GENERAL DISCUSSION

Epilepsy surgery is preceded by various investigations and imaging techniques that constitute the presurgical evaluation. Presurgical evaluation is in need of further improvement, because the epileptogenic zone is currently located adequately in only two-thirds of all patients undergoing surgery (Kwan et al., 2010; Sander, 2003). Therefore, the main aim of this dissertation is to improve the outcome of epilepsy surgery by improving the presurgical evaluation. The discussion is structured by relating each research question stated in the introduction to the greater research question is: Can we improve the identification of the epileptogenic zone?

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

In order to improve the localization of the epileptogenic zone, the current situation is examined as a first preparatory step. With the current clinical analysis tools, the majority of the MEG recordings that contain epileptiform abnormalities can be analyzed successfully, i.e. a hypothesis about the location of the epileptogenic zone is generated (Chapters 1 and 2). MEG is mainly applied to clinically complex cases, where previous presurgical evaluation findings are inconclusive (Chapter 1). Often, MEG is recommended for the analysis of MRI-negative patients (Iida et al., 2015; Jung et al., 2013), but those patients also present a challenge for MEG (Chapter 1). MEG could be applied more selectively by only utilizing this modality for less complex cases, but this is not justified given that MEG potentially adds information about the location of the epileptogenic zone. The fact that it is safe and inexpensive compared to invasive recordings (although expensive relative to EEG) makes MEG worthwhile. MEG is still underused in the clinic today, the main reasons being the high costs of a system (2-3 million euros) and high running costs (relative to EEG), lacking awareness of the added clinical value, no access to an MEG system nearby, technically challenging acquisition and analysis, and no health care reimbursement in most countries (De Tiege et al., 2017; Stefan and Trinka, 2017). Nonetheless, MEG is a mature clinical technique for presurgical evaluation (De Tiege et al., 2017). However, a considerable number of MEG recordings contain only normal activity, i.e. they do not display any epileptiform abnormalities (Chapter 1) (Englot et al., 2015b). In clinical practice, some approaches to minimize the number of recordings without epileptiform activity are to require patients to be sleep-deprived or to let them hyper-ventilate during the recording (Giorgi et al., 2013; Guaranha et al., 2005; Holmes et al., 2004; Malow, 2004). None of the recordings that only contained normal activity resulted in adequate localization of the potential epileptogenic zone with the current
clinical tools (Chapter 1), which underscores the need for new localization methods.

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

The localization of the epileptogenic zone can be improved by improving the localization of spikes (i.e., epileptiform activity). One way is to improve the precision of the localization by enhancing the detection of spikes in the recording, for example by using automatic spike detection to support manual detection (Brown et al., 2007; Gaspard et al., 2014; Ossadchii et al., 2004). Another way is to use an alternative method for localizing the spikes in source space. One such alternative method is kurtosis beamforming (Chapter 2). In our study, kurtosis beamforming localized epileptiform activity successfully, but it did not significantly improve localization to the resection area compared to the standard ECD analysis. Nevertheless, it identified spikes in patients in whom ECD analysis did not result in adequate localization. Therefore, kurtosis beamforming should rather complement the current ECD analysis than replace it, as it can be applied favorably in patients with few spikes in the recording or with widespread ECD localizations. The drawback of kurtosis beamforming is its sensitivity to artefacts in the recordings, therefore it is necessary to visually inspect the time series.

An improvement in spike localization could arise from the simultaneous recording of scalp EEG and MEG, which detects more spikes than the individual modalities separately, and thereby improves the precision of spike localization (Aydin et al., 2015; Chowdhury et al., 2015). Another potential method is the localization of HFOs, which localize the seizure onset zone more specifically than spikes (Jacobs et al., 2008; Melani et al., 2013). HFOs often co-occur with spikes, but they can also appear alone (Zijlmans et al., 2017). However, HFOs are found in fewer patients than spikes (Chapter 4) (van Klink et al., 2015). Other methods that are independent of spikes would be able to localize the epileptogenic zone in more recordings than the current methods as they would also be able to analyze recordings without epileptiform abnormalities or focal slowing (Chapter 1).

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

The most important improvement in localizing the epileptogenic zone would be the development of a method that does not rely on the presence of interictal spikes in the MEG recordings. Several studies have shown that recordings can contain information about the epileptogenic zone even in the absence of spikes (Coito et al., 2016; Soriano et
Similarly, the measures we evaluated showed that there is information about the epileptogenic zone in data without spikes (Chapter 3). However, the extension of the study in Chapter 3, which included more patients and measures (Chapter 5), did not indicate that the epileptogenic zone can be located adequately on the basis of MEG recordings without spikes. Several measures localized the resection area in significantly more patients than chance level, but the localization of the epileptogenic zone (the resection area in seizure-free patients) was unsuccessful. Localizing the resection area through non-invasive and interictal recordings is a first step, and a level of performance that corresponds to the current situation where the resection area is based on the decision of experts that use a variety of presurgical evaluation findings. Nonetheless, we would like to go further than the current state and localize the resection area in seizure-free but not in not seizure-free patients, thereby improving the expert decision. Possibly, the goal (to combine resection area and surgery outcome) was too ambitious and an intermediate step is required, such as finding measures that distinguish between seizure-free and not seizure-free surgery outcome (Englot et al., 2015b; Goodfellow et al., 2016; He et al., 2017; Sinha et al., 2017) or identifying measures that serve as red flags in the presurgical evaluation (e.g. the absence of hubs ins, or nearby, the planned resection area). Subsequently, surgery outcome and the location of the resection area can be combined to evaluate promising measures. Another possibility is that some patients might not have a confined epileptogenic zone. Some causes are more diffuse, such as gray matter heterotopia, which often presents with drug-resistance and poor surgery outcome (Battaglia et al., 2006). Patients with diffuse causes might benefit from a network approach, which might offer alternative resection strategies that are spatially not related to the location of the malformation. Such alternative resection strategies could be tested in an individualized computer model, on which seizure activity can be simulated and terminated with a successful strategy.

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

Network theory offers a promising framework for the development of a new localization method (Bartolomei et al., 2017; Engel et al., 2013b; Ponten et al., 2007; van Diessen et al., 2013a). Applying network theory, we investigated the role of hubs in the localization of the epileptogenic zone. The results show that hubs are indicating the epileptogenic zone in some patients (Chapter 3 and 7), but not in others (Chapters 3 and 7). Also, hubs seem to indicate the resection area irrespective of surgery outcome and therefore not exclusively the epileptogenic zone (Chapter 5). This result is in agreement with the finding that the
average hub status is elevated in the irritative zone (Chapter 4), as the irritative zone does not take surgery outcome into account (Lüders et al., 2006). Taken together, it is not yet clear how hubs are related to the epileptogenic zone. However, hubs have been shown to be changing over temporal as well as spatial scales, and the changes were not necessarily related to the occurrence of seizures (Carbo et al., 2017; Geier and Lehnertz, 2017). Even though it is accepted that hubs play an important role in epilepsy (Kramer and Cash, 2012; Stam, 2014), it remains to be shown which role they play.

One role of hubs can be to facilitate the spread of seizure activity (Morgan and Soltesz, 2008; Tomlinson and Venkataraman, 2017). In the model presented in the introduction (see Figure 3 in the introduction), the epileptogenic zone is connected to a hub, which spreads seizure activity to the rest of the network. Such a pathological hub might be located in the epileptogenic zone in some patients but not in others. Furthermore, the model contained a ‘seizure-switch’, which means that a connection is established between the epileptogenic zone and pathological hub in the ictal state but not in the interictal state. Our studies analyzed interictal recordings, and we found that it is possible to find hubs in, or near, the epileptogenic zone in the interictal state (Chapter 3, 4, 5, 7). This suggests that a connection might exist between the epileptogenic zone and the pathological hub even in the interictal state. In this sense, the connection might exist all the time and be responsible for the local spread of interictal activity (which might help explain why the irritative zone is generally larger than the epileptogenic zone (Lüders et al., 2006)) as well as ictal activity (Zubler et al., 2015) (which might – opposed to interictal activity – be suppressed by a surrounding zone of inhibition). The proposed ‘seizure-switch’ might instead be contained within the epileptogenic tissue and determine whether interictal or ictal activity is generated, as interictal epileptiform activity has a different morphology than ictal activity in MEG and SEEG recordings. Along these lines, a pathological hub is just a relay station that conveys the activity it receives (interictal as well as ictal) to its connected neighbors.

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

As discussed in the previous paragraph, centrality measures indicating hubs are potential measures to identify the epileptogenic zone. Our results from Chapter 3 led to the hypothesis that the epileptogenic zone itself is a hub. However, further investigations showed that hubs are not in the center of, but near the irritative zone – the center had consistently low hub values (Chapter 4). Furthermore, the hub value (betweenness centrality) showed a negative correlation with the presence of interictal epileptiform activity: the more spikes and HFOs, the lower the hub status (Chapter 4) (van Diessen et
These results are in agreement with the finding that hubs are not sufficient to indicate the epileptogenic zone (Chapters 5 and 7). However, this also implies that hubs might be located close to the epileptogenic zone, as the average of a wider area (all ROIs contained fully or partly in the resection area) showed an increased hub status compared to the rest of the brain (Chapters 3, 4, 5). Such a hub could still be responsible for seizure spread and therefore be pathological, if it is well-connected to the epileptogenic zone (see Figure 3 in the Introduction). The model presented in the introduction does not require the hub to be close by the epileptogenic zone, but the findings from Chapter 3, 4, 5 imply that such a pathological hub is located in spatial proximity. To conclude, hubs seem to be located outside the center of the epileptogenic zone (at the rim in some patients) and do therefore not function as an accurate marker for the epileptogenic zone (even though they might mark the surrounding area or the lobe containing the epileptogenic zone). Rather, hubs might constitute a negative marker – if hubs are absent in, and nearby, the planned resection area, the area might not be the epileptogenic zone. However, this concept of the hub as a negative marker remains to be tested in future studies.

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

One specific aspect of improving the localization of the epileptogenic zone relates to the differentiation between neocortical and mesial temporal lobe epilepsy (Dolezalova et al., 2016). A recent study showed that MEG can infer simulated hippocampal activity using generative models (Meyer et al., 2017b). Our results demonstrated that MEG is able to detect spikes originating in the hippocampus in humans by the use of virtual electrodes (Chapter 6). Virtual electrodes constitute an improvement of MEG analysis to achieve a higher signal-to-noise ratio, thereby allowing for the reconstruction of activity in deeper brain structures such as the hippocampus (Mills et al., 2012; van Klink et al., 2015). Furthermore, other structures or areas of interest can be targeted by the specific placement of virtual electrodes (Chapters 4 and 7). The use of virtual electrodes for the clinical analysis of MEG recording is now standard in our center, where they are placed in the irritative zone, around lesions, within the mesial structures, or according to the clinical hypothesis. This has led to numerous cases with improved spike detection and localization, with some examples shown in (Chapter 6) Additionally, previous MEG recording have been re-analysed using the virtual electrode approach for several patients that were scheduled for SEEG recordings, in order to guide the placement of the depth electrodes (unpublished).
7. How comparable are MEG virtual electrodes estimates to SEEG recordings? Could MEG reduce the use of SEEG in the future?

Virtual electrodes can be placed to identify the epileptogenic zone on a more local scale than when using the pre-set locations of a cortical atlas, similar to the placement of depth electrodes compared to scalp EEG (Gavaret et al., 2016). They allow to zoom in and to achieve a higher signal-to-noise ratio in the hypothetical epileptogenic zone or other areas of interest (van Klink et al., 2015). Our results show that spectral and functional connectivity measures, but not network measures, quantified from MEG virtual electrodes do not differ from those quantified with SEEG on a different occasion (often more than several months apart), and under different conditions (e.g. off medication for dEEG) (Chapter 7). The use of virtual electrodes at the locations of the planned SEEG trajectories could possibly predict whether useful information will be obtained with SEEG measurements at those locations, which might influence and improve SEEG placement. MEG virtual electrodes will probably not make SEEG recordings obsolete in the near future, but they could possibly reduce the number of patients undergoing SEEG and the number of implanted electrodes. Similar to the discussion of research question 3, a comparison of MEG and SEEG should initially focus on either the seizure onset zone or the resection area. Once MEG virtual electrodes have been shown to reproduce SEEG results rigorously, the subsequent step is the improvement of MEG beyond SEEG by accounting for surgery outcome as well.

LIMITATIONS

Frequency bands

Traditionally, neurophysiological signals are divided into several frequency bands based on biological significance (Chang et al., 2011). For example, the prominent alpha band (8-13 Hz) is associated with relaxed wakefulness while having the eyes closed and appears at the posterior regions (Chang et al., 2011). The use of six narrow frequency bands and one broadband results in a seven-fold increase of the number of statistical tests. Those tests need correction for multiple comparisons (using e.g. the false-discovery rate, family-wise error or Bonferroni correction), which greatly reduces the power of the individual statistical tests. For each additional statistical test, the likelihood to correctly reject a null-hypothesis decreases. It is therefore desirable to select one, or a few, frequency bands based on the hypothesis or on previous results (Gross et al., 2013). In epilepsy, comparable studies have found significant results mainly in the theta, lower alpha and upper alpha band (Carbo et al., 2017; Douw et al., 2010b; van Dellen et al., 2014; van Diessen et al., 2013b) but also in other frequency bands (Douw et al., 2013; Wilke et al., 2011). Of
specific interest in epilepsy is the delta band, because focal slowing is an indicator of the epileptogenic zone (Baayen et al., 2003; Tao et al., 2011). All frequency bands, including a high frequency band (80-250 Hz), were analyzed in Chapter 4, with significant findings in all frequency bands (though fewer in the high frequency band). Interestingly, the observed trends were consistent across frequency bands. However, the comparison of the epileptogenic versus the contralateral region showed significant differences for both the betweenness centrality and PLI in the delta band (Chapter 4), but those differences did not remain significant after correcting for multiple testing across the number of frequency bands using the false-discovery rate (Benjamini and Hochberg, 1995). It is unclear whether those differences reflect a statistical variation or a true difference that ceased to be significant after the multiple comparison correction. The lack of a clear hypothesis about a certain frequency band in epilepsy led to the analysis of the broadband (0.5-48 Hz) in Chapters 3, 5, and 7, as it encompasses all classic frequency bands. This choice results in a greater statistical power to detect true significances, with the downside that interpretation of broadband phase-phase connectivity is ambiguous (Gross et al., 2013) and that effects with opposite direction in different narrow bands could cancel each other out in the broadband. To conclude, a limited a priori choice of narrow frequency band is recommended, despite the chance of missing potentially useful information in the omitted frequency bands. Alternatively, multilayer networks can be utilized to represent one frequency band per layer, thereby allowing for interactions between frequency bands. Another option is to include measures from several frequency bands in a machine learning classifier, which bypasses the need for multiple comparisons correction (Fornito et al., 2013).

**Interictal recordings as a marker for the epileptogenic zone**

MEG recordings are mainly interictal and only infrequently capture ictal activity (Chapter 1). The evaluation of new localization methods for the epileptogenic zone using interictal MEG makes two inferences: 1) interictal epileptiform activity is informative about the epileptogenic zone and 2) interictal recordings without epileptiform activity are informative about the epileptogenic zone. The validity of those two inferences is discussed below.

Interictal epileptiform activity indicates the irritative zone, which is more extensive than, and not necessarily coincides with, the seizure-onset zone and the epileptogenic zone (Lüders et al., 2006). Interictal spikes (Chapters 1, 2 and 4) and HFOs (Chapter 4) might therefore not overlap with the epileptogenic zone (Bartolomei et al., 2016; Ray et al., 2007). This is important to keep in mind when evaluating new methods based on interictal epileptiform activity, such as the kurtosis beamformer (Chapter 2). The method might be able to accurately localize the irritative zone, but the localization might be outside the
epileptogenic zone (Lüders et al., 2006) in contrast with ictal activity. For this reason, it is important to evaluate the method against the gold standard: the resection area plus surgery outcome (Rosenow and Lüders, 2001) (Chapters 2, 3, 5, 7). Nonetheless, correct localization, though to a broader area, is still helpful for surgical planning, as other methods can subsequently be employed to narrow down the surgical excision (e.g. SEEG).

SEEG has a higher spatial resolution and can measure ictal activity and therefore the seizure-onset zone (Chapter 7). MEG is accordingly not only helpful for establishing a hypothesis about the location of the epileptogenic zone, but also for guiding the placement of SEEG (Agirre-Arrizubieta et al., 2014; Stefan et al., 2011a).

The analyzed brain networks are constructed based on interictal recordings (Chapters 3, 4, 5, 7), assuming that those recordings carry information about the epileptogenic zone, or at least the irritative zone, also without the presence of interictal epileptiform abnormalities. This assumption might not hold true, although several studies have shown that connectivity and network measures are indicative of the epileptogenic zone in recordings without spikes or without prior selection of epochs (Coito et al., 2016; Tomlinson et al., 2017; van Mierlo et al., 2014). If interictal recordings without epileptiform activity would not contain any information about the epileptogenic zone, those studies and the studies presented in Chapters (3, 4, 5) would not be able to indicate the epileptogenic zone better than random guessing. Therefore, our results indicate that interictal recordings without epileptiform activity do carry information about the epileptogenic zone.

Some MEG recordings contain ictal activity (Chapter 1), allowing for the localization of the seizure onset zone, which is smaller than the irritative zone. Recording more seizures with MEG would allow for a stronger hypothesis about the location of the epileptogenic zone, having localized both the irritative zone and seizure onset zone. However, patient movement often restricts the recording of longer seizure segments. A flexible MEG system would allow the capturing of entire seizures (see optically pumped magnetometers in the Future directions section), thereby extending the use of MEG from interictal to ictal recordings.

Classification of surgery outcome
The classification of patients into seizure-free and not seizure-free was done based on the Engel classification, which classifies surgery outcome into four categories (Engel Jr et al., 1993). In this dissertation, only class 1 (free of disabling seizures) was considered as 'seizure-free'. However, class 2 (rare disabling seizures) and even class 3 (worthwhile improvement) constitute a decrease in seizure-frequency and might therefore be considered as a favorable surgery outcome (Engel et al., 2003). Therefore, some studies
divide the patient cohort into class 1 and 2 (good outcome) and 3 and 4 (poor outcome) (Gonzalez-Martinez et al., 2007; Jacobs et al., 2010). And from the surgeons point of view, the outcome classes 1-3 all mean that the surgery was worthwhile (Lee et al., 2005). In this sense, most surgeries (88% of the 94 patients in Chapter 5) constitute an improvement for the patient. Our criterion for surgery outcome is more conservative, because even rare persistent seizures imply that the epileptogenic zone was not removed entirely. Research into new approaches should generally use stricter definitions in order to accomplish a substantial improvement above the status quo. Nonetheless, sometimes those definitions can be too strict for the current state of technology and knowledge, and perhaps less strict definitions would have resulted in clinically valuable insights.

Patient reports of seizures are often inaccurate (Bidwell et al., 2015). Often patients underreport the number of seizures (Bidwell et al., 2015; Blachut et al., 2017), especially at night (Hoppe et al., 2007; Kerling et al., 2006). The self-assessment of seizure frequency of the patients could have influenced the Engel score classification, resulting in misclassifications. Nonetheless, our studies distinguished between entirely seizure-free and reoccurring seizures, which means that the detection of seizures was more important than seizure frequency.

Another consideration about the surgery outcome classification concerns the time point of the classification assessment. The classification was performed at least one year following the surgery (Chapters 2, 3, 5, 6, 7). This commonly chosen point in time is arbitrary and could also be chosen earlier or later. Some patients present with initial seizures in the first months post-surgery, but eventually achieve seizure freedom afterwards; others are initially seizure-free several months or years after surgery, but experience seizure recurrence later (de Tisi et al., 2011; Najm et al., 2013). The percentage of patients who are seizure-free after surgery diminishes with increasing follow-up time after surgery (de Tisi et al., 2011; Kelemen et al., 2006; McIntosh et al., 2012; McIntosh et al., 2004). In this sense, the used classification (and thereby the classification of epileptogenic zone versus not epileptogenic zone) might have changed if we had considered another time point of follow-up. Additionally, the classification (successful versus unsuccessful surgery) might be incorrect because it does not regard the underlying cause of recurrent seizures. For example, patients with a tumor (17% of 379 patients in Chapter 1) might initially be seizure free, implying that the epileptogenic zone was removed. However, tumor growth might induce seizures after several months (Pallud et al., 2014). Such a patient will be classified at one year post-surgery as not seizure-free, even though the resection area was in the correct location and should be classified as the epileptogenic zone. As such, the Engel classification does not consider dynamic brain networks, resulting in possible miss-classifications especially in the group with
Reproducibility of connectivity and network measures

Lately, there have been several studies on the reproducibility of connectivity and network measures (Colclough et al., 2016; Fornito et al., 2010; Hincapie et al., 2017; Mahjoory et al., 2017). The overall conclusion is that most measures show considerable variability for different choices in the processing pipeline, and that some choices lead to more variability than others. This could result from the many choices to be made in the pipeline from the initial recording to the final measure. The recordings have undergone several transformations, including going from time-series in sensor space to time-series in source space, spatial and temporal filtering, phase extraction, connectivity estimation, thresholding the connectivity values (the MST is also a form of thresholding), and reconstruction of network measures. All of these steps could influence the final measures in a non-trivial way, and different research groups have different opinions about the optimal choices for each step, which therefore lead to low reproducibility across studies. However, there is another explanation for the low reproducibility. One assumption of reproducibility testing is that all measures stay invariant over time and conditions. However, the brain is highly variable and dynamic, as it is a complex system (Siegelmann, 2010; Telesford et al., 2011). Connectivity and network measures have been shown to change across the life span (Smit et al., 2016). Therefore, a simple explanation for low reproducibility is that the brain itself is dynamic and not reproducible. Nonetheless, a measure that reliably indicates the epileptogenic zone should be robust to different processing choices, recording times, and conditions. For example, to test the dynamics of hubs and whether they remain a good indicator of the epileptogenic zone remains to be shown within a recording (Carbo et al., 2017; Geier and Lehnertz, 2017) and in a longitudinal study.

MEG as one part of the presurgical evaluation

MEG is only one modality out of several others that are part of the presurgical evaluation (Bagic et al., 2009; Stefan et al., 2009). Even more, MEG does not belong to the standard evaluation, but is mainly used if previous EEG and MRI assessments were inconclusive or contradictive (Chapter 1). Hence, any improvement in localizing the epileptogenic zone using MEG is restricted by 1) the limited application of MEG in the presurgical evaluation, 2) MEG being only one investigation out of many, and 3) often applied in the most difficult clinical cases. A greater improvement of surgery outcome would require research that combines all modalities, and that would additionally investigate how the combination of these different modalities contribute to clinical decision making. Nonetheless, every small step towards an improvement counts and there is potential in improving MEG
localization (Chapter 1 and 2). In conclusion, even though MEG only constitutes one part of several presurgical evaluation modalities, an improvement in MEG localization methods contributes to the improvement of epilepsy surgery.

FUTURE DIRECTIONS

Study improvements

A rigorous method to identify the epileptogenic zone using network measures is still lacking. Several options are available to create a stronger foundation for a hypothesis-driven search for potential network measures that may identify the epileptogenic zone. One option is to select a more homogeneous patient cohort, which could result in more consistent results across patients. In our studies, we included all patients irrespective of resection location or underlying etiology. Instead, one choice could be to only include patients with temporal lobe epilepsy, possibly even discriminating between left and right onset, or mesial and neocortical. Similarly, the patient cohort available at our center is large enough to allow for the categorization of frontal lobe patients. Furthermore, patients with a tumor could be excluded, to avoid a change in brain network due to tumor growth. Choosing a homogeneous cohort with known epileptogenic zone would greatly increase the chances to find a network measure that indicates the epileptogenic zone, because other possible confounding factors can be excluded, such as misclassification of patients with persistent seizures, different brain networks for different etiologies or for different locations. However, such a successful approach might be limited to the chosen patient group and not be valid in other patient groups.

A second strategy would be to first identify measures that can distinguish seizure-free patients from patients with persistent seizures after surgery. For group comparisons, global network measures can be included to predict surgery outcome. Subsequently, potential local measures could be evaluated for differentiating between the resection area and non-resection areas. As a last step, the information gained from the group comparison and area comparison could be combined to identify markers for the epileptogenic zone. Group differences in surgery outcome are easier to detect than identifying the epileptogenic zone, although less helpful in the presurgical evaluation. Predicting the surgery outcome is a valuable evaluation of a planned resection, but it does not help in generating a hypothesis about the location of the epileptogenic zone. Alternatively, for this strategy only seizure-free patients can be included to assure a localization of the epileptogenic zone (although the resection area is often larger than necessary and contains healthy as well as epileptogenic tissue), after which the strategy can be evaluated on both seizure-free and not seizure-free patients. This approach has a greater chance to be successful compared to using both seizure-free and not seizure-free patients, on the other hand it might indicate
the resection area in not seizure-free patients as well, instead of indicating an alternative resection area.

A third option is to include all frequency bands in a machine learning or multilayer network approach to circumvent the multiple comparisons problem. Multilayer networks take interactions between frequency bands into account, which could contain information regarding the location of the epileptogenic zone. Machine learning algorithms detect patterns in the data without the need of a prior hypothesis. Both approaches could reveal previously hidden information that aids the localization of the epileptogenic zone. However, given the heterogeneity of our patient cohort and that the machine learning algorithms applied to this cohort did not yield additional insights, the chances of detecting entirely new markers is not very high. In addition, a multilayer approach might result in a complex result which might not be generalizable to other cohorts.

A fourth strategy is to carefully select epochs that are free of artefacts and spikes (Coito et al., 2016). Alternatively, only epochs containing spikes could be analyzed, as they carry information about the irritative zone, which is used for the clinical standard ECD analysis. As we used spike- and artifact-free epochs with moderate success (Chapter 3), and epochs containing spikes and artefacts with less success (Chapter 5), careful selection of epochs might improve the localization results. Most recordings in our patient cohort did not contain many spikes (Chapter 1 and 2), therefore the important factor might be the avoidance of artifacts and not the absence of spikes. Data with no artefacts and only few noisy channels are better suited for connectivity and network analysis, therefore it might be an advantage to only include patients with clean recordings.

Last, less conservative methods can be used than in our studies. The PLI is robust to volume conduction, which comes at the cost of neglecting true zero-lag connections (Stam et al., 2007). Alternatively, independent component analysis or different connectivity measures can be used that can be orthogonalized to limit the influence of volume conduction. The MST forms the backbone of the original network, but it only consists of a fraction of the original number of links (Kruskal, 1956), thereby disregarding a lot of information contained in the original network (even though MST measures are as sensitive to network topology alterations as conventional network measures (Tewarie et al., 2015). Other options are to consider networks with more links than the MST, such as weighted networks with all links or binary networks with less stringent thresholds. The MSTs in our brain networks are much smaller networks than the networks for which many of the network measures were originally developed. Instead of including more links, the number of nodes could be increased by using a more refined and possibly MEG-based atlas. All method choices have advantages and drawbacks, while there are often no optimal choices but sometimes preferable options. However, reproducibility decreases as the number of
choices increases (Colecgh et al., 2016; Forntito et al., 2010; Hincapie et al., 2017; Mahjoory et al., 2017). Therefore, making the same or similar methodological choices as reported in the literature might increase the chance of positive findings. This also means that an unsuccessful approach might become successful (or vice versa) when using different choices regarding, for example, connectivity and network measures. Standardizing methodological pipelines across the research field would maybe not offer relief on some methodological issues, but would make studies comparable between centers.

**Optically pumped magnetometers**

A recent exciting development around MEG scanners is a new type of sensor: the optically pumped magnetometer (OPM) (Knappe et al., 2014). Those sensors measure the strength of an external magnetic field by detecting the change of light, which is shone through a gaseous chamber heated to around 150 degrees Celsius. The advantage of OPMs compared to the currently used superconducting quantum interference devices (SQUIDS) is that they operate without cryogenic cooling. This saves the costs of helium, reduces the depletion of the last dwindling helium resources, and allows the sensors to be placed closer to the scalp at a 6mm distance, which increases the signal-to-noise ratio (Boto et al., 2017; Iivanainen et al., 2017). Single OPMs are already commercially available, and the next step is to develop a whole-head system based on OPMs. This has several implications for the recordings of patients with epilepsy. The higher signal-to-noise ratio would improve the detection of interictal spikes, which could improve both ECD analysis and kurtosis beamforming. Additionally, the localization accuracy would increase, because of the improved spatial resolution. Another advantage of OPMs is that they do not need to be fixed in a helmet, but they can be placed in a flexible and moveable system, such as a headcast (Meyer et al., 2017a). This would allow the recording of seizures inside a shielded room. A single sensor can also be placed inside the mouth against the back of the upper mouth cavity, which brings it in close proximity to the hippocampus and improves the signal-to-noise ratio greatly compared to the SQUID-based MEG. However, the improved signal-to-noise ratio of the OPMs necessitates a precise measurement of the location of the sensors to ensure accurate localization results (Boto et al., 2017).

**Computer model**

Computer models constitute a way to combine the localization intention of presurgical evaluation with the conclusion from this dissertation (see conclusion and discussion of research question 3) to distinguish between surgery outcome as a first step. A computer model can be build that simulates seizure activity and is adjusted to the brain network of
the individual patient. The models can be used to integrate structural, functional, and pathophysiological data about neuronal networks (Wendling et al., 2016). As such, the underlying structural and functional pathways can be modelled and make the model patient-specific. For example, Proix et al. based a model on structural pathways and found that the model accounted for seizure propagation in the individual patient (Proix et al., 2017). Such models can also be used to for patient-specific resection strategies that may lead to a better outcome than standard surgery (Hutchings et al., 2015; Taylor et al., 2014). Recent research showed that resection of the pathological network node is not necessarily the best approach to alleviate seizures (Hebbink et al., 2017). The model parameters can be adjusted such that the seizure onset and spread is simulated in the individual patient (Jirsa et al., 2017). Subsequently, resection strategies can be tested by performing virtual resections on the model and predicting the surgery outcome (Goodfellow et al., 2016; Sinha et al., 2017). A virtual resection that ceases seizure activity in the model suggests seizure freedom after surgery in the patient. Such models based on electrocorticography (ECoG) recordings have predicted surgery outcome with 81% accuracy using interictal segments (Sinha et al., 2017) and 88% accuracy using ictal segments (Goodfellow et al., 2016). The advantage of such models is that multiple resections can be tested, before the surgeons decide on the final resection strategy. Additionally, many different markers of the epileptogenic zone can be tested without the multiple comparisons problem.

CONCLUSION

MEG is a valuable part of the presurgical evaluation and can easily be applied to all patients undergoing epilepsy surgery because it is non-invasive and relatively cost-efficient. It can detect information about the location of the epileptogenic zone also in interictal recordings. The use of virtual electrodes makes the application of MEG versatile and able to investigate both entire brain networks as well as hypothesis-driven subsets or regions. Virtual electrodes should become a standard tool in the clinical analysis, as they are easy to implement and aid the detection of spikes in suspected regions. Both virtual electrodes and kurtosis beamforming are supplementary analyses to the current clinical standard (ECD analysis), even though they rely on the presence of spikes. Improving the localization of the epileptogenic zone independent of spikes with network theory is still a task for the future. Several network measures were tested for localization of the epileptogenic zone, but they were not successful in all patients. Network hubs play an important role in epilepsy, but are not consistently indicating the epileptogenic zone. The challenge is to predict surgery outcome in combination with the resection area. Network theory is possibly more suited for group comparisons than for localization purposes in the individual patient. Therefore, taking a step back and answering questions about markers...
for seizure-freedom might lead to better hypotheses for finding a localization marker for the epileptogenic zone. Alternatively, computer models can simulate epileptogenic networks in individual patients, enabling the determination of measures that localize the epileptogenic zone, which could be further aided by machine learning. In addition, multilayer networks can reveal new potential network measures in simulated data and patient recordings. Finally, network theory can lead to a better understanding of the mechanisms behind epilepsy and seizure freedom, where the epileptogenic zone is seen as part of a wider epileptogenic network.