of SEEG with non-invasive alternatives (such as MEG) would be preferable to minimize the risk and burden for the patient. The level of spatial resolution of SEEG is hard to match by MEG, but the use of virtual electrodes increases the spatial resolution and allows for the reconstruction of activity at user-defined locations. Virtual electrodes can thus be placed at the contact points of SEEG and the two modalities can be compared directly.

**AIM AND RESEARCH QUESTIONS**

The outcome of epilepsy surgery is not optimal, as one out of three patients continues to experience seizures after surgery (Englot et al., 2015b; Jobst and Cascino, 2015; Spencer and Huh, 2008). The goal of this dissertation is to improve the outcome of epilepsy surgery. This can be achieved by an improved hypothesis about the location of the epileptogenic zone before surgery, resulting in more patients undergoing surgery by assuring the hypothesized epileptogenic zone. Here, we focus on the improvements that can be accomplished by one of the techniques used during presurgical evaluation: non-invasive MEG measurements.

The specific research questions are:

1. How successful is the current clinical MEG analysis in localizing epileptiform abnormalities?

The answer allows for a characterization of the patients in whom MEG is not successful. Ensuing research can be targeted to develop alternative analysis methods for that specific patient group. A potential method emerges from the concept of hubs that are connected to the epileptogenic zone, as described in Figure 3. This is addressed by the following
research questions:

2. Can we improve the localization of epileptiform activity by using alternative methods?

3. Can we extend MEG localization of the hypothetical epileptogenic zone from being spike-dependent to being independent of spikes?

4. Can we localize the epileptogenic zone using network theory? More specifically, are hubs indicative of the epileptogenic zone?

The use of MEG beamforming not only allows localization to a predefined atlas or grid, but also to user-defined placements of virtual electrodes. The placement can be guided by specific hypotheses or research questions, enabling a more refined sampling of areas of interest. In this sense, we can zoom in on the irritative zone (displaying interictal epileptiform activity) and investigate the location of hubs and the relation between hubs and the irritative zone (see concept in Figure 3):

5. What is the spatial relationship between network hubs and the location of interictal epileptiform activity (i.e. spikes and HFOs)?

We can also place virtual electrodes in the hippocampus, which is a critical area for epilepsy surgery, to answer the following question:

6. Can MEG measure signals from the hippocampus and detect hippocampal spikes?

Additionally, we can place MEG virtual electrodes at the locations of SEEG contact points, to compare the two modalities:

7. How comparable are MEG virtual electrodes estimates to SEEG recordings? Could MEG reduce the use of SEEG in the future?

OUTLINE OF DISSERTATION

Chapter 2 describes the patient population that is referred to our center for an MEG as part of their presurgical evaluation. Most patients are referred because of previous inconclusive findings, with the aim of generating a hypothesis about the epileptogenic zone and for the localization of eloquent cortex. Patient characteristics are used to predict
beforehand whether MEG would be able to localize the irritative zone. In addition, the patients without clinical MEG localization are characterized, as they stand to benefit the most from new localization methods. One such new localization method is described and tested on a clinical patient cohort in Chapter 3: the kurtosis beamformer. Similar to the standard clinical ECD analysis, the kurtosis beamformer is dependent on the presence of interictal spikes in the recordings. Therefore, Chapter 4 sets out to describe a spike-independent localization method based on network theory. The hypothesis that network hubs indicate the epileptogenic zone is tested in the same clinical patient cohort. The spatial location of network hubs are compared to other markers of the irritative zone, namely interictal spikes and HFOs in Chapter 5. MEG allows the placement of beamformer-based virtual electrodes, which are utilized to zoom in on the irritative zone and investigate whether network hubs are present in the center or close by the irritative zone. Network hubs are investigated further on a larger patient cohort in Chapter 6, where hub measures alongside spectral and connectivity measures are evaluated as indicators of the epileptogenic zone. Virtual electrodes are also employed in Chapter 7 and placed in the hippocampus, to measure interictal spikes that had been recorded in the hippocampus using SEEG. This idea is taken further in Chapter 8, where virtual electrodes are placed at all contact points of SEEG electrodes. This allows for a comparison of MEG and SEEG, with the goal to potentially replace invasive SEEG recordings with MEG.