FUNCTIONAL BRAIN NETWORKS AND PREDICTION MODELS IN CHILDHOOD EPILEPSY

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Per i miei

"Gutta cavat lapidem, non vi, sed saepe cadendo"

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(met een samenvatting in het Nederlands)

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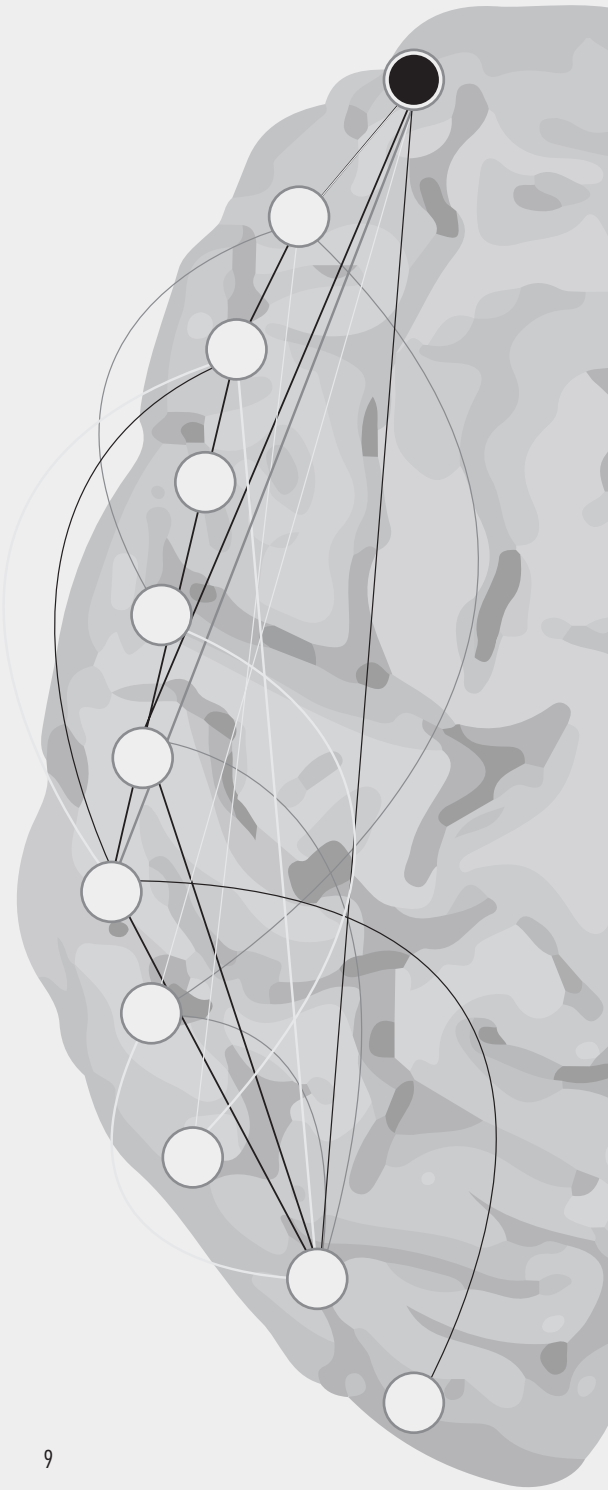
CHAPTER 1

GENERAL INTRODUCTION, AIMS AND OUTLINE



GENERAL INTRODUCTION

Background

Epilepsy is one of the most common chronic brain disorders in children. The clinical hallmark of epilepsy is the occurrence of seizures, which are defined as transient clinical signs and symptoms due to abnormal (synchronous) neuronal brain activity. Recently, the International League Against Epilepsy (ILAE) proposed a practical definition for epilepsy (Fisher et al., 2014). Epilepsy is currently seen as a disease of the brain defined by any of the following conditions: i) at least two unprovoked or reflex seizures occurring within 24 hours, ii) one unprovoked or reflex seizure and a probability of further seizures similar to the general recurrence risk after two unprovoked seizures (occurring over the next ten years), iii) diagnosis of an epilepsy syndrome. From the more than fifty million people who suffer from active epilepsy worldwide (Leonardi and Ustun, 2002), an estimated 25 percent are children (Forsgren, 2004). Population-based studies on childhood-onset epilepsy indicate higher annual incidence rates in low-income countries (61-124 per 100.000) than in high-income countries (51-60 per 100.000) (Ba-Diop et al., 2014; Forsgren, 2004; Ngugi et al., 2011). The higher incidence of childhood epilepsy in low-income countries is probably due to region specific endemic parasitic diseases (Carter et al., 2004; Quattrocchi et al., 2012) and increased perinatal medical complications (Ngugi et al., 2013).

The clinical prognosis of childhood-onset epilepsy strongly depends on the underlying cause. In self-limiting (or 'benign') epilepsies, in which epileptic seizures are easily treated or require no treatment, seizure remission is usually achieved before adulthood. Self-limiting epilepsies are generally not associated with permanent cognitive or behavioral disturbances (Guerrini and Pellacani, 2012). Roughly one third of all children with new-onset epilepsy will develop pharmacoresistant (or refractory) epilepsy (Schuele and Luders, 2008). Potential factors that are predictive for refractory epilepsy in children include structural abnormalities, such as cortical dysplasia, mesial temporal sclerosis or dual pathology, underlying neurological deficit, focal seizures or multiple seizure types, high initial seizure frequency, neonatal seizures and failure to respond to first antiepileptic drugs (for review see (Wirrell, 2013)). In a subset of these children who have refractory epilepsy, surgery could be a successful treatment strategy. Its primary aims are seizure-freedom – or at least a significant reduction of seizure load – and the improvement of cognitive and behavioral performance,

particularly in young children in whom epileptic activity itself interferes with normal development (Ryvlin et al., 2014; van Schooneveld and Braun, 2013).

Irrespective of the eventual outcome, early and adequate treatment is desirable. It has been suggested that recurrent seizures hamper the developing brain in some types of epilepsies (Berg et al., 2014; O'Callaghan et al., 2011) and negatively influence the child's health-related quality of life (Sabaz et al., 2001). Otherwise, diagnostic inaccuracy could lead to a false diagnosis of epilepsy resulting in unnecessary antiepileptic drug treatment. To this end, an accurate decision-making tool for diagnosing epilepsy readily available at first consultation would be highly valuable.

Diagnosis of epilepsy

Accurate description of the epileptic seizures (also called 'seizure semiology') is an essential part of clinical history taking when the patient is suspected of epilepsy (Guerrini, 2006). In the majority of childhood-onset epilepsies, seizures are the first overt clinical symptom. However, the great variety in seizure manifestations obfuscates early recognition of the disorder by caregivers, the children and the consulted physicians (Berg et al., 2014). An accurate description of seizure semiology guides the diagnostic process and can be considered as a first attempt to localize and identify the underlying cause (Luders et al., 1998). To facilitate this, the ILAE proposed a seizure classification enabling a clinical translation between seizure semiology and different types of epilepsy. Traditionally, epileptic seizures were classified into generalized and focal seizures (ILAE, 1981). Generalized seizures usually consist of bilateral involuntary motor manifestations, loss of consciousness, or both and were believed to originate from bilateral engaging networks. Focal seizures manifest as typically unilateral motor or somatosensory symptoms and originate from one hemisphere (ILAE, 1981). Based on this dichotomy, epilepsies were similarly classified into focal and generalized epilepsies (ILAE, 1989). Although this traditional classification acknowledged that not all seizures and types of epilepsies could be classified as either generalized or focal, it is increasingly perceived to be too imprecise. The classification fails to reflect the clinical situation in many cases and disregards the underlying pathology. Therefore, the classification of seizures and the differentiation of epilepsy syndromes has been recently revised based on a more scientific and etiological rationale (Berg et al., 2010).

Besides clinical history taking, an electroencephalography (EEG) recording is often necessary to support the clinical diagnosis of epilepsy or for classification of the epilepsy syndrome. The initial EEG is often recorded interictally, which means during a seizure-free period, and has a limited sensitivity to discriminate children with and without epilepsy, ranging between 25 and 56% (Hamiwka et al., 2007; King et al., 1998; Shinnar et al., 1994; Smith, 2005). As a result, additional electrophysiological investigations, such as a prolonged or sleep-deprived EEG recordings, are often performed to increase the diagnostic yield (Smith, 2005; Wirrell, 2010). When the diagnosis remains inconclusive, clinical follow-up or other ancillary investigations such as neuroimaging, genetic and metabolic screening methods are needed to obtain a final etiological diagnosis.

A network perspective in epilepsy

In epilepsy research, there is a growing tendency to comprehend the brain as a complex interplay of structural and functional networks (Engel et al., 2013; Kramer and Cash, 2012; Richardson, 2012). Perceiving the brain as a network provides a theoretical framework to better understand, for example, the widespread cognitive problems (Vlooswijk et al., 2010) and structural brain abnormalities (Otte et al., 2012b) found in focal epilepsy, the fast spreading activity during secondary generalization of a focal seizure (Lopes da Silva et al., 2003), and the general cognitive improvement after successful surgical intervention in patients with focal refractory epilepsy (van Schooneveld and Braun, 2013).

To operationalize the concept of brain networks, a research paradigm characterizing these networks is needed (see box 1). An appealing method to quantify brain networks in a meaningful way was offered by Watts and Strogatz (Watts and Strogatz, 1998). In their seminal paper they describe a mathematical model using a network analytical approach. This model explained the efficient organization of complex systems such as the C. Elegans' neural network. Within a short time, the network analytical approach was widely adopted in the field of neurosciences to investigate the network organization, both in healthy as in the diseased brain (Bullmore and Sporns, 2009; Sporns et al., 2004; Stam, 2004). In epilepsy, network studies revealed novel insights into seizure dynamics and showed a gradual reorganization of functional brain networks before, during and after a seizure (Ponten et al., 2007; Schindler et al., 2007). Additionally,

brain areas associated with the seizure onset zone were identified as central network nodes during seizure generation (Ortega et al., 2008b; Wilke et al., 2011). In the interictal situation, pathological brain network alterations were associated with cognitive decline and language impairments (Vaessen et al., 2012; Vlooswijk et al., 2011) in children with chronic epilepsy. Together, these studies provided novel insights into abnormal brain organization and provided new opportunities for disease monitoring and early diagnosis in epilepsy (Douw et al., 2010a).

Aims and outline

The objective of this thesis is two-fold. First, we investigated if a network analytical approach could increase our understanding of the pathological mechanisms underlying epilepsy, and possibly contribute to the diagnostic process, particularly in newly onset (focal) childhood epilepsy. To that aim, we used interictal and intracranial EEG recordings that were part of clinical investigations in children suspected of having (focal) epilepsy or in patients with refractory focal epilepsy. More specifically, we characterized functional network alterations to investigate how these global and local network changes coincide with clinical signs and symptoms in epilepsy. The second aim of this thesis concerned the development of prediction models for diagnosing epilepsy in children in two different ways: i) based on network analytical data and ii) by means of routinely available clinical information and EEG report at first presentation. The research topics that we will address are based on the results from previous network analytical studies, which are extensively discussed in chapter 3 of this thesis, and will be provided in different parts:

PART I: Systematic reviews of networks in epilepsy. When constructing functional brain networks derived from EEG and magnetoencephalography recordings, several technical and methodological challenges are frequently encountered. These challenges are discussed in chapter 2. A qualitative review in chapter 3 introduces the epileptologist to the clinical application of a network analytical approach in epilepsy, and summarizes the most important network analytical results of previous research. Recommendations for future studies are provided as well. Considering the inconclusive results on global network alterations in focal epilepsies compared to healthy subjects, the systematic review and meta-analysis in chapter 4 pools

the results from different studies to clarify this. We specifically aim to characterize global disturbances in brain network topology in focal epilepsies, which may relate to the wide-range cognitive deficits in these patients.

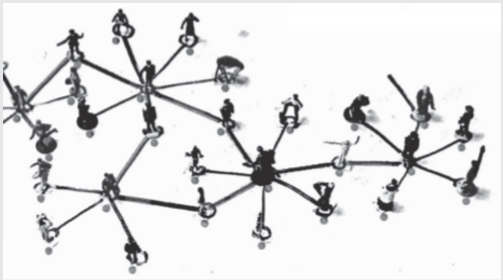
PART II: Brain network studies in epilepsy. The purpose of **chapter 5** is to assess whether specific focal epileptic EEG abnormalities, so-called high frequency oscillations, coincide with local network alterations. Intracranial EEG recordings from patients with refractory focal epilepsy are used to construct functional brain networks and are related to local network analytical metrics. In **chapter 6** the minimum-spanning tree (MST) approach is used to investigate if functional brain network alterations – derived from EEG recordings before and after sleep deprivation – are associated with an increased sensitivity of the EEG recording after sleep deprivation. A shift towards a more epileptic network configuration after sleep deprivation might explain the increased diagnostic yield of sleep deprived EEG recordings. The MST approach overcomes specific methodological limitations of conventional network analysis and focuses on the 'connectivity backbone' of the original network. A similar approach is used in **chapter 7** to investigate if functional EEG networks differences are present at an early stage of the disease and how they differ between focal and generalized epilepsies. A difference in network configuration between both types of epilepsies can relate to distinct processes that support the generation of focal or (primary) generalized seizures.

PART III: Prediction models in children with suspected epilepsy. The information provided by functional EEG network metrics from standard interictal EEG recordings at time of first consultation is used to early diagnose children with new-onset focal epilepsy (**chapter 8**). Network metrics are combined into a robust multivariate prediction model and its performance is compared with conventional EEG evaluation to investigate if a network analytical approach can improve the early diagnosis of focal epilepsy. In **chapter 9** individual clinical and EEG characteristics of a large cohort of children who visited a first seizure clinic are used to build a multivariate logistic regression model and tested to provide a reliable prediction of the diagnosis epilepsy. Here, the aim is to create a prediction model to assist the clinician in reliably predicting the diagnosis of epilepsy after a possible seizure.

PART IV: Discussion and summary. In chapter 10 the main findings from this thesis are summarized and discussed. Finally, a vision is given on how future network studies can be used to be of additional value in clinical practice.

Box 1: A brief history of network analysis

Network analysis is a branch of graph theory, which studies graphs in a simplified representation of nodes (vertices) and connections (edges). Its roots date back to the 18th century, when Leonard Euler solved 'the crossing of the seven bridges of Königsberg' by representing the problem as a network – stripped of irrelevant details – and focusing on the pattern of connectivity (Euler, 1741). Doing so, Euler proved that it was impossible to walk through the city of Königsberg from side-to-side crossing each of its seven bridges only once. Two centuries later, Stanley Milgram took a similar approach and confirmed experimentally that social networks contain properties of local clustering that enables short social distances between individuals (Milgram, 1967). This so-called 'small-world' phenomenon implicated an efficient organization of complex systems (see figure). At the turning of the century, two experimental models provided scientific support that efficient organization relies on a brittle balance between local segregation and global integration (Watts and Strogatz, 1998), and on the presence of centrally connected nodes, so-called 'hubs' (Barabási and Albert, 1999). Over the past decade, the information derived from these mathematical and social networks has been increasingly applied to the brain. As a result, we currently better understand that the balance on which efficient organization of brain networks relies, is disturbed in numerous neuropsychiatric disorders including epilepsy.



Organization of social networks' (adapted from milgram, 1967)
This schematic illustration of a social network depicts local clustering around persons that are centrally connected, leading to an efficient organization in terms of information flow from person to person. In essence, network analytical studies in neurosciences construct brain networks in a similar way. Brain regions are the nodes in a network, and structural and functional connections describe the communication flow between those brain regions.

CHAPTER 2

OPPORTUNITIES AND METHODOLOGICAL CHALLENGES IN ELECTROENCEPHALOGRAPHY AND MAGNETOENCEPHALOGRAPHY RESTING STATE FUNCTIONAL BRAIN NETWORK RESEARCH

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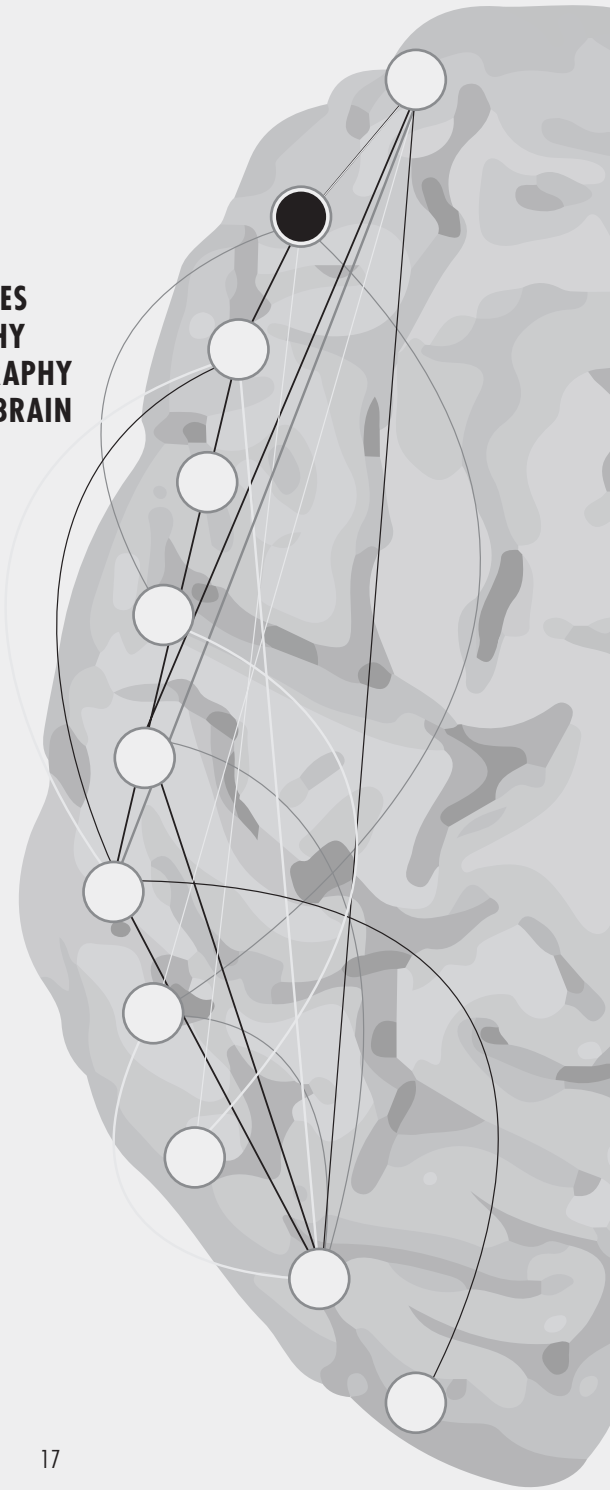

ABSTRACT

Electroencephalogram (EEG) and magnetoencephalogram (MEG) recordings during resting state are increasingly used to study functional connectivity and network topology. Moreover, the number of different analysis approaches is expanding along with the rising interest in this research area. The comparison between studies can therefore be challenging and discussion is needed to underscore methodological opportunities and pitfalls in functional connectivity and network studies. In this overview we discuss methodological considerations throughout the analysis pipeline of recording and analyzing resting state EEG and MEG data, with a focus on functional connectivity and network analysis. We summarize current common practices with their advantages and disadvantages; provide practical tips, and suggestions for future research. Finally, we discuss how methodological choices in resting state research can affect the construction of functional networks. When taking advantage of current best practices and avoid the most obvious pitfalls, functional connectivity and network studies can be improved and enable a more accurate interpretation and comparison between studies.

INTRODUCTION AND RATIONALE

In recent years, there has been a growing interest in characterizing the functional network of the brain 'at rest'. This so-called 'resting state' paradigm is believed to reflect intrinsic activity of the brain, which may reveal valuable information on how different brain areas communicate (Birn, 2012; Deco et al., 2011; Greicius et al., 2003). It has linked spontaneous – task independent – fluctuations in neural activity to diseases, cognitive decline, and disturbances in consciousness (Bassett and Bullmore, 2009; Bullmore and Sporns, 2009; Greicius, 2008; Stam, 2014).

This interest in the 'resting state' is associated with several breakthroughs in functional magnetic resonance imaging (fMRI) research (Raichle, 2009). The claim, however, that valuable information on communication between brain areas can be inferred from intrinsic activity – obtained with neurophysiological techniques – is much older (for a comprehensive overview see (Pinneo, 1966; Snyder and Raichle, 2012)). The high spatial resolution might be a favourable feature of fMRI; still this technique only provides an indirect measurement of brain activity and has a limited temporal resolution. Information processing in the brain, however, acts on multiple time-scales, depending on the specific cognitive or behavioural function (Lopes da Silva, 2013). A considerable part of the information processed in the brain at rest is encoded on time scales from milliseconds to seconds (Koenig et al., 2005), a time scale that better suits techniques such as electroencephalography (EEG) and magnetoencephalography (MEG).

In the last decade, EEG and MEG connectivity and functional brain network studies have gained considerable interest resulting in a yearly growing number of published studies on this subject (Figure 1). These studies have provided valuable information on the deviant organisation in the diseased brain, such as in Alzheimer's disease (Dubovik et al., 2013; Stam et al., 2007a), epilepsy (Bartolomei et al., 2006; Ibrahim et al., 2013), schizophrenia (Hinkley et al., 2010; Siebenhuhner et al., 2013), multiple sclerosis (Schoonheim et al., 2013; Van Schependom et al., 2014), Parkinson's disease (Fogelson et al., 2013), as well as in the healthy brain on topics as aging (Smit et al., 2012; Vecchio et al., 2014), gender differences (Boersma et al., 2011) and a healthy lifestyle (Douw et al., 2014). Furthermore, connectivity and functional brain network

studies can be used in the clinical setting. For example, in epilepsy it has been shown to prompt early diagnosis (van Diessen et al., 2013c) and to improve accuracy of epilepsy surgery by removing aberrant network nodes (Wilke et al., 2011). In Alzheimer's disease, EEG connectivity studies were used to monitor the success of novel interventions (de Waal et al., 2014). Similarly, progression of cognitive deficits in Parkinson's disease was correlated with functional brain network changes (Olde Dubbelink et al., 2014). Together these examples clearly underline the importance and additional value of connectivity and brain network analyses in EEG and MEG research.

When performing these analyses, one makes several assumptions and choices that may influence the eventual results. Moreover, the literature on functional connectivity and functional network studies is rapidly evolving, with an increasing number of analysis methods becoming available. Discussion is needed to obtain uniformity and comparability between different studies (Duncan and Northoff, 2013; Gross, 2014). The present paper therefore aims to highlight challenges, problems, and opportunities that are encountered when performing this type of research. As there are only few methodological studies that address these issues systematically, our review can be seen as a reflection of the current state of the field. We provide an overview of the methodological issues that should be considered when performing functional connectivity and network studies with EEG or MEG, and highlight the advantages and disadvantages of different approaches. Although we specifically focus on resting state EEG and MEG studies, most of the information provided is also applicable to task-related studies and other imaging techniques such as fMRI.

We start with subject-related methodological issues that are of interest when conducting a resting state study. What is resting state, and how does the heterogeneous experience of subjects affect resting state studies? Furthermore, we explain how the state of vigilance and an eyes open versus eyes closed condition might influence the resting state recording. We then summarize analysis-related methodological choices that can strongly influence the eventual results of a functional connectivity or network study, namely: choice of EEG reference, source space analysis, artifact handling and filtering, epoch selection, choice of frequency bands, and test-retest reliability. For each methodological choice we introduce current common practice, explain why these choices are important for the eventual data analysis and summarize the advantages and disadvantages of

each choice. Furthermore, we discuss issues that are still unresolved or subject of debate and give some general recommendations. Subsequently, a concise overview of currently used functional connectivity measures is provided and how these connectivity measures relate to various methodological choices and assumptions. Finally, we discuss current challenges in functional network analysis based on EEG and MEG recordings.

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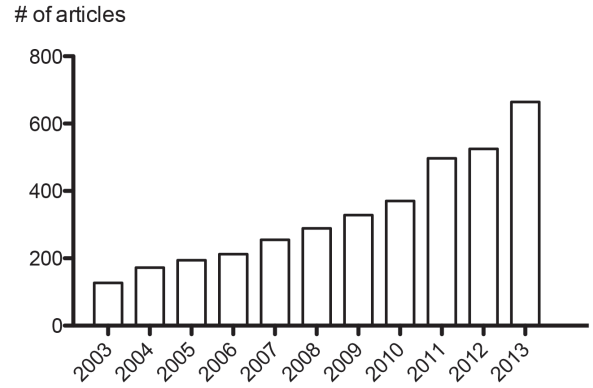

Increase of EEG and MEG network studies

Number of articles per year from Pubmed search using keywords "(EEG OR MEG) AND (connectivity OR brain networks OR functional networks OR graph theory OR network analysis)" in the period 2003-2013.

SUBJECT-RELATED METHODOLOGICAL ISSUES

What is 'resting state' and how does it affect the recording?

Resting state is the state in which a subject is awake and not performing an explicit mental or physical task. Traditionally, the 'resting state' condition was commonly used in EEG research – besides event-related potential studies – to study patterns of brain activity, whereas fMRI research was mainly focused on alterations in activity during task performance. Early EEG studies, including the first EEG recordings performed by Berger (Berger, 1929), already provided evidence for patterns of brain activity when subjects were not performing a task (Collura, 1993). Ironically, it was not until Biswal and colleagues revealed

a distinct fMRI pattern of interacting brain regions when not performing a task that the resting state condition became a research paradigm for the study of interconnectivity of brain regions (Biswal et al., 1995). Since then, many studies have identified sets of brain regions that share a common activation pattern during the resting state (Damoiseaux et al., 2006; Greicius et al., 2003) including the 'default mode network' (Raichle et al., 2001; Shulman et al., 1997) and other so-called resting state networks (Rosazza and Minati, 2011). These resting state networks have been replicated and validated both in neuroimaging and neurophysiological studies (Brookes et al., 2011; Hipp et al., 2012; Miller et al., 2009), suggesting that resting state patterns of connectivity are the result of robust and specific intrinsic neural activity.

In contrast to task related activity, it is difficult to control the behaviour of subjects during a resting state experiment. Differences in the specific instructions for the resting state period may at least partly influence the activity of the default mode network (Benjamin et al., 2010). Commonly, subjects are asked to close the eyes and not to fall asleep. As a result, thoughts are drifting and thereby creating stimulus independent thoughts (Andreasen et al., 1995; Teasdale et al., 1995). A recent study investigated what kind of thoughts patients experienced during a resting state recording and found several phenotypes of resting state cognition (Diaz et al., 2013). Even when similar instructions were given to subjects, the subjective experience during a resting state recording varied greatly, thereby potentially confounding the results. In addition, the cognitive state before the recording could also influence resting state dynamics (Lopez Zunini et al., 2013). These findings underline the heterogeneous experience of subjects during the resting state condition. Instructing a resting state condition might thus not be as straightforward as it seems. Controlling spontaneous thoughts is difficult, however, it might not be necessary as the experienced random episodic spontaneous thoughts seems to activate similar brain regions as resting state networks (Andreasen et al., 1995). Furthermore, consistent activation patterns of resting state networks in healthy controls among studies suggest only a limited influence (Damoiseaux et al., 2006). To reduce the externally induced heterogeneity of the resting state condition, we recommend the use of a priori defined instructions throughout a study, which should be reported in the method section of the eventual manuscript. Furthermore, subjects should have a similar pre-experimental procedure, to avoid introducing variance in cognitive state.

Therefore, researchers should consider the order of neuropsychological testing and recording of resting state. We suggest to first record a resting state condition after which an experimental session can be performed to minimize disturbances of the resting state condition. Finally, longer registration increases the stability of resting state networks, as with time it becomes more likely that the complete repertoire of resting state networks has been activated (Honey et al., 2012), but this also increases the risk of drowsiness in subjects when no precautions are taken. The exact relation between duration of recording and stability in EEG and MEG resting state functional connectivity remains complex and is discussed further in section 'Number and length of epochs and sample frequency'.

State of vigilance

During the day, the brain is constantly shifting between different levels of activation, also called 'states of vigilance'. In EEG and MEG research, we distinguish several vigilance states. These different states can be identified by visual inspection or spectral analysis of the EEG (Minkwitz et al., 2011; Olbrich et al., 2009). The three most studied vigilance states are wake, sleep, and sleep deprived. The state 'drowsiness' is often avoided in resting state research, because alertness or wakefulness is reduced during a drowsiness state and may vary and influence measurements greatly. However, recognizing drowsiness in resting state recordings can be difficult and requires a systematic approach (Koenis et al., 2013; van Diessen et al., 2014b). Each state of vigilance has specific characteristics that contribute to differences in spectral power (Cantero et al., 1999; Niedermeyer and Lopes da Silva, 1987) and functional connectivity (Kuhnert et al., 2010; Piantoni et al., 2013). Several factors have been identified that influence state of vigilance: circadian rhythm (Kuhnert et al., 2010), task performance before the recording (Klimesch et al., 1999), including neuropsychological testing, medication use, or even caffeine intake (Barry et al., 2011; Siepmann and Kirch, 2002; Tal et al., 2013). Body posture and recording environment may also affect vigilance and, consequently, functional connectivity measures. For example, drowsiness is more likely to occur in a dimly lit and sound attenuated room with the subject in supine position, compared to a noisy environment with the subject sitting in daylight. Also, the amplitude of the EEG recording changes as a result of different body postures due to shifts in cerebrospinal fluid layer thickness (Rice et al., 2013). The effect size

of these possible confounders in resting state studies is unknown. It is therefore recommended to eliminate or record and correct for these possible confounders as much as possible.

Eyes open versus eyes closed

Whether a recording is performed with eyes open or eyes closed influences the resting state condition greatly. Evidence from fMRI, MEG and EEG studies has revealed differences between eyes open and eyes closed conditions for functional connectivity measures and functional networks (Horstmann et al., 2010; Jin et al., 2014; Tan et al., 2013; Xu et al., 2014). Irrespective of the condition, eye movements affect neurophysiological recordings, particularly the frontal channels (Allen et al., 2004; Davidson, 1988) and are thus a potential confounder in connectivity analyses. Both eyes open and eyes closed are associated with specific eye movements. For example, eye blinks are more prevalent during eyes open condition, whereas rolling of the eyes might particularly influence the eyes closed condition. Rolling of the eyes during eyes closed condition is often due to drowsiness, which is normally not a state of vigilance where resting state studies are interested in and, as a result, discarded from further analysis (section 'State of vigilance'). Furthermore, the eyes closed condition, is more stable over sessions when quantifying EEG parameters than the eyes open condition (Corsi-Cabrera et al., 2007) and standardization of the procedure is relatively straightforward even in subjects who are difficult to instruct such as children, or patients with behavioral or cognitive problems. Together with the robust topographic effect posteriorly in the alpha frequency band when eyes are closed, thereby giving good guidance for selecting resting state epochs, we advocate to use the eyes closed condition during resting state recordings. Methods to automatically remove eye movements from the EEG and MEG recordings are discussed in section 'Signal versus source space'.

ANALYSIS-RELATED METHODOLOGICAL CHOICES

Choice of reference

In contrast to MEG, the electric potentials measured by EEG electrodes are defined with respect to a reference. Besides bipolar recordings, in which EEG activity is defined by the electric potential difference between two electrodes, EEG recordings often use a single common reference such as auricular, mastoid or central electrode

as reference. These conventional reference montages are confounded by brain activity that will eventually affect further analysis. As a result, recordings are often re-referenced offline to compute reference montages that are electrophysiologically more silent (Hu et al., 2010; Kayser and Tenke, 2010; Nunez et al., 1997; Pivik et al., 1993). The common average reference has previously been suggested as a practical compromise to reduce the confounding effect of brain activity that is picked up by the reference (Nunez et al., 1997). The advantage of the common average reference of approximating a zero sum reference is, however, increasingly lost in low-density EEG recordings (Nunez and Srinivasan, 2006; Schiff, 2005). To this end, several other methods have been proposed. These include the infinity reference, which tries to estimate a time-varying constant that is removed from the recorded data (Yao, 2001), and the surface Laplacian, which represents a truly reference-free transformation (Hjorth, 1975; Tenke and Kayser, 2012). Similar to the conventional and average common reference, the infinity reference is reversible to the original reference scheme, whereas the surface Laplacian involves the estimation of radial current flow at the scalp, which cannot be undone (Tenke and Kayser, 2012). Both methods, in particular surface Laplacian, have been used empirically in various basic and applied contexts (Nunez and Srinivasan, 2006). Recently, a statistically robust method has been proposed to adequately mitigate the influence of neural activity in the common average reference (Lepage et al., 2014). More investigations are needed to further explore the performance of this theoretically appealing method.

A related question is to what extent reference choice will affect the computation of connectivity measures. For example, Qin and colleagues demonstrated that infinity reference has a superior performance compared to other reference montages when estimating functional connectivity by means of coherence (Qin et al., 2010). Correlation measures such as coherence, however, are increasingly abandoned in connectivity studies, as they fail to include information on the intrinsic nonlinearity of brain activity (section 'Connectivity measures') and it is currently unclear whether the superior effect of the infinity reference is maintained when using nonlinear connectivity measures. We await future studies that critically evaluate possible biases due to reference as was illustrated for effective connectivity measures (van Straaten et al., 2014).

In the light of the ongoing discussion on reference choice (Kayser and Tenke, 2010; Nunez, 2010) and its effect on connectivity measures (Qin et al., 2010;

van Straaten et al., 2014) we encourage researchers to explore the effects of different types of references when computing connectivity measures.

Signal versus source space

Many resting state EEG and MEG studies use the activity at the electrode level to infer how brain regions are (functionally) interconnected. This analysis is performed in so-called 'signal space' as neural activity is directly inferred from signals measured at the EEG electrode or MEG sensor. When performing connectivity analysis in signal space, several factors should be considered. Firstly, multiple electrodes pick up activity from a single source due to the nature of the signal, also called 'field spread' (Sarvas, 1987). A second problem is related to volume conduction: the 'blurring' effect due to the electrical conduction properties of the human head (van den Broek et al., 1998). Together, these factors can result in an erroneous estimate of the actual connectivity between brain areas. To obtain more reliable information on the communication between brain areas, studies project the activity measured at the electrode or sensor (signal space) back to the underlying sources, the so-called 'source space'. The mapping from signal space to source space is known as the inverse problem (Niedermeyer and Lopes da Silva, 1987). Unfortunately, no unique solution exists to this problem (Helmholtz, 1853), unless constraints and assumptions are made. These assumptions concern, for example, the number of possible sources or the non-linearity between sources (Hillebrand and Barnes, 2005; Michel and Murray, 2012). Furthermore, analyzing neurophysiological signals in source space does not completely overcome the problems of field spread and volume conduction, and it has therefore been suggested to combine source space analysis with a robust connectivity measure (Hillebrand et al., 2012). These robust measures include, for example, imaginary coherence, phase slope index or phase lag index (section 'Connectivity measures'). Secondly, the mixture of signals arising from spatially separated sources at a single electrode also hampers the interpretation of connectivity estimate in signal space (Nunez and Srinivasan, 2006; Schoffelen and Gross, 2009). Source space analysis could be helpful in demixing signals (Michel and Murray, 2012). Various approaches for source space analysis have been offered (Baillet et al., 2001), including low resolution brain electromagnetic tomography (LORETA) in EEG research (Pascual-Marqui et al., 1994) and the beamforming approach for EEG and MEG recordings

(Hillebrand and Barnes, 2005; van Drongelen et al., 1996). In general, the accuracy of source localization increases with the number of recording sites (Lantz et al., 2003) if the signal quality remains constant, although a noiseless recording condition may also allow source localization with a standard 10-20 system (Laarne et al., 2000). A high channel density recording might even become a disadvantage for source reconstruction approaches that rely on an accurate description of the lead fields. Lead field is defined as the electrode or sensor signal that is produced by a source of unitary strength. Incorrect source and head models lead to deviations from the 'true' lead fields, and subsequently to source reconstruction errors (Hillebrand and Barnes, 2003; Hillebrand and Barnes, 2011). These errors in the lead fields are more discernible when using good quality data. Counterintuitively, recordings with higher signal-to-noise ratio and higher density may therefore degrade source reconstructions in the presence of these modeling errors. Finally, increased channel density can result in bridging, the incidental spread of electrolyte gel between adjacent electrodes, thereby negatively influencing the recording and subsequent inverse solutions and/or connectivity estimates (Alschuler et al., 2014).

Artifact handling and filtering

To minimize the influence of artifacts on the results, visual inspection and automatic detection of artifacts are often used to remove artifacts or to select artifact-free data segments. Many attempts have been made to reject or mitigate eye movement artifacts, to reduce interobserver variability, and to improve efficiency in visible inspection of the data (Cassani et al., 2014; Croft and Barry, 2000). Blind source separation, such as independent component analysis, is increasingly used for the detection and removal of ocular artifacts (Gao et al., 2010; LeVan et al., 2006), although it is unknown to what extent these artifact reduction methods influence functional connectivity and network metrics. Furthermore, a number of techniques is available for automatic removal of muscle artifacts; however, none of them guarantees muscle artifact free data (Muthukumaraswamy, 2013). Improvement of software-based muscle artifact recognition is therefore needed (Delorme et al., 2007; Whitham et al., 2007). Currently, most studies use visual inspection to eliminate epochs with myogenic or eye-movement artifacts, although the precise procedure is often not described. Some studies mention the removal of frontopolar and auricular channels to reduce the influence of artifacts (de

Haan et al., 2009; van Dellen et al., 2014b). Although these artifacts will also affect other channels due to volume conduction, this influence is reduced when excluding frontopolar and auricular channels prior to offline re-referencing. It is, however, preferable to select artifact free epochs. In addition, visual recognition of any EEG artifact depends on the chosen reference montage. This means that a possible artifact on the reference channel could influence all other recording sites and could lead to the undesirable rejection of an epoch. Although this problem is relatively easy resolved by replacing (e.g. interpolating) the original reference, it underlines the importance that visual inspection should be done by a well-trained researcher.

In EEG and MEG recordings analogue filtering is needed to prevent from aliasing and eliminate the direct current (DC) component. Besides the analogue filtering, software programs have digital filtering options that can be useful to improve inspection and selection of epochs. It is important to realize, however, that this digital filtering may affect amplitude and phase of EEG and MEG recordings. It is essential to know how the data is exported from the recording device and whether a digital filter is contaminating the signal. To avoid any possible influences of the digital filters on the recorded signal, it is therefore important to consider elimination of software based filtering or to use zero-phase filtering, for example by a forward and reversed filtering approach.

Epoch selection

Standardization and interobserver variability in epoch selection

Selection of epochs based on visual inspection is a subjective approach, which may result in inter-observer variability. The effect of epoch selection on functional connectivity has never been investigated systematically. A few studies, however, assessed the stability of their outcome measures by repeating the analysis with a different number and selection of epochs, showing minimal changes between conditions (Douw et al., 2013; Olde Dubbelink et al., 2014; van Dellen et al., 2014b). This may indicate a modest subjective influence on epoch selection when sufficient epochs are selected. Still, automated analysis could be helpful for researchers and clinicians in evaluating and improving epoch selection in EEG and MEG recordings (Lodder et al., 2014). The current complexity and limited transparency of automated detection systems demotivate researchers to use it on a larger scale (Anderson and Doolittle, 2010) although recent

advances are promising (Shibasaki et al., 2014). Also, it is recommended to define the selection criteria prior to the epoch selection. Selection by two or more experienced researchers or clinicians could improve the reliability of the epoch selection. For example, when a researcher selects epochs according to predefined criteria, a second researcher can be asked to independently evaluate the selected epochs. When both researchers agree on the quality, the epoch can be included. Epochs without consensus can be replaced by new epochs. Another option is the use of an automatic software-based rejection procedure (section 'Artifact handling and filtering') or a random selection among all selected artifact-free epochs (Shibasaki et al., 2014). Beside the elimination of artifacts in the included data, it is important to define the time segments in the recording from which epochs are selected. As the variance of vigilance increases with the length of the EEG recording (Maltez et al., 2004), we recommend selecting the first artifact free epochs of sufficient quality after the start of the resting state recording. In this way, selection bias of EEG epochs will be minimalized.

Number and length of epochs and sample frequency

Different epoch lengths and number of epochs are currently used in resting state functional connectivity studies, ranging from one second (Chu et al., 2012; Knyazeva et al., 2010) to a few minutes (Tahaei et al., 2012) or even a day (Kuhnert et al., 2010) for epoch length; and from one epoch (Ahmadlou and Adeli, 2011) to over 100 epochs containing the entire EEG recording (Knyazeva et al., 2010). Previous studies have investigated epoch length in relation to connectivity stability (Chu et al., 2012; David et al., 2004; Honey et al., 2007) and showed that the length of epochs to obtain stable connectivity measures is highly dependent on the type of connectivity measure. Recent studies have used a more pragmatic approach and extended the original analysis to investigate connectivity stability for the included subjects, for example, by determining the minimum number of epochs that are needed using a leave-one-out analysis (Douw et al., 2013). Similarly, varying the epoch length revealed that a longer epoch length does not automatically imply a more stable connectivity value. For phase synchronization measures, longer epochs could result in lower connectivity values based on the asymmetrical distribution of the phase difference (van Dellen et al., 2014b). From this perspective, we recommend to use epochs of an identical length within a study, as epoch length can influence the connectivity measure

(van Dellen et al., 2014b), and to choose an epoch length in accordance with the connectivity measure of choice. Furthermore, we recommend computing connectivity values per epoch and consequently an average value (over epochs) per subject to increase the stability of connectivity values. In some studies, it might be particularly interesting to investigate temporal dynamics of functional connectivity and networks. The use of sequential short epochs might be useful to study these dynamical properties (Singer, 2013).

An often neglected issue when choosing epoch length and number is the sample frequency. Increasing sample frequency will result in a higher temporal resolution and, consequently, a higher number of samples in one epoch. It is unclear how sample frequency influences connectivity measures exactly, although it is reasonable to assume that a lower sample frequency leads to a reduced sensitivity and a larger variability over epochs for detection of coupling between signals. Sample frequencies commonly used in functional connectivity EEG and MEG studies range between 250 and 512 Hz. Analysis of connectivity measures in higher frequency bands requires higher sample frequencies to fulfill the Nyquist-Shannon sampling theorem (Candès, 2006). Otherwise, the spectral resolution is only dependent on the length of the epochs. We recommend comparing epochs with a similar sample rate even if this would mean down sampling for some epochs.

Choice of frequency bands

Factor analysis revealed that the classification of EEG recordings into distinct frequency bands, namely delta (0.5 – 4 Hz), theta (4 – 8 Hz), alpha (8 – 13 Hz), beta (13 – 30 Hz) and gamma (30 – 90 Hz), is statistically sound (Lopes da Silva, 1998). Furthermore, each frequency band is associated with distinct cognitive functions (Basar et al., 2001). Nevertheless, considerable disagreement exists whether traditional frequency bands are indeed fixed entities. For example, spectral band limits seems to depend on factors such as age (Aurlien et al., 2004; Boersma et al., 2011). In addition, it has been argued that the lower (8 – 10 Hz) and higher (10 – 13 Hz) alpha frequency band are involved in different cognitive processes (Klimesch, 1999). The reliability of the gamma frequency band is under debate as it remains questionable whether gamma oscillations can be reliably inferred from surface recordings (Lopes da Silva, 2013). Although various research fields have consistently identified gamma activity being related

to tasks (e.g., visual attention, perception and memory (Lopes da Silva, 2013)) or diseases (e.g., epilepsy, autism, ADHD and schizophrenia (Herrmann and Demiralp, 2005)), studies have suggested that EEG oscillations >20 Hz from surface recordings reflect myogenic artifact (Pope et al., 2009; Whitham et al., 2007). These artifacts can, however, be removed using surface transformations of the EEG (Fitzgibbon et al., 2013). Besides myogenic artifacts, saccadic spike potentials are also known to affect the gamma frequency band (Yuval-Greenberg and Deouell, 2009; Yuval-Greenberg et al., 2008). Finally, various diseases of the brain have been associated with abnormal slowing in specific frequency bands and thereby acting as disease specific modifiers, for review see (Kaplan and Rossetti, 2011). These disease specific spectral changes might become a problem when evaluating connectivity measures in these patients, particularly when connectivity measures are strongly dependent on amplitude. Connectivity measures based on phase differences are therefore recommended, as they are not or less sensitive to amplitude differences (section 'Connectivity measures'). In addition, we suggest performing a power spectral analysis along with connectivity and functional network analyses to disentangle disease specific spectral changes from disease related connectivity and network changes in the frequency bands (de Haan et al., 2009; van Diessen et al., 2013c). To overcome previously described frequency band-related problems, some studies have used a broadband approach. This could be an option when exploring an undirected hypothesis and to avoid inflated type I error rates due to multiple comparisons across frequency bands, but it will probably fail to disentangle simultaneous (opposing) changes in different frequency bands.

Test re-test reliability

To evaluate changes in resting state EEG recordings over time or the effects of an intervention, it is important to understand the effects of multiple recordings over time on the outcome measures. Previous literature showed a moderate to high intra-individual correlation in EEG spectral analysis, with correlation coefficients ranging between 0.4 and 0.9 (Fingelkurts et al., 2006; Gasser et al., 1985; Gudmundsson et al., 2007; Kondacs and Szabo, 1999; Napflin et al., 2007). These correlations do not seem to depend on the time interval between the recordings (Olde Dubbelink et al., 2014; Salinsky et al., 1991). When the interval between two tests are separated by a few months or years, one should

also be aware of the effects of aging, especially in pediatric studies (Boersma et al., 2011; Smit et al., 2012).

CONNECTIVITY MEASURES

To investigate functional interactions between brain regions, EEG and MEG studies have used different connectivity measures, for an overview see (Bonita et al., 2014; Pereda et al., 2005; Stam, 2005). The quantification of interacting brain regions can be subdivided into functional and effective connectivity measures (Friston, 1994; Friston, 2011). Connectivity measures are based on statistical interdependencies between signals (Aertsen et al., 1989). The extent to which brain regions are connected is defined by the strength or consistency of this statistical interdependency (Varela et al., 2001), also called synchronization. In dynamical systems the term synchronization generally refers to (phase) coupling of two or more harmonic oscillators (Boccaletti et al., 2002), but it is currently used in a more liberal way in brain connectivity analyses (Daffertshofer and van Wijk, 2011). Thus, a stronger synchronization, often reflected by a high coupling or consistency of oscillating systems, leads to a stronger connection. In contrast to functional connectivity measures, which only give information about the temporal correlation, effective connectivity measures also provide information on causal and therefore directed information flow between brain regions (Friston, 2011). Increasing evidence suggests that information processing in the brain follows a complex, directional pattern between brain regions (Stephan and Roebroeck, 2012). Besides effective connectivity, directed functional connectivity measures can reveal disturbances in the normal directionality of information flow in the diseased brain, for example, in dementia (van Straaten et al., 2014), epilepsy (Korzeniewska et al., 2014), or when consciousness is disturbed (Lee et al., 2013). These directed functional measures do not provide information about the causal relation between brain areas.

Deciding on the most appropriate connectivity measure can be arduous, as several issues should be considered. This includes the consideration of linear or nonlinear relations, analysis in time or frequency domain, using an amplitude or phase-based measure, obtaining directed or undirected information, and whether to include indirect relations or not (i.e., multivariate or bivariate). Linear correlations to investigate connectivity of the brain have been used for

several decades and are relatively straightforward in terms of computation and interpretation, for a review see (Shaw, 1984). Since these linear methods are not able to take into account the intrinsic nonlinearity of neuronal activity, various nonlinear connectivity measures have been introduced (Pereda et al., 2005; van Mierlo et al., 2014; Vindiola et al., 2014; Wendling et al., 2009), including several phase-based connectivity measures, such as the phase locking value (Lachaux et al., 1999) and phase lag index (Stam et al., 2007b). Besides considering nonlinearity, novel multivariate connectivity measures are aiming to differentiate between direct and indirect interrelations (Friston, 2011). Whereas bivariate measures disregard the influence of other signals when computing the interaction between two signals of interest, multivariate measures try to disentangle this information in a meaningful manner. Obviously, all these different connectivity measures have their unique advantages and disadvantages. In Table 1 we provided an overview of the currently most often used measures, including their main advantages and disadvantages.

In addition to computational differences between connectivity measures, it is important to consider related methodological issues that could be encountered when choosing, and interpreting the results of, a connectivity measure. This includes, for example, the problem of field spread, volume conduction (section 'Signal versus source spaces') and specific reference montages (section 'Choice of reference'), which for many measures leads to an erroneously high estimate of connectivity between two recording sites (Nunez et al., 1997; Schoffelen and Gross, 2009; Stam et al., 2007b). It is possible, however, to remove these biases prior to computing connectivity measures (Brookes et al., 2012; Hipp et al., 2012) or to estimate the influence of the bias on the connectivity measure through simulations (Brookes et al., 2011). A more straightforward approach is to use phase-based connectivity measures that are less sensitive to these spurious interactions. Typically, these phase-based measures, such as the imaginary part of coherence (Nolte et al., 2004), phase-slope index (Nolte et al., 2008) and phase lag index (Stam et al., 2007b) ignore the zero phase interaction that are the result of volume conduction/field spread (at the expense of ignoring true zero phase interactions). The phase lag index has the additional advantage that it does not depend directly on the amplitude of the signal (Muthukumaraswamy and Singh, 2011).

T1

Connectivity measure	Property measured	Advantage(s)	Disadvantage(s)	Key reference(s)
Correlation	The linear relation between the amplitude of two signals in the time domain	Commonly used and straightforward method	Nonlinearity not considered Not possible to make a distinction between direct and indirect relations Sensitive to volume conduction	(Brazier and Barlow, 1956)
Coherence	The linear relation between the amplitude of two signals in the frequency domain	Commonly used and straightforward method	Nonlinearity not considered Not possible to make a distinction between direct and indirect relations Sensitive to volume conduction	(Adey et al., 1967)
Granger causality	The future of signal X can be predicted more precisely when the past of signal Y is included and vice versa	Estimates causal interaction, and therefore directionality is assessed Well established and widely used in many fields of research	Nonlinearity not considered Methodological choices (e.g., choice of reference in EEG), as well as other confounders (e.g., volume conduction) could interfere with the actual causality	(Bressler and Seth, 2011b; Granger, 1969; Hesse et al., 2003)
Directed coherence	The directed linear relation between two signal in the frequency domain based on the Granger causality principle	Directionality of information flow	Nonlinearity not considered Not possible to make a distinction between direct and indirect relations Sensitive to volume conduction	(Wang and Takigawa, 1992)
Directed transfer function	Gives the causal relation between the outflow of node X towards node Y in the frequency domain based on Granger causality principle, normalized by all inflows towards node Y	Directionality of information flow Distinction between common source and interconnectedness Insensitive to volume conduction	Nonlinearity not considered Not possible to make a distinction between direct and indirect relations Noisy channels affect the directionality Difficult to estimate an optimal order for the multivariate model	(Kaminski and Blinowska, 1991)

Table 1. Continued

Connectivity measure	Property measured	Advantage(s)	Disadvantage(s)	Key reference(s)
Partial directed coherence	Gives the causal relation between the outflow of node X towards node Y in the frequency domain based on Granger causality principle, normalized by all outflows from node X	Directionality of information flow Insensitive to volume conduction	Nonlinearity not considered Not possible to make a distinction between direct and indirect relations No conclusion about the strength of coupling, due to normalization Difficult to estimate an optimal order for the multivariate model	(Baccala and Sameshima, 2001)
Imaginary part of coherency	Based on coherency*, but excluded the influence of volume conduction by including only the imaginary part of the coherency	Less sensitive to volume conduction	Nonlinearity not considered Imaginary part is mostly small, thereby risking to miss meaningful interactions Not possible to make a distinction between direct and indirect relations	(Nolte et al., 2004)
Mutual information	Gives the amount of information in signal X that can be explained by signal Y and vice versa, based on the probability distribution of X and Y, and the joint probability distribution of X and Y	Mutual information is sensitive in narrow-frequency band analysis	No directionality of the interaction Weak coupling could be missed Complicated computational measure to obtain from experimental time series Not possible to make a distinction between direct and indirect relations	(Fraser and Swinney, 1986)
Synchronization likelihood	Describes the normalized strength of the mutual information between signal X with signal Y in state space	Adequately deals with complexity caused by interacting systems Sensitive to nonlinear relations	Sensitive to volume conduction Not possible to make a distinction between direct and indirect relations	(Stam and Van Dijk, 2002)

Table 1. Continued

Connectivity measure	Property measured	Advantage(s)	Disadvantage(s)	Key reference(s)
Phase locking value	Gives the modulus of the averaged instantaneous phase differences between two time series	Nonlinearity is taken into account	No directionality of interaction Sensitive to volume conduction The size of the instantaneous phase difference is included, however, there is no evidence that the size of the phase difference is important for the coupling strength Not possible to make a distinction between direct and indirect relations	(Lachaux et al., 1999)
Phase slope index	Estimates the direction of information flow, based on the slope of the phase difference of the cross spectral density between signal X and Y	Directionality of information flow Weighs the contribution of different time series Not affected by mixture of independent sources (e.g., background activity)	Not possible to make a distinction between direct and indirect relations Complicated computational method Not possible to make a distinction between direct and indirect relations	(Nolte et al., 2008)
Phase lag index	The asymmetry of the distribution of phase differences between two signals	Less sensitive to volume conduction, common sources, and montage	The risk to miss linear but functionally meaningful interactions The instantaneous phase differences are binarized, therefore, small phase differences may also be missed under noisy conditions Not possible to make a distinction between direct and indirect relations	(Stam et al., 2007b)

Table 1. Continued

Connectivity measure	Property measured	Advantage(s)	Disadvantage(s)	Key reference(s)
Weighted phase lag index	The contribution of the observed phase leads and lags is weighted by the magnitude of the imaginary part of the coherency	Reduced sensitivity to noise (cf. PLI) Improved detection of phase synchronization changes (cf. PLI)	The size of the instantaneous phase difference is included, however, there is no evidence that the size of the phase difference is important for the coupling strength Relative insensitive to phase differences around 0 and 180 degrees Mixes information about consistency and magnitude of phase differences, hampering interpretation Not possible to make a distinction between direct and indirect relations	(Vinck et al., 2011)
Directed phase lag index	The probability that the instantaneous phase of signal X was smaller than the phase of signal Y (modulo π) over time	Directionality of information flow Less sensitive to volume conduction and common sources (cf. PLI)	Directionality can be ambiguous as leading with a small differences is similar to lagging with a large phase difference Not possible to make a distinction between direct and indirect relations	(Stam and van Straaten, 2012a)

Connectivity measures in EEG and MEG research

Overview of different connectivity measures used in EEG and MEG research, with their advantages and disadvantages. *Coherency between two time signals is the linear relation at a specific frequency, an imaginary-valued measure containing information about the magnitude and phase between the signals. Coherence is the absolute value of coherency, containing only information about the magnitude.

FUNCTIONAL NETWORKS

Resting state EEG and MEG data can be used to construct connectivity matrices and, consequently, functional networks by using network analysis (Bullmore and Sporns, 2009; Sporns et al., 2004; Stam, 2010). In contrast to connectivity measures, which only provide information on how pairs of different brain regions are (functionally) connected, network analysis characterizes the organization of networks (Stam and van Straaten, 2012b). Complex network analysis, a branch of graph theory, reduces the brain into a collection of 'nodes' and 'edges' and allows quantitative characterization of these networks. In EEG and MEG research, nodes correspond to the recording sites (electrodes or sensors), or specific brain regions when using a source space analysis. Edges are connections between nodes and represent (functional) connectivity values. Together, nodes and edges form the basic elements of a network, and from these elements various global and local network measures can be inferred, for an overview see (Rubinov and Sporns, 2010) and section 'Global and local network metrics'. Providing an overview on the vast literature on functional networks and its statistical challenges (Zalesky et al., 2010) is beyond the scope of this paper; rather, we discuss relevant issues that need to be considered when preparing and analyzing a resting state EEG or MEG study.

Network construction

Functional networks are based on the strength or consistency of functional interactions between the network nodes. In a weighted network, the strength of this interaction is taken into account, whereas in an unweighted network only the existence or absence of an interaction is taken into account. Such a binary network is obtained by setting a threshold for the functional connectivity, above which a functional connection is considered to be present. A motivation to use a binary network could be to discard spurious connections that are potentially influenced by, for example, noise (Bullmore and Sporns, 2009). Selecting the value for the threshold is, however, arbitrary and may vary between individuals and groups (van Wijk et al., 2010). Although a weighted network overcomes the problem of this subjective factor and provides a more realistic representation of functional networks, spurious weak connections are also taken into account, potentially influencing network metrics. Besides weighted or unweighted, a

network can be directed or undirected. In order to construct a directed network, one should use an effective connectivity measure to infer information on the directionality of communication. Although this could potentially provide useful additional information on network functioning, most studies use undirected networks (van Wijk et al., 2010).

Network density

Network density refers to the number of connections in a network and is influenced by the size of the network. When comparing networks between subjects, the number of nodes should therefore be equal, as it will directly influence network density and various network metrics (van Wijk et al., 2010). A straightforward approach to correct for network density is by using a binary network. This, however, will lead to a data reduction wherein valuable information of the network is not taken into account (van Wijk et al., 2010). Other ways to reduce the effect of network density on network metrics is by using weighted networks in combination with normalization procedures that use network metrics based on surrogate data for comparison (Rubinov and Sporns, 2010; Stam and van Straaten, 2012b). Although these steps reduce the influence of network density on the eventual network metrics, it is still difficult to make an unbiased comparison between networks (Stam et al., 2014; van Wijk et al., 2010). Furthermore, each of these steps can potentially influence the computation of network metrics and should therefore be included in the methodological section of the study.

Minimum spanning tree

We have briefly mentioned several strategies to improve comparison between networks. Some of these strategies involve specific methodological choices, such as epoch selection, artifact handling, specific frequency bands, and connectivity measures (section 'Analysis-related methodological choices'). Other solutions include normalization procedures and the use of weighted networks (van Wijk et al., 2010). Despite these efforts, traditional network metrics will remain sensitive to the effect of network density. A new approach to overcome the problem of network density in network analytical studies involves computation of the minimum spanning tree (MST). The MST is a unique acyclic sub graph that contains most of the strongest connections of the original undirected, weighted graph, for review see (Stam et al., 2014). As the communication in the original graph follows the

most efficient paths (Van Mieghem and van Langen, 2005), the MST can be considered the backbone of the functional brain network (Van Mieghem and Magdalena, 2005; Wang et al., 2008). During the process of constructing the MST graph, connections that will lead to loops in the network will be excluded from the eventual network. By doing so, the number of connections in an MST graph will always correspond to the number of nodes minus one. As a result, MST networks with the same number of nodes will automatically have the same number of connections, thereby facilitating the comparison of networks. Moreover, normalization procedures with surrogate networks are not necessary anymore. A possible disadvantage of the MST approach is that it may miss information about the network topology that is contained in the weaker connections of the network. An increasing number of studies have evaluated the practical utility of the MST approach for network analysis in resting state EEG (Boersma et al., 2013; Lee et al., 2010; Ortega et al., 2008b; Schoen et al., 2011; van Diessen et al., 2014b) and MEG (Olde Dubbelink et al., 2014; Tewarie et al., 2014a; van Dellen et al., 2014a) recordings.

Global and local network metrics

Several metrics exist to characterize the organization of networks (Rubinov and Sporns, 2010). The two most commonly used network metrics are the average path length, a measure of global integration of the network, and the average clustering coefficient that defines local segregation of a network. Both average path length and average clustering coefficient are considered to be global network metrics as they provide global information of the network and are commonly used to describe the network topology. An optimal network organization is characterized by a short average shortest path length and a high average clustering coefficient, also called a 'small-world' configuration (Watts and Strogatz, 1998). Although these global network metrics are appealing to use, as they have been widely used in network analytical studies, they fail to explain the diversity found at node level (Bullmore and Sporns, 2009; Stam and van Straaten, 2012b).

To explain this diversity at node level, local network metrics are used. Network metrics like degree, betweenness centrality, and eigenvector centrality are used to specify the level of importance of a specific node in a network (Rubinov and Sporns, 2010). Nodes with many connections and a central position within the network are considered 'hubs'. Removal of a hub-node will have a

considerable impact on the network (Bullmore and Sporns, 2009; Stam and van Straaten, 2012b). Often, a network is built out of smaller subnetworks, also called 'modules'. These modules are formed by groups of nodes that are highly connected to each other (Newman, 2006), but much less so to other nodes in the network. Provincial hubs are the most highly connected nodes within a module. Connector hubs are the important nodes connecting one module with other modules in the network (van den Heuvel and Sporns, 2013b). Each local network metric captures specific information about the network topology, making it appropriate for certain analysis, depending on the specific research question. For example, the eigenvector centrality gives a more accurate estimation of the centrality of a specific node in the network than degree (Batool and Niazi, 2014), and has lower computational costs than betweenness centrality (Lohmann et al., 2010), it is less sensitive for the detection of hubs in modules (Joyce et al., 2010) and has specific normalization problems (Ruhnau, 2000).

When making choices for specific network metrics, it is recommendable to take into account the chosen connectivity measure. Connectivity measures that depend on linear correlations or the level of synchronization are more susceptible to volume conduction (Table 1). As a result, particularly the interaction between two nearby network nodes will be overestimated (Stam et al., 2007b), which might result in an overestimation of (local) clustering. Another important issue to consider when choosing network metrics, is the influence of network density (van Wijk et al., 2010). Although it is possible to reduce the effect of network density, the MST approach offers an elegant manner to control for differences in network density. Like in conventional networks, MST metrics are inferred from MST graphs. For example, MST diameter and leaf number are global metrics that provide information on network integration and segregation, similarly to average path length and average clustering coefficient. Furthermore, various other local MST metrics can be computed (Boersma et al., 2013; Stam et al., 2014; Tewarie et al., 2014a). Finally, it is important to realize that some network metrics are highly correlated (Li et al., 2011; van Diessen et al., 2013c), which means that some combinations of network metrics are redundant. It is difficult to recommend specific network metrics, as this will eventually depend on the specific research question.

CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

We have summarized several problems and challenges by reviewing current practice in resting state functional connectivity EEG and MEG research. First, performing a resting state recording might not be as straightforward as it seems; behavior during, and perception of, a stimulus independent condition may vary greatly between subjects despite similar instructions (Diaz et al., 2013). In our overview, we differentiated subject-related from measurement-related methodological issues. For future research we suggest to explicitly mention the instructions given to the subject and to control for factors that might influence the state of vigilance of subjects. Second, we mentioned technical issues that are important to consider when collecting data from resting state EEG and MEG recordings (Figure 2 for summary). Since the current literature is too diverse to provide a uniform methodological guideline, we suggest including different methodological approaches in resting state studies to better understand the influence of these approaches on study results. Some recommendations are, however, useful irrespective of the chosen approach (Gross et al., 2013). We summarized these recommendations in Table 2.

Since resting state EEG and MEG recordings are increasingly used for a network analytical approach (Bullmore and Sporns, 2009; Stam and van Straaten, 2012b), we briefly introduced some relevant topics. It is recommendable to decide on the connectivity measure and network metrics simultaneously as they are mutually dependent. We provided suggestions to overcome some limitations that are inherent to conventional network analysis (van Wijk et al., 2010) and offered a new approach to overcome the influence of network density on network metrics: the minimum spanning tree (Stam et al., 2014).

Finally, methodological studies are needed to systematically investigate the influence of various choices that researchers have to make when conducting a functional connectivity resting state experiment. Particularly the process of selecting data and artifact handling needs a more evidence-based approach. Regarding the choice of an appropriate connectivity measure, more information on the advantages and disadvantage is available (Pereda et al., 2005; van Mierlo et al., 2014; Vindiola et al., 2014; Wendling et al., 2009). Increasing evidence exist that some connectivity measures are more vulnerable to volume conduction (see Table 1), leading to unreliable connectivity values, and consequently,

unreliable network estimations. Future studies should use this knowledge to make appropriate decisions. We await methodological studies wherein different methodological issues are investigated systematically. This would be invaluable to the field of functional connectivity and network studies.

Acknowledgments

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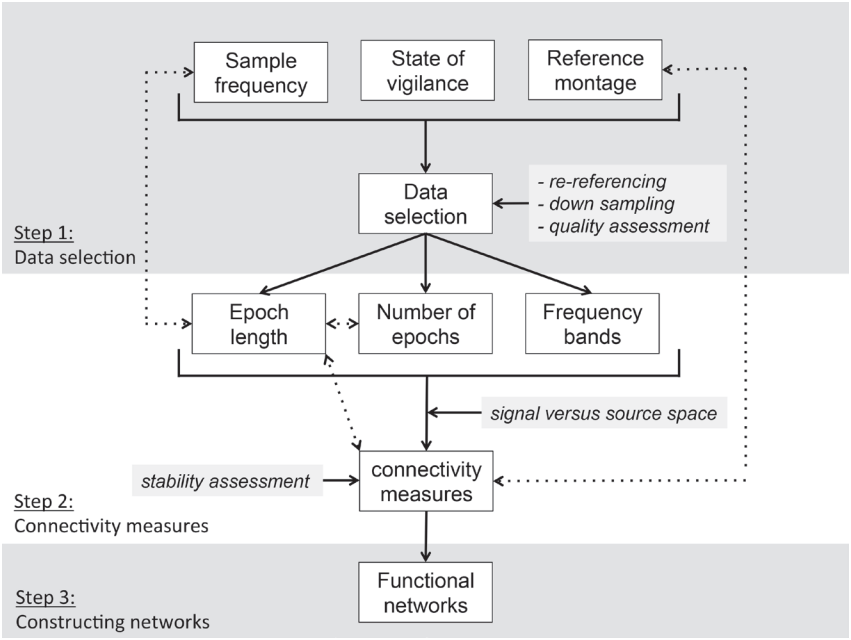
F2



Methodological choices throughout analysis pipeline

Schematic illustration how various methodological choices will influence resting state data and, consequently, the construction of functional networks. Note that choice of connectivity measures is highly depended on methodological choices. Furthermore, several methodological options exist during step 3. These options are discussed in the section on functional networks.

T2

Subject-related methodological issues

Heterogeneity of resting state condition

- Use a priori defined instructions throughout a study to reduce the external induced heterogeneity
- Eliminate or control for possible confounders such as time of the day, intake of caffeine or medication, task or physical performance prior to recording and state of vigilance

Measurement-related methodological issues

Choice of reference

- Conventional reference montages will influence EEG measures, both a reference-free and (robust) common average reference are reasonable choices
- Various reference montages will influence the estimated strength and directionality of information flow and thereby the outcome of functional and effective connectivity

Epoch selection

- Select the first artifact free epochs after the start of the resting state recording to minimize any potential selection bias
- Re-inspect selected epochs by an independent researcher to avoid any selection or systematic bias
- Include extra epochs to perform a leave-one-out analysis to investigate stability of connectivity and network measures
- Length and sample frequency of epochs should be equal and appropriate for the connectivity measure that is used

Filtering and artifact handling

- Select epochs without eye-movement or muscle artifacts. If not possible, use an automatic artifact reduction approach and describe these procedures or exclude affected channels (but maintain an equal number of channels per subject)
- Use zero-phase filtering to eliminate the phase shift of digital filters on the signal

Frequency bands

- Differentiate neurophysiological signals in separate frequency bands when an effect is expected in specific bands. Broadband analysis could be used when testing an undirected hypothesis
- Avoid gamma band when impossible to control for myogenic influences
- Perform a spectral power analysis along with your connectivity analysis

Connectivity measures

Volume conduction

- Avoid connectivity measures that are susceptible for volume conduction
- Compute connectivity values per epoch and consequently an average value per subject to increase the stability of connectivity values

Functional networks
Network construction
- Use weighted networks to avoid subjectivity in unweighted network analysis or approaches not influenced by network density (e.g., minimum spanning tree)
- Use directed networks in combination with effective connectivity measures when information is needed on the directionality within the network
Network density
- When using traditional network metrics use a combination of weighted networks and normalization procedures with surrogate data to correct for the influence of density or use the minimum spanning tree approach

Summary recommendations

General recommendations for methodology of functional connectivity resting state EEG and MEG research.

CHAPTER 3

FUNCTIONAL AND STRUCTURAL BRAIN NETWORKS IN EPILEPSY: WHAT HAVE WE LEARNED?

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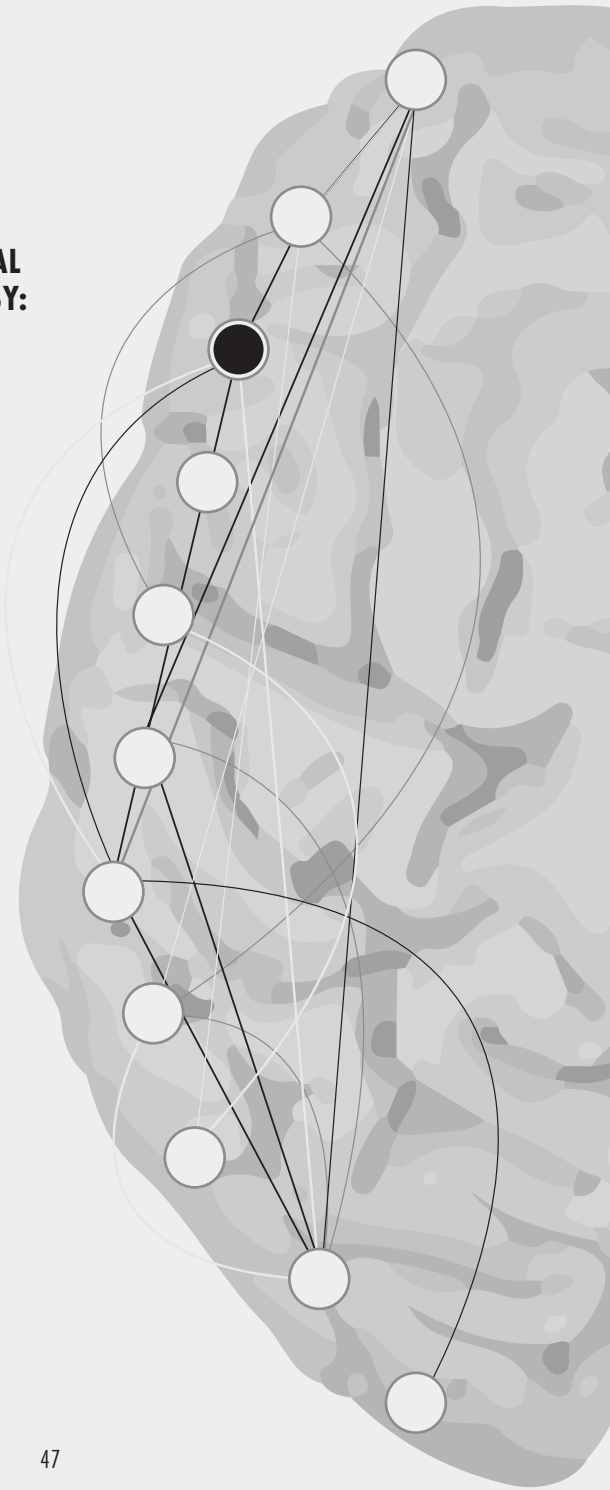

ABSTRACT

Brain functioning is increasingly seen as a complex interplay of dynamical neural systems that rely on the integrity of structural and functional networks. Recent studies investigating functional and structural networks in epilepsy have revealed specific disruptions in connectivity and network topology and, consequently, have led to a shift from 'focus' to 'networks' in modern epilepsy research. Disruptions in these networks may be associated with cognitive and behavioral impairments often seen in patients with chronic epilepsy. In this review, we aim to provide an overview that would introduce the clinical neurologist and epileptologist to this new theoretical paradigm. We will focus on the application of a theory, called 'network analysis', to characterize resting-state functional and structural networks and discuss current and future clinical applications of network analysis in patients with epilepsy.

INTRODUCTION

Connectivity measures are increasingly used to investigate the integrity of brain networks in epilepsy. Based on the level of synchronous neural activity or structural correlates between different brain areas, studies revealed disease specific patterns of increased or decreased connectivity. Although these studies provided useful information on how different brain areas are connected, they fail to clarify how these complex systems are organized and tell us little about the underlying mechanism that drives these changes in connectivity. In the study of epilepsy, network analysis (or network theory) is gaining more interest as it offers a framework to characterize the organization of brain networks (Bullmore and Sporns, 2009; Ponten et al., 2007; Reijneveld et al., 2007; Rubinov and Sporns, 2010; Stam, 2010; Stam and van Straaten, 2012b). Network analysis reduces complex systems – such as the brain – to a collection of "nodes" and "edges". Nodes represent functional or structural elements of the network (recording sites or brain areas). Edges are any type of relation between brain areas; representing either a structural or functional connection. Together, these two building blocks enables characterization of the organization of brain networks.

The application of network analysis in epilepsy has provided valuable information on seizure onset, propagation and termination (Kramer et al., 2008; Ponten et al., 2007; Schindler et al., 2008), on the interictal state of functional networks in epilepsy (Chavez et al., 2010; Horstmann et al., 2010; Liao et al., 2010; van Dellen et al., 2009; van Dellen et al., 2012) and on alterations in structural networks (Bernhardt et al., 2011; Raj et al., 2010). Network analysis has also been applied to improve our understanding of comorbidities of chronic epilepsy, such as behavioral problems and cognitive decline (Vaessen et al., 2012; Vlooswijk et al., 2011).

As the current literature on functional and structural networks in epilepsy expands rapidly (Engel et al., 2013; Kramer and Cash, 2012; Richardson, 2012) and the concepts involved are new to most clinicians, we aim to provide an overview to introduce modern network analysis, as applied to epilepsy, to the clinical epileptologist. To start, we introduce some basic concepts in network studies to put recent findings into perspective: what is functional and structural connectivity and how do we use these concepts for network construction? Then we summarize and interpret the results of functional and structural network studies in epilepsy,

focusing on resting-state investigations to facilitate comparison between studies. Finally, we consider clinical implications of functional and structural network studies in epilepsy that could bridge the gap between theoretical studies and the clinical need to understand epileptogenesis, and to predict its clinical outcomes more reliably.

CONCEPTS IN FUNCTIONAL AND STRUCTURAL NETWORKS

Functional connectivity

A central concept to study the integrity of functional networks is functional connectivity. In clinical neuroscience, this concept denotes statistical associations between physiological recordings of different brain areas (Aertsen et al., 1989). In a comprehensive review, Varela and colleagues stated that the extent to which different brain areas are functionally connected depends on the level of synchronous temporal activity, irrespective of signal amplitude (Varela et al., 2001), also called 'synchronization'. Functional connectivity can either be studied in a task-related paradigm or in a so-called resting-state condition. During a resting-state condition, the subject is in a non-active, awake state and task related activity is absent. This condition allows the detection of intrinsic activity of the brain (Deco et al., 2011; Greicius, 2008; van den Heuvel and Hulshoff Pol, 2010).

In the study of epilepsy, neurophysiological techniques, such as (intracranial) electroencephalography (EEG) and magnetoencephalography (MEG), are widely used to localize epileptiform activity and to provide information on how brain areas are functionally connected. Over the past decades, different methods have been described to determine functional connectivity between brain areas (Lemieux et al., 2011; Pereda et al., 2005; Stefan and Lopes da Silva, 2013). Initially, connectivity studies focused on linear correlations between two signals as a function of the frequency during seizure propagation (Brazier, 1972; Gotman, 1983). Later, complex, non-linear correlations were introduced to investigate functional coupling between different brain areas (Bartolomei et al., 2001; Pijn et al., 1990). Examples of non-linear correlation measures currently used in epilepsy studies include the non-linear correlation coefficient (Wendling et al., 2001); synchronization likelihood, that detects both linear and nonlinear interdependencies between two (time) signals (Stam and Van Dijk, 2002); phase lag index, that overcomes volume conduction as a confounding effect (Stam

et al., 2007b); granger causality, a method that denotes causality between interacting signals (Bressler and Seth, 2011a) and partial directed coherence, an effective connectivity measure that is able to distinguish both direct and indirect causality flows (Baccala and Sameshima, 2001). All these methods have their unique advantages and deal with specific limitations of neurophysiological recordings (Pereda et al., 2005; Stam and van Straaten, 2012b). Wendling and colleagues performed a modeling study, comparing different connectivity measures, and concluded that the ideal method eventually depends on the studied model (Wendling et al., 2009). Furthermore, functional connectivity in neurophysiological studies is usually analyzed in separate frequency bands: delta band (0-4 Hz), theta band (4-8 Hz), alpha band (8-13 Hz), beta band (13-30 Hz) and gamma band (>30 Hz) as each frequency band is associated with distinct networks and functions (Basar et al., 2001).

Using spontaneous low frequency fluctuations in the blood oxygenation level-dependent (BOLD) signal, functional MRI (fMRI) enables functional connectivity investigations with a higher spatial but lower temporal resolution compared to neurophysiological recordings. Since BOLD signal changes relate to underlying neural activity, fMRI provides only an indirect measure of functional connectivity. The various methods used in this paradigm include a seed or region-of-interest (ROI) based approach and the independent component analysis (ICA). The ROI based approach is used to determine temporal correlations from a selected region (or multiple regions) with all other brain areas. Although this method is relatively easy to apply, it requires an a priori, investigator driven, definition of seed regions and has some statistical limitations (Fox and Raichle, 2007). In contrast to the ROI-based approach, the ICA is a data-driven method, which decomposes the BOLD signal into temporally correlated and spatially independent brain areas (Beckmann et al., 2005).

Structural connectivity

A pivotal question in studying functional connectivity is to what extent functional networks are constraint by structural connectivity (van den Heuvel and Hulshoff Pol, 2010). Where functional connectivity is considered as an on-going physiological process of 'communication' between different brain areas, structural networks can be considered as the supporting hardwire. This assumption is repeatedly confirmed by studies revealing a positive correlation between structural and

functional connectivity (Damoiseaux and Greicius, 2009), although the exact relation between structural and functional connectivity is complex.

Most commonly, structural connectivity is inferred from diffusion tensor imaging (DTI). This imaging technique is based on the directionality of diffusion of water molecules in the brain. The diffusivity of water molecules is facilitated along the axons in the white matter, and is restricted in the direction perpendicular to axonal tracts (Basser and Jones, 2002). When carefully considering the pitfalls in the analysis of DTI (Jones and Cercignani, 2010), it offers the opportunity to reliably construct whole brain white matter networks from fiber tractography. To assess the integrity of axonal tracts, measures of diffusivity, tract volume and fractional anisotropy are often used as markers to identify white matter connections between brain areas.

Alternatively, structural connectivity can be inferred from more standard anatomical MRI sequences. In a set of brain regions, either cortical thickness, or (sub) cortical gray matter volumes are quantified on three-dimensional T1-weighted MR images. These brain regions are considered connected when they show morphological correlations across subjects (He et al., 2007; Wen et al., 2011). Weakness of this approach is that it requires analysis of networks at the level of large groups. Recently, Tijms and others have proposed an alternative method to study structural networks based upon grey matter organization in individual subjects (Tijms et al., 2012)

Network organization

Whereas functional and structural connectivity answers the question if – and to what extent – different brain areas are connected, network analysis offers a framework to characterize the organization of functional and structural networks. As mentioned in the introduction, nodes (i.e. brain areas) and edges (i.e. structural connections or functional symmetric or asymmetric interactions between brain areas) form the most elementary building blocks of a network. These two building blocks are indispensable for further characterization of network organization (or topology). Network characteristics often used to describe global organization of whole brain networks are "clustering coefficient" and "average path length" (figure 1). The average clustering coefficient is a measure of segregation, which defines connection probability of nearest neighboring nodes and is therefore seen as a measure of the local

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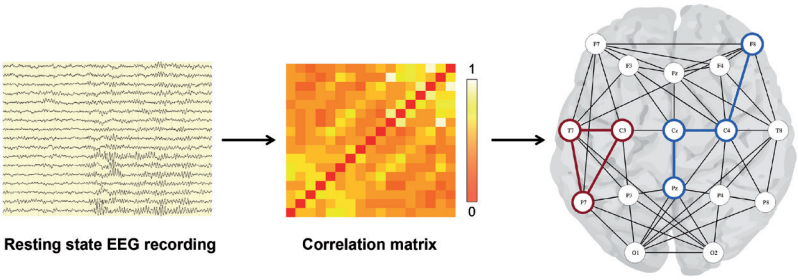

Illustration of a network based on EEG data

(average network based on children with idiopathic epilepsy (n=25); unpublished data). After selecting resting state EEG epochs, we computed a correlation matrix based on the linear and nonlinear interdependencies between two channels (in this case we used the synchronization likelihood (SL)). In the matrix, light colored boxes indicate a high correlation. The diagonal was set on 0. After constructing a correlation matrix, various software programs allow the construction of visual graphs (figure on the right). For illustration purposes, we applied a threshold (k=4) to obtain a binary network. Two elementary building blocks of the network are highlighted: clustering coefficient (in red) and shortest path length (in blue). Recording specifications: broadband frequency (0.5 – 45Hz). Electrodes Fp1, Fp2, A1 and A2 were left out of the network due to myogenic- and (eye) movement artifacts.

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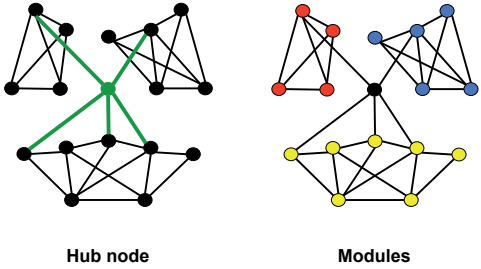

Schematic illustration of network node characteristics

In the left figure, an example of hub node is highlighted in green: it has a central position in the network, has many connections with other nodes and connects different modules. Several network measures exist to quantify this hub node. The simplest form is "degree", which simply counts the number of connections of a node. "Strength" also takes into account the weight of the connections (in this figure, all connections have the same weight). More advanced calculations include betweenness centrality and eigenvector centrality. Betweenness centrality takes into account the fraction of all shortest path length that needs to pass through a specific node. Eigenvector centrality determines the importance of a node on the basis of the number and weight of connections to other nodes and how those other nodes are connected. In the right figure, three groups of nodes (red, yellow and blue) represent so-called modules. Modules are groups of nodes that are more strongly connected with each other than with nodes from different modules.

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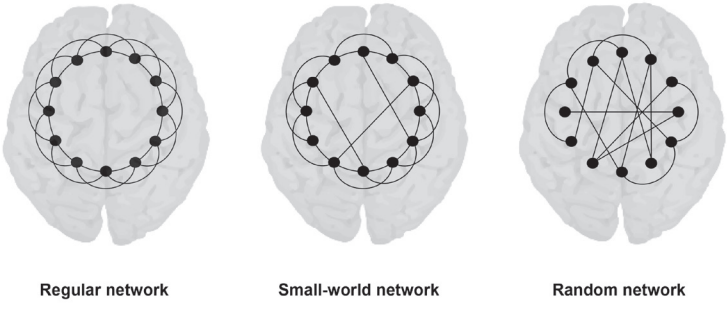

Three basic network topologies: regular, small-world and random

A regular network (left figure) has a characteristic high average clustering coefficient (on average, there is a high connection probability of nearest neighboring nodes) and a long average path length (on average, many connections must be traversed to travel from one node to another) resulting in a good local connectedness. The opposite is true for a random network (right figure) (low average clustering coefficient and short average path length). In a small-world network configuration (middle figure), the advantages of a regular and random network are combined into an efficient network topology.

connectedness within a network. A network characteristic to compute the global connectedness is the average path length. Path length refers to the number of edges that must be traversed to go from one node to another. The average path length is based on all possible pairs of nodes in a network and is inversely related to network integration (lower averaged path length means a better integration). Together, average clustering coefficient and averaged path length are frequently used to distinguish three basic network configurations: regular, small-world and random (Watts and Strogatz, 1998). A regular (or ordered) network has many local connections (high clustering coefficient), but a limited number of distant connections (and thus a high average path length). The opposite is true for a random network, which has limited local connections and many distant connections. A small-world network combines the advantages of a regular network (good local connectedness) with the advantages of a random network (good global connectedness) and is considered to be the most efficient network topology (figure 3).

Apart from clustering coefficient and path length, various other network characteristics can be inferred from networks that could prove useful in further characterizing network organization (Rubinov and Sporns, 2010). Although

a full overview on all network characteristics would go beyond the scope of this review, two networks characteristics are worthwhile mentioning as they are increasingly studied in relation to epilepsy: "hubs" and "modules" (figure 2). Hubs are nodes with many connections and with a central position within a network. Examples of network measures to quantify hub nodes are degree, strength, betweenness centrality and eigenvector centrality. Hub nodes are crucial for efficient communication due to their extensive connectivity and are, therefore, associated with an increased synchronized network. As will follow, studies have repeatedly identified a spatial association between pathological hubs, or clusters of increased synchronization, and the epileptogenic zone (Ortega et al., 2008a; Palmigiano et al., 2012; Schevon et al., 2007; Wilke et al., 2011). Otherwise, hub nodes have the ability to connect different modules within a network (connector hubs). Modules are subnetworks that can be defined as a group of nodes that are more connected to each other than to other parts of the network (Stam and van Straaten, 2012b).

SEIZURE GENERATION AND ICTAL NETWORK TOPOLOGY

The study of seizure dynamics based on intrinsic neurophysiological activity in epilepsy has a long history. In the past, many studies have focused on seizure propagation and localization of the epileptogenic zone. In this section, however, we will focus on studies using a network analytical approach. Pre-ictal, ictal and post-ictal network topology have been studied with (intracranial) EEG recordings to gain insight in the temporal evolution of epileptic networks. More specifically, we will focus on how seizures influence network organization.

Intracranial recordings during the ictal period revealed a network shift towards a more regular topology (with increased clustering coefficient and path length) compared to the pre-ictal period in partial seizures (Kramer et al., 2008; Ponten et al., 2007; Schindler et al., 2008). Network analysis based on surface EEG recordings during generalized seizures also found an ictal network with a more regular topography compared to the interictal state (Ponten et al., 2009). Having zoomed in on the ictal period (ictal onset, mid-ictal and ictal termination) in patients with temporal lobe epilepsy, variations were seen as functional connectivity fluctuates over time (Ponten et al., 2007). An increased average clustering coefficient and path length, reflecting a more regular network

configuration of the epileptic brain, characterizes the ictal onset. During the mid-ictal period, the number of connections drops to pre-ictal values, resulting in a less synchronizable network, and eventually a normalization of network configuration occurs after ictal termination (figure 4) (Ponten et al., 2007; Schindler et al., 2008).

Kramer and others described changes in functional network configuration during a focal seizure in terms of formation and dissolution of subnetworks (or modules). At seizure onset, one dominant and highly regular subnetwork is formed out of highly connected nodes. Towards seizure termination this dominant subnetwork disintegrates into smaller subnetworks and network topology becomes more random again (Kramer et al., 2010). Both the formation of one dominant subnetwork and the shift, during a seizure, towards a more regular network topology could rely on decreased centrality of hubs and an altered synchronizability during the ictal state (Ponten et al., 2007; Schindler et al., 2008; Wilke et al., 2011).

INTERICTAL NETWORKS

Neurophysiological studies

In patients with epilepsy comorbid cognitive and behavioral deficits are often found. Explanations are sought and investigated in terms of altered neural synchrony, differentiating pathological interictal networks from healthy brains (Uhlhaas and Singer, 2006). Studies using either EEG and/or MEG recordings in patients with partial epilepsy repeatedly revealed an increased functional connectivity and a subsequent loss in network efficiency, particular in the theta frequency band (Bartolomei et al., 2006; Bettus et al., 2008; Bosma et al., 2009; Douw et al., 2010a; Douw et al., 2010b; Horstmann et al., 2010; van Dellen et al., 2012). Different studies, however, reported conflicting results with respect to changes in network efficiency. Where some studies reported a decreased clustering coefficient and/or path length in patients with partial seizures due to brain tumors (Bartolomei et al., 2006; van Dellen et al., 2012), others found an increased clustering coefficient and path length in patients with partial epilepsy (Horstmann et al., 2010). These contradicting findings could reflect differences in methodology, etiology or duration of disease (see section "Potential modifiers and methodological considerations in functional and structural network studies"). Additionally, network characteristics such as

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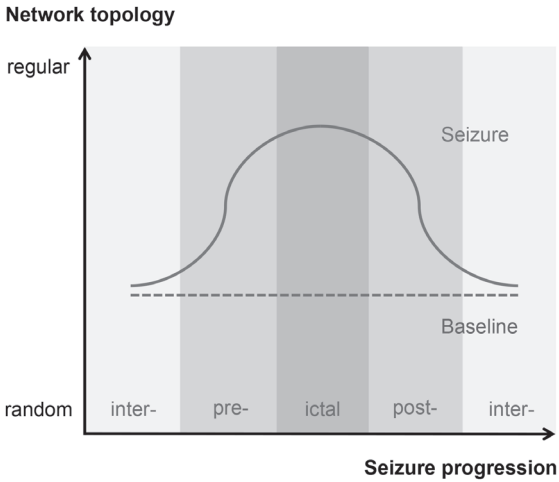

Schematic representation of ictal network changes

An increase in ordered network topology (solid line) is observed during a seizure, compared with baseline (dashed line) with a gradual increase during the pre-ictal state and a gradual decrease in the post-ictal state.

clustering coefficient and path length might be insufficient to fully understand the relation between network organization and brain functioning in epilepsy. Studies are therefore increasingly focusing on other network characteristics to yield additional information on the interictal network organization. For example, van Dellen and others found that a less modular organization, decreased clustering coefficient, and a lower synchronizability (i.e. a measure for the stability of the synchronous state in a network) were associated with an increased seizure occurrence and with cognitive decline in brain tumor patients suffering from clinical seizures (van Dellen et al., 2012).

Functional MRI studies

A considerable number of fMRI studies have used a resting-state paradigm to investigate functional connectivity in generalized and partial epilepsies. In patients with TLE, studies have repeatedly identified a general decrease of functional connectivity (Laufs et al., 2007; Liao et al., 2010; Luo et al., 2011),

most prominently in the ipsilateral hemisphere (Bettus et al., 2009; Pittau et al., 2012) and already visible at an early stage of TLE (Mankinen et al., 2011). Furthermore, TLE is associated with a disrupted interhemispheric connectivity in subcortical structures (Morgan et al., 2011; Pereira et al., 2010). A reduced functional connectivity (Luo et al., 2012; Masterton et al., 2012; McGill et al., 2012; Yang et al., 2012) and an increased interhemispheric connectivity (Bai et al., 2011) was found in patients with generalized epilepsies.

Few of these fMRI studies further explored network organization by means of network analysis. In a pioneering study, Liao and others investigated how changes in functional connectivity were related to network organization in TLE patients. Besides widespread decreased connectivity in – and between – occipital, temporal, parietal and frontal lobes, increased connectivity was found within the temporal lobe. Network analysis revealed a decreased clustering coefficient and path length and a lower number of hub nodes primarily within the default mode network (Liao et al., 2010). Together, these findings illustrate the widespread alterations in network topology in partial epilepsy. Similarly, Vlooswijk and others investigated whole brain network organization and its relation with cognitive decline in patients with cryptogenic epilepsy. In contrast to Liao and others, a decreased clustering coefficient and a trend towards an increased average path length was found in patients. These network alterations were associated with cognitive decline (Vlooswijk et al., 2011). Possibly, the differences between both fMRI studies are related to differences in patient population and duration of disease.

Structural networks in epilepsy

Cortical thickness analysis has been applied to explore structural networks in TLE patients, and was found to be useful in distinguishing patients from controls (Raj et al., 2010). Bernhardt and others investigated whether structural network alterations – based on cortical thickness – progressively increase over time in TLE patients. Although small-world topology was largely preserved in patients, an increased clustering coefficient and path length were observed. To further explore network organization in TLE, the authors investigated the distribution of hub nodes. In contrast to controls, in whom hub nodes were evenly distributed over the different lobes, hub nodes in patients were primarily found in the limbic and temporal association cortices. The authors speculate that this pathological

distribution of hub nodes in TLE patients could be related to the disturbed connectivity between temporolimbic and extratemporal neocortical structures (Bernhardt et al., 2011). These speculations were partly verified in a recent DTI study that revealed structural reorganization of the limbic areas, leading to aberrant connections and epileptogenicity (Bonilha et al., 2012).

To date, only one study has investigated whole brain structural networks in epilepsy by means of DTI. Vaessen and others investigated network integrity of patients with non-symptomatic partial epilepsy. Interestingly, a decreased clustering coefficient and increased path length was only observed in patients with cognitive decline. Network organization in epilepsy patients without cognitive decline did not differ from that in healthy controls (Vaessen et al., 2012). This study convincingly illustrates how network alterations can be related to cognitive profiles of patients with epilepsy.

METHODOLOGICAL CONSIDERATIONS IN NETWORK STUDIES

Various studies have identified potential modifiers that could influence results from functional and structural networks, irrespective of the underlying disease. In addition to general modifiers, such as age (Khundrakpam et al., 2012; Micheloyannis et al., 2009; Smit et al., 2012), gender (Boersma et al., 2011), state of vigilance (Kuhnert et al., 2010) and intellectual performance (Li et al., 2009; van den Heuvel et al., 2009), more specific modifiers in epilepsy include, for example, antiepileptic drug (AED) use (figure 5). It remains unclear to what extent AEDs influence global functional networks properties (Horstmann et al., 2010) and whether cognitive impairments associated with disrupted functional networks in partial epilepsies are related to chronic use of AEDs (Vlooswijk et al., 2011). In addition, in epilepsy patients with underlying structural brain lesions, it is unknown whether alterations in networks arise from epilepsy per se, or from the associated structural lesions, as investigated in patients with brain tumors (Douw et al., 2013; Douw et al., 2010b; van Dellen et al., 2012). Other potential modifiers when studying network organization in epilepsy include duration of epilepsy (Liao et al., 2010; van Dellen et al., 2009) and seizure frequency (Douw et al., 2010b; van Dellen et al., 2012). To avoid heterogeneity of patients or lesion type and the possible effects of AEDs, animal models may help to further understand how disease duration and seizure frequency affect overall

brain network topology. In a model of focal neocortical epilepsy, induced in previously healthy rats that have no significant structural cortical brain lesions, whole brain resting-state fMRI network analysis revealed an increased clustering coefficient and decreased path length, suggesting a more regular functional topology compared to healthy control animals (Otte et al., 2012a).

As mentioned, results from interictal studies have often been contradictive. In general, neurophysiological studies show an increased connectivity whereas fMRI studies often report an opposite, decreased connectivity. These differences might reflect possible alterations in neurovascular coupling secondary to epilepsy (Bettus et al., 2011). Otherwise, differences in spatial and temporal resolution between neurophysiological and fMRI studies make it difficult to compare results. More simultaneous EEG and fMRI studies are therefore needed to further explore the relation between neurophysiological and BOLD associated connectivity. Furthermore, comparing studies using a network analytical approach can be challenging. Studies have consistently identified a less efficient network organization in epilepsy, albeit either a more regular or a more random network topology. In a comprehensive review, several methodological considerations were raised that could be accountable for these opposite results (van Wijk et al., 2010). Size of networks (i.e. number of nodes) or edge-density, connectivity measures used, thresholding of unweighted networks, and normalization of network characteristics all influence the eventual network topology and should be considered when comparing different network studies.

CLINICAL IMPLICATIONS OF NETWORK STUDIES

Network studies in patients with epilepsy can have multiple clinical implications. First, they can be used to investigate the mechanisms underlying comorbid cognitive decline. Both structural and functional network studies have shown that cognitive decline in epilepsy patients is associated with a loss of network efficiency (Vaessen et al., 2012; Vlooswijk et al., 2011). As a result, the authors speculated that network studies could potentially identify patients at risk to develop cognitive impairment. Other studies have explored the diagnostic use of network analytical approach. The predictive value of conventional EEG reports was compared with functional connectivity analysis of the same EEG recording in a univariate model to distinguish patients, from those in whom the diagnosis

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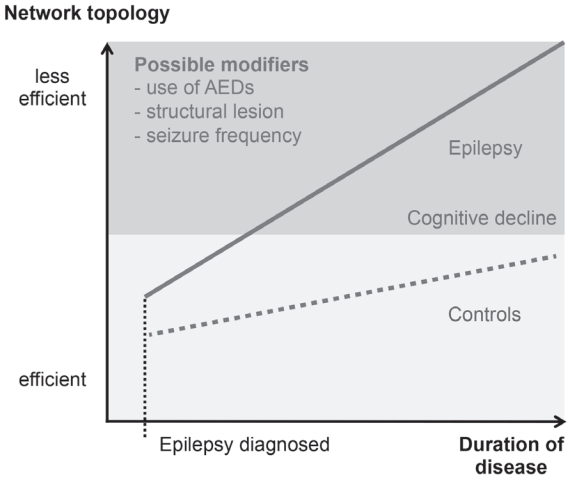

Schematic representation of interictal network changes

As time progresses, network topology in epilepsy (solid line) exhibits a gradual increase in orderedness and synchronization compared to healthy controls (dashed line), which may be associated with cognitive decline. Possible modifiers of this effect are reported in the figure: use of anti-epileptic drugs (AEDs), structural lesions and/or seizure frequency.

epilepsy was eventually rejected, after a first suspected seizure. Particularly in the theta frequency band, increased functional connectivity was found to be useful as a diagnostic tool with a superior sensitivity and specificity compared to conventional EEG report (Douw et al., 2010a). Similarly, we showed that by combining several network characteristics into a sophisticated model, we were able to predict the diagnosis of epilepsy more reliable than based upon the presence of epileptiform transients (van Diessen et al., 2013c). More recently, expression of epilepsy related proteins in brain tumor patients with clinical seizures was found to be related with specific network alterations, such as the connectivity between brain modules (Douw et al., 2013). The authors therefore suggested that network analysis could be used as a non-invasive diagnostic biomarker for microscopic tumor characteristics.

The development of functional network biomarkers (Zhang et al., 2012) to predict the risk of seizure recurrence is another possible clinical application of

functional network analysis. Although network properties fail to reliably predict seizure onset (Takahashi et al., 2012), they may improve prediction of outcome (seizure recurrence) after epilepsy surgery (Negishi et al., 2011). Ortega and others retrospectively analyzed intracranial corticography data in patients with TLE and found that resection of cortical areas with clusters of highly synchronized activity was related to an increased seizure control (Ortega et al., 2008a; Palmigiano et al., 2012). Similarly, Wilke and others revealed that resection of hub nodes that were active during a seizure was associated with seizure freedom in patients with neocortical epilepsy (Wilke et al., 2011). Together, these studies indicate the potential use of network analysis to improve the outcome of epilepsy surgery.

CONCLUSIONS AND FUTURE DIRECTIONS

Functional and structural network studies have thus far provided valuable insights into ictal and interictal network alterations in epilepsy. Ictal studies have repeatedly identified a more regular network topology and changes in modularity when the epileptic brain goes into a seizure. Towards the end of a seizure, network topology returns to its baseline configuration. In contrast, results from interictal studies are more contradictive. We have summarized different potential modifiers and methodological consideration that could turn helpful when interpreting contradicting results from ictal and interictal functional and structural network studies. Throughout the review, we discussed the strengths and weaknesses of each modality used in network analytical studies and summarized them in table 1. In addition, 'conventional' network characteristics such as clustering coefficient and path length might be insufficient to clearly pinpoint epilepsy specific changes in the interictal network topology. Advanced network characteristics, such as measures of hub-nodes, modules and synchronization could turn out to be useful. Interestingly, studies using these measures have already revealed disease-specific changes in hub distribution and/or modularity of the epileptic network (Bernhardt et al., 2011; Douw et al., 2013; Ortega et al., 2008a; van Dellen et al., 2012; van Diessen et al., 2013b; Wilke et al., 2011). Otherwise, recent network analytical studies are using novel approaches to overcome methodological shortcomings, such as the minimum spanning tree (MST). The MST approach considers the smallest possible subnetwork, which

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Modality	Strengths	Weaknesses
Functional networks		
EEG	- Widely used in clinical practice - High temporal resolution - Suitable to study ictal networks	- Low spatial resolution (less for high definition EEG) - Sensitive to volume conduction artifacts
Intracranial recordings	- Direct electrical recordings of neuronal activity - High temporal and spatial resolution - No myogenic artifacts	- Only available in a surgical setting - No whole brain network analysis possible
MEG	- High temporal and spatial resolution - Source space analysis allows identification of anatomical network specification	- High temporal and spatial resolution - Source space analysis allows identification of anatomical network specification
fMRI	- High spatial resolution - Allows the study of subcortical networks separately - Widely available	- Low temporal resolution - Assumption of BOLD changes in respect to electrophysiological changes in the epileptic brain
Structural networks		
Cortical thickness	- Inferred from standard MRI sequences - High spatial resolution	- Analysis of individual networks complicated - Analysis of subcortical structures not possible
DTI	- Physical network connections can be studied - Both cortical and subcortical structures and their interconnectedness can be studied	- Several technical pitfalls when analyzing DTI data - Many arbitrary choices in the process of data extraction

Strength and weaknesses

Overview on strengths and weaknesses of different modalities used in network analytical studies.

still connects all the nodes in a network without forming cycles, and reduces the connection costs in a network. This approach might be particularly valuable when comparing patients with controls (or different types of epilepsy); MST networks have the unique advantage that networks from different groups contain the same number of nodes and connections, thereby facilitating comparisons between groups, particularly in neurophysiological studies. Several neuropsychiatric studies have already successfully applied this technique (Alexander-Bloch et al., 2010; Boersma et al., 2013; Lee et al., 2006; Lee et al., 2010; Ortega et al., 2008a; Schoen et al., 2011). Studies using more advanced network characteristics and/or MST approach are needed to further explore their potentials in the

study of epileptogenic networks. In addition, these novel measures can be used with different neurophysiological and neuroimaging techniques; this allows the construction of more detailed networks on different temporal and spatial scales that could potentially result in a more accurate visualization and quantification of epileptogenic networks.

Another question that remains unanswered is to what extent functional networks are constrained by structural networks (Otte et al., 2012a; Voets et al., 2012; Wang et al., 2012). Longitudinal studies combining both functional (fMRI, MEG) and structural networks (based on cortical thickness associations or DTI) simultaneously are crucial to understand the integration of these networks and their implication for clinical outcome of patients with epilepsy. The combination of these modalities could potentially elucidate specific network alterations in epilepsy and possibly clarify why in some patients behavioral and cognitive deficits arise (Vaessen et al., 2012; Vlooswijk et al., 2011).

Finally, despite the contradictive results as described above, characterization of networks in the dynamical process of seizure generation and in the interictal state has advanced our understanding of epilepsy as a network disease. In addition, a network analytical approach could become valuable in the diagnostic process. Standard EEG recordings performed in the initial diagnostic work-up have proven that a network analytical approach can be used to differentiate patients from controls (Douw et al., 2010a; van Diessen et al., 2013c). Larger EEG studies with external validation are needed to fully explore the true diagnostic value of functional networks. Furthermore, invasive neurophysiological recordings and neuroimaging studies in the pre-surgical work-up have already shown a potential value of a network approach (Bartolomei et al., 2013; Morgan et al., 2012; Ortega et al., 2008a; Varotto et al., 2012; Wilke et al., 2011) and should be further exploited. Earlier identification of network alterations may lead to an accelerated and improved intervention in (refractory) epilepsy to reduce future burdens, such as cognitive decline or behavioral impairments.

CHAPTER 4

BRAIN NETWORK ORGANIZATION IN FOCAL EPILEPSY: A SYSTEMATIC REVIEW AND META-ANALYSIS

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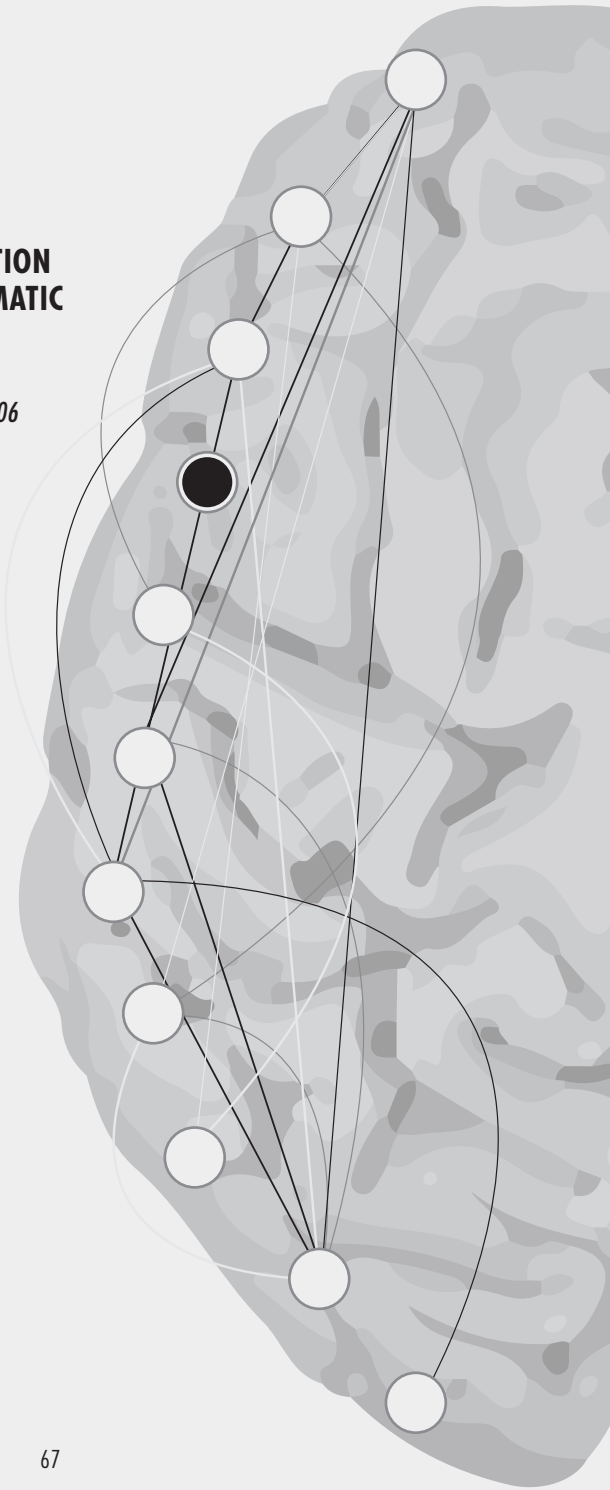

ABSTRACT

Objective: normal brain functioning is presumed to depend upon interacting regions within large-scale neuronal networks. Increasing evidence exists that interictal network alterations in focal epilepsy are associated with cognitive and behavioral deficits. Nevertheless, the reported network alterations are inconclusive and prone to low statistical power due to small sample sizes as well as modest effect sizes.

Methods: we therefore systematically reviewed the existing literature and conducted a meta-analysis to characterize the changes in whole-brain interictal focal epilepsy networks at sufficient power levels. We focused on the two most commonly used metrics in whole-brain networks: average path length and average clustering coefficient.

Results: twelve studies were included that reported whole-brain network average path length and average clustering coefficient characteristics in patients and controls. The overall group difference, quantified as the standardized mean average path length difference between epilepsy and control groups, corresponded to a significantly increased average path length of 0.29 (95% confidence interval (CI): 0.12 to 0.45, $p = 0.0007$) in the epilepsy group. This suggests a less integrated interictal whole-brain network. Similarly, a significantly increased standardized mean average clustering coefficient of 0.35 (CI: 0.05 to 0.65, $p = 0.02$) was found in the epilepsy group in comparison with controls, pointing towards a more segregated interictal network. Sub-analyses revealed similar results for functional and structural networks in terms of effect size and directionality for both metrics. In addition, we found individual network studies to be prone to low power due to the relatively small group differences in average path length and average clustering coefficient in combination with small sample sizes.

Significance: the pooled network characteristics support the hypothesis that focal epilepsy has widespread detrimental effects, that is, reduced integration and increased segregation, on whole brain interictal network organization, which may relate to the co-morbid cognitive and behavioral impairments often reported in patients with focal epilepsy.

INTRODUCTION

Traditionally, the brain has been perceived as a set of brain areas with highly specialized functions. However, there is increasing evidence that brain functioning is emerging from a complex interplay of different brain areas. This shift from a 'location specific' to a more 'network oriented' approach has revealed novel insights into the physiological functioning of the brain and further clarified neurological diseases, including epilepsy (Bullmore and Sporns, 2012; Power et al., 2011). Several studies have shown a relation between epilepsy and disrupted functional and structural brain networks. This relation could, at least partly, be attributed to the cognitive and behavioral impairments often found in patients with epilepsy (Engel et al., 2013; Kramer and Cash, 2012; Richardson, 2012; Vlooswijk et al., 2010).

To quantify and characterize brain networks, studies are increasingly using network analysis as a mathematical paradigm (Bullmore and Sporns, 2009; Rubinov and Sporns, 2010; Stam, 2010; Stam and van Straaten, 2012b). Network analysis reduces complex systems to a collection of 'nodes' (that is, brain areas) and 'edges' (that is, connections between brain areas). From these elementary network building blocks various quantitative metrics can be inferred. Two very informative metrics, the path length and clustering coefficient, have been widely used to characterize brain network organization and changes herein in healthy and epileptic brains (Figure 1). Path length refers to the minimal number of edges that must be traversed to travel from one node in the network to another. The average path length of a network is inversely related to the level of network integration. The clustering coefficient is defined as the connection probability of nearest neighbor nodes and the average clustering coefficient represents network segregation. A highly segregated network has a high average clustering coefficient. A short average path length and a high average clustering coefficient characterize healthy brain networks: a so-called small-world configuration (Bullmore and Sporns, 2009; Stam, 2010). A small-world configuration is considered optimal for network functioning as the number of long distance connections is minimized while high average clustering of neighboring nodes is retained. This reduces the network's 'building and maintenance costs' without compromising fast exchange of information (Bullmore and Sporns, 2012).

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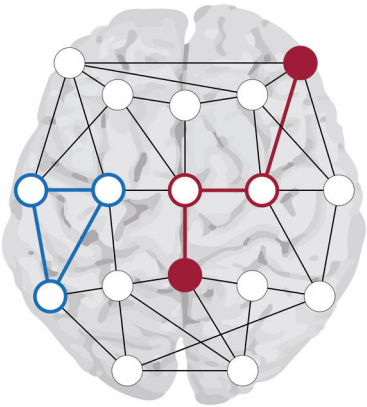

Clustering coefficient and path length

Explanation of the clustering coefficient and path length using a schematic whole-brain binary network representation of nodes (circles) and binary edges (black lines). The clustering coefficient is based on triangles (one triangle shown in blue) and is equivalent to the fraction of the node's neighbors that are also neighbors of each other. The average clustering coefficient is a global measure of network segregation and reflects the clustered connections around individual nodes. The path length in a binary network is the minimal number of edges that must be traversed to travel from one node to another. In red an example path is given which contains the minimal number of edges (i.e. three) between the two uniform red nodes. The average path length, a global measure of network integration, is this average minimal number of edges of all possible node connections.

It is important to accurately quantify changes in brain network organization to increase our understanding of epilepsy and its associated cognitive and behavioral comorbidities. Both clinical and experimental neurophysiological and imaging studies have, in general, reported a less optimal interictal network organization in patients with epilepsy. Nevertheless, these case-control studies have reported contradictive results. Increased, decreased or unchanged average path length and/or average clustering coefficient have been described in patients with epilepsy as compared to controls; for overviews see (Bernhardt et al., 2013; Kramer and Cash, 2012; van Diessen et al., 2013a). A possible explanation for the inconclusive network characterization, as found in the individual network studies comparing focal epilepsy with controls, is a lack of sufficient statistical power. The number of included subjects is typically in the range of 20-40 per group. As a consequence the chance of detecting a true effect might be reduced and, consequently, results less reliable (Ioannidis, 2005).

In this meta-analysis we first aimed to obtain an accurate estimate of the changes in interictal network organization in patients with epilepsy as compared to healthy individuals using existing literature. The advantage of a meta-analysis over a qualitative review is that it enables a quantitative analysis of the current literature and provides data on the between-study heterogeneity. We were

exclusively interested in network studies including patients with focal epilepsy as these often have cognitive and behavioral impairments that are not necessarily directly associated with the epileptogenic focus (Bell et al., 2011; Risse, 2006). This implicates that brain areas outside the epileptogenic focus are affected as well, which is supported by the widespread changes of white matter structure that have been found in focal epilepsy using diffusion tensor imaging (Otte et al., 2012b). Network analysis offers a unique perspective to investigate the complex involvement of the different brain areas in focal epilepsy outside the epileptogenic zone. Second, to help to increase reproducibility of – and reduce spurious findings in – future focal epilepsy network studies, we aimed to estimate the statistical power of existing literature and proposed the minimal sample size required to have sufficiently powered future network studies comparing focal epilepsy with healthy individuals.

METHODS

Information sources and search strategy

Studies were identified by searching the online databases Pubmed (NCBI), ISI Web of Science (Thomson Reuters) and Embase (Excerpta Medica Database), and reviewing the reference lists of selected studies. Language was restricted to English. Search terms related to epilepsy, different neurophysiological and imaging studies, and network analysis were used. Details on the search strategies are presented in the Supplementary Table S1 in File S1. The search was conducted January 16th, 2014.

Selection criteria

We included studies that compared brain networks of focal epilepsy patients with controls using the average path length, average clustering coefficient or both, in networks not restricted to a specific part of the brain (whole-brain networks), irrespective of acquisition technique.

We allowed included studies to differ in various ways, such as in acquisition technique and network construction. Multiple clinical neurophysiology and imaging techniques are available to infer functional or structural connectivity between brain regions and construct networks. Functional connectivity may be derived from synchronized neuronal activity recorded from distinct brain regions using electroencephalography (EEG), magnetoencephalography

(MEG) or resting-state functional MRI. Diffusion tensor imaging is an MRI technique that enables the characterization of directional water diffusion in white matter bundles. It might be exploited to map structural connectivity between remote brain regions using tractography (Ciccarelli et al., 2008). Another means to infer structural connectivity from MRI data is to correlate cortical thicknesses between functional fields (He et al., 2007).

When network analysis was introduced in the field of neuroscience, most studies relied on methods that calculated the average path length and average clustering coefficient without taking the strength (or 'weight') of network connections into account. This is called a binary analysis. The construction of binary networks depends on an arbitrary threshold. A high threshold results in a network with a high level of sparsity (that is, only the strongest connections are retained), and vise versa. More recently threshold-free methods are favored that use the connection strength to calculate these two network metrics, which is called weighted analysis (Rubinov and Sporns, 2010). In addition to these methodological differences a subset of studies normalized their network metrics for statistical comparison, using null models (Sporns, 2011). This normalization might enhance statistical comparability between groups within studies (van Wijk et al., 2010). In our statistical analysis we took these differences between studies as much as possible into account using *i*) a standardized mean difference measure, *ii*) a random-effect model, *iii*) sensitivity analyses and *iv*) a leave-one-out validation method (the analysis is explained below).

Study exclusion criteria were: network analysis of a specific part of the brain only (that is, no whole brain networks; this includes corticography studies), no data on average path length and average clustering coefficient, no comparisons with controls, brain surgery in the focal epilepsy group, or different studies using an identical patient population.

Data extraction

Two authors independently performed the literature search and screened all study titles and abstracts. From the eligible articles, full-text versions were retrieved and reviewed. Data were extracted by one author [WZ] and checked by another [EvD]. For missing data the corresponding authors were contacted. Disagreements on study inclusion and data extraction were resolved by reaching

consensus through discussion with a third author [WMO]. The following data was extracted:

Study population: group sizes, sex distribution, age at data acquisition, age at epilepsy onset, duration of epilepsy, antiepileptic drug usage and presumed etiology.

Acquisition: technique specific information such as modality, field strength (for imaging studies) and sampling frequency (for neurophysiological studies).

Network construction: network size, connectivity measure, binary or weighted connections and sparsity level if binary connections are used.

Network metrics: the mean and standard deviations (SD) of the average path length and average clustering coefficient are used. If SDs were not provided we calculated those from the standard error or 95% confidence intervals (CIs). If a range of sparsity levels was given, values corresponding to the lowest, middle and highest level were extracted. For studies reporting on both binary and weighted networks, all data were extracted (we used the binary data for the sensitivity analysis and included the weighted data for the summary estimates). Normalized and non-normalized measures were extracted if both were reported. If network metrics were reported for subgroups only, they were combined into a single value using weighted mean calculation. The average path length was defined as the reciprocal of the network efficiency if studies reported the latter only (Vlooswijk et al., 2011). We did not differentiate between slightly different mathematical formulations of the average clustering coefficient that exist in literature (for instance, the global (Luce and Perry, 1949) or the averaged local (Barrat et al., 2004) clustering coefficient).

EEG and MEG network studies often report network metrics for different frequency bands. For those studies the average path length and average clustering coefficient values were only extracted for the theta frequency band networks, as several studies have shown that network alterations in patients with focal epilepsy are most prominent in the theta frequency band (Bartolomei et al., 2006; Bettus et al., 2008; Douw et al., 2010a; Douw et al., 2010b; Horstmann et al., 2010; van Dellen et al., 2012; van Diessen et al., 2013b).

Quality assessment

Study quality was assessed independently by two authors [WZ, WMO] with the adjusted Newcastle-Ottawa scale method (Stang, 2010). Disagreements were

resolved by consensus. This scale is developed for non-randomized studies and allocates stars to the following domains: the selection and comparability of study groups, and the ascertainment for the exposure of interest for case-control or the outcome of interest for cohort studies. A star system is used in the Newcastle-Ottawa scale for semi-quantitative analysis wherein high quality studies get more stars, with a maximum of seven in total.

Methods of analysis

All data were entered in RevMan version 5.2.6 for analysis (http://ims.cochrane.org/revman). Average path length and average clustering coefficient summary group differences, and their CIs, were calculated by fitting random-effect weighted standardized mean difference models using restricted maximum likelihood estimation (Schmidt et al., 2009). A random-effect model was used because this provides a more conservative effect than a fixed-effect model as it takes heterogeneity between studies into account. We used the standardized mean difference because we expected that scale differences of the average path length and average clustering coefficient values between studies might be affected by modality, connectivity measure, network construction (for example, binary or weighted, normalization or no normalization) and sparsity level. The standardized mean difference model is a well-excepted method to calculate summary values if scale variation is present. It has the advantage of resulting in a scale-free summary estimate (that is, free of the original measurement scale) (Cummings, 2011). Significant heterogeneity between studies was defined by p value <0.1 using the $\chi 2$ test or an $I^2>75\%$. An I^2 value below 40% suggests negligible heterogeneity, between 40 and 75% moderate heterogeneity, and above 75% considerable heterogeneity (Higgins et al., 2003). In the latter case, summary estimates are unreliable. To detect a reporting bias in the included studies, we visually inspected funnel plots for asymmetry and excluded outliers (Higgins et al., 2008). Furthermore, we checked whether individual studies had a significant effect on the heterogeneity by means of a leave-one-out analysis (that is, omitting one study at the time with recalculation). We excluded studies with significant effects on summary estimations. Furthermore, we performed meta-regression to explain the potential heterogeneity in the standardized mean differences of the average clustering coefficient and average path length. We regressed the individual study standardized mean difference values against the

i) mean age of the patients, control subjects and ages from patients and controls combined, and the *ii*) mean duration of epilepsy.

Subgroup and sensitivity analyses

We distinguished overall changes in functional and structural networks using sub-analyses based on modality: functional synchronization for functional networks and white matter or cortical thickness connectivity for structural networks. Considering the brain network alterations during lifespan (Cao et al., 2014; Uddin et al., 2010), we performed separate sub-analyses comparing children with adults. To investigate the influence of different types of epilepsy on network alterations, we conducted separate analyses for temporal lobe epilepsy, extratemporal epilepsy and patients with epilepsy secondary to brain tumors. The robustness of the summary estimates was tested by means of sensitivity analysis. In this analysis, changes in point estimates and CIs were determined using recalculations at different network sparsity levels for binary or weighted networks.

Statistical power

We calculated the power of each individual study using the estimated summary effect obtained from the random-effect analysis to which it contributed. Power calculations were done with the *pwr* package (http://cran.r-project.org/web/packages/pwr) of the R software. The individual study powers for the average path length and average clustering coefficient are presented as mean ± SD.

RESULTS

Article selection

The literature search and study selection flow chart is given in Figure 2. We found no previous systematic literature reviews or meta-analyses on network organization in focal epilepsy. From 65 manuscripts, full text was reviewed and reference lists were inspected. Eventually, thirteen studies were initially included (Bartolomei et al., 2006; Bernhardt et al., 2011; Bonilha et al., 2012; Bosma et al., 2009; Horstmann et al., 2010; Liao et al., 2010; Quraan et al., 2013; Raj et al., 2010; Vaessen et al., 2013; Vaessen et al., 2012; van Dellen et al., 2012; van Diessen et al., 2013c; Vlooswijk et al., 2011). Study characteristics are summarized in Table 1; more detailed methodological information on the included electrophysiological and functional MRI studies is provided in

T1

Study	Characteristics of study population							Imaging characteristics		Network properties				
	Group	Group size (N)	Female / Male	Age in years (±SD)	Duration in years (±SD)	Type of lesion	Drug treatment	Modality	Field strength or sample frequency	Network size (template)	Connectivity measure	Binary / weighted	Sparsity level	Used network characteristics
Bartolomei et al., 2006	Tumor	17	9 / 8	40 (12)	n.r.	Mixed	n.r.	MEG	312.5 Hz	149	SL	Binary	k=10	γ, λ
	Control	15	7 / 8	31 (8)										
Bernhardt et al., 2011	TLE	122	70 / 52	36 (10.5)	20.5 (12)	Hippocampal atrophy	n.r.	MRI	1.5 Tesla	52 (AAL)	Cortical thickness correlation	Binary	5-40%	C, L, γ, λ, σ, BC
	Control	47	24 / 23	32 (12)										
Bonilha et al., 2012	TLE	12	8 / 4	37.5 (9.8)	n.r.	Hippocampal atrophy	n.r.	DTI	3.0 Tesla	20 (AAL)	Fiber density	Binary	5-55%	C, Eglobal, Elocal, BC
	Control	26	16 / 20	34.3 (8.8)										
Bosma et al., 2009	Tumor	17	n.r.	42.7 (11.2)	n.r.	LGG	Mono- or polytherapy	MEG	312.5 Hz	149	PLI	Binary	k=10	C, L, γ, λ, σ, K
	Control	17	n.r.	42.6 (12.7)										
Horstmann et al., 2010	Symptomatic focal epilepsy	21	12 / 9	38 (11)	21.3 (11)	Mixed	Mono- or polytherapy	EEG	254.31 Hz	29	Time-resolved phase synchronization	Binary & weighted	k=3-7; lowest connectedness	C, L, R, γ
	Control	23	11 / 12	33 (9)										
Liao et al., 2010	TLE	18	7 / 11	23.9 (8.5)	13.2 (10)	Hippocampal sclerosis	Mono- or polytherapy	fMRI	1.5 Tesla	90 (AAL)	Pearson's correlation coefficient	Binary	0.22-0.386	C, L, γ, λ, σ, K
	Control	27	8 / 19	25.6										
Quraan et al., 2013	TLE	9	2/7	42 (13)	n.r.	Mixed	Polytherapy	EEG	500 Hz	64	Mean phase coherency	Binary	0.3<T<0.6; 18.9<K<37.8	γ, Eglobal, σ
	Control	15	n.r.	33 (10)										

Table 1. Continued

Study	Characteristics of study population							Imaging characteristics		Network properties				
	Group	Group size (N)	Female / Male	Age in years (±SD)	Duration in years (±SD)	Type of lesion	Drug treatment	Modality	Field strength or sample frequency	Network size (template)	Connectivity measure	Binary / weighted	Sparsity level	Used network characteristics
Raj et al., 2010	TLE	27	17 / 10	38.5 (7.7)	n.r.	Mixed	Mono- or polytherapy	MRI	4.0 Tesla	64	Cortical thickness correlations	Binary	15%	C, K, Entropy
	Control	30	20 / 10	38.3 (9.7)										
Vaessen et al., 2012	Focal epilepsy	39	30 / 19	40 (12)	17.8 (11.1)	Mixed	Mono- or polytherapy	DTI	3.0 Tesla	90 (AAL)	Structural connection strength	Weighted	Voxels transversed by > 50 tracts	C, L
	Control	23	14 / 9	40 (13)										
Vaessen et al., 2013	FLE	28	n.r.	11.3 (1.5)	n.r.	Mixed	n.r.	fMRI	3.0 Tesla	82	Pearson's correlation coefficient	Binary & weighted	0.35-0.75	C, L
	Control	37	n.r.	10.5 (1.3)										
Van Dellen et al., 2012	Tumor	35	15 / 20	42.2 (9.5)	7.4 (6.5)	Mixed	n.r.	MEG	625 Hz	136	PLI	Weighted	Connection strength	γ, λ, Q
	Control	36	18 / 18	43.9 (11.9)										
Van Diessen et al., 2013	Focal epilepsy	35	11 / 24	10.1 (3.4)	0	Mixed	none	EEG	512 Hz	17	SL	Weighted	Connection strength	C, L, BC, K, CC, EC
	Control	35	11 / 24	9.9 (3.1)										
Vlooswijk et al., 2011	Focal epilepsy	41	21 / 20	40 (12)	17.8 (11)	Mixed	Mono- or polytherapy	fMRI	3.0 Tesla	893 (AAL-adapted)	Pearson's correlation coefficient	Binary	0.87-1	C, L, γ, λ, Eglobal, Elocal
	Control	23	14 / 9	40 (12)										

Sample characteristics and network properties of the studies included in the systematic review

TLE = temporal lobe epilepsy, FLE = frontal lobe epilepsy, n.r. = not reported, LGG = low-grade glioma, AAL = anatomical automatic labeling, SL = synchronization likelihood, PLI = phase lag index, C = average clustering coefficient, L = average path length, γ = normalized clustering coefficient, λ = normalized average path length, Q = modularity, BC = betweenness centrality, K = degree, CC = closeness centrality, Eglobal = global efficiency, Elocal = local efficiency.

Supplementary Table S2 in File S1. Two of the included studies only reported on the average clustering coefficient and not the average path length (Bosma et al., 2009; Raj et al., 2010).

The quality of included studies was variable with a range in Newcastle-Ottawa scores between five and seven stars (see Supplementary Table S3 in File S1). Based on the asymmetry in the average path length funnel plot, as shown in Supplementary Figure S1 in File S1, we excluded two obvious outliers from the average path length data from the final analysis (Bartolomei et al., 2006; Quraan et al., 2013). No obvious outliers were detected in the average clustering coefficient funnel plot, shown in Supplementary Figure S2 in File S1. We tested the remaining studies on their individual contribution to heterogeneity. All data are provided in the Supplementary Information (Data S1).

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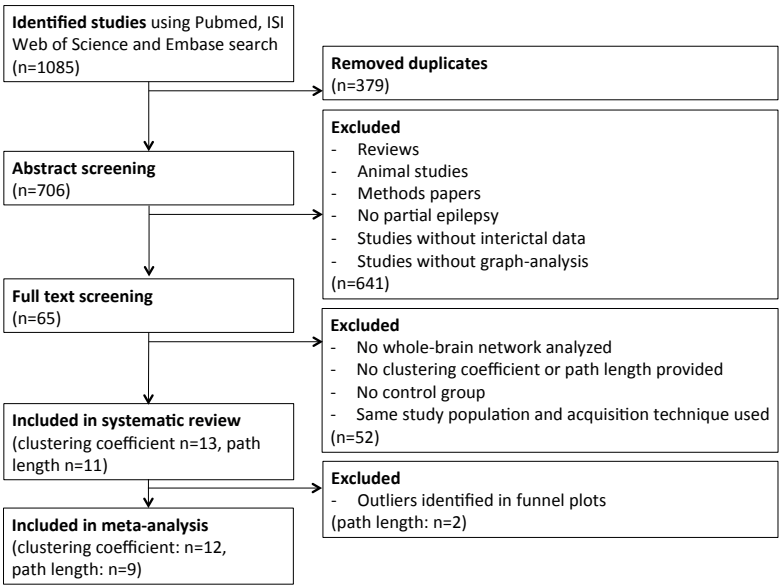


Flow chart

Flow chart of the literature search and identification of studies for inclusion in the meta-analysis.

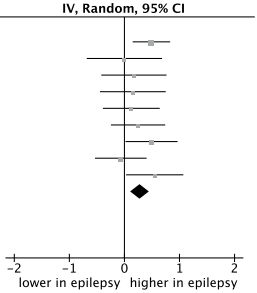
Average path length

Average path length summary estimates were based on data from nine studies (Bernhardt et al., 2011; Bonilha et al., 2012; Horstmann et al., 2010; Liao et al., 2010; Vaessen et al., 2013; Vaessen et al., 2012; van Dellen et al., 2012; van Diessen et al., 2013c; Vlooswijk et al., 2011). The standardized mean difference summary estimate, based on 351 patients with focal epilepsy and 277 control networks was 0.29 (CI: 0.12 to 0.45, p = 0.0007; Figure 3). In other words, the network organization in patients with focal epilepsy was characterized by an increased average path length as compared to controls. There was no evidence for heterogeneity: I^2 = 0%, p = 0.57 (Table 2). Data for the summary estimate was taken from normalized weighted network analysis, or the highest sparsity level if the study used binary networks only. Sensitivity analysis revealed only very small differences (that is, in the order of 1-10%) in summary estimates and CIs and *p* values if we changed the type of network or sparsity level (see Supplementary Table S4 in File S1).

F3

Study or Subgroup	Focal epilepsy patients Mean	SD	Total	Controls Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Bernhardt et al. 2011	2	0.104	122	1.94	0.16	47	23.3%	0.49 [0.15, 0.83]
Bonilha et al. 2012	1.34	0.03	12	1.34	0.03	26	5.8%	0.00 [-0.68, 0.68]
Horstmann et al. 2010	1.06	0.028	21	1.055	0.029	23	7.7%	0.17 [-0.42, 0.76]
Liao et al. 2010	2.095	0.36	18	2.037	0.38	27	7.6%	0.15 [-0.44, 0.75]
Vaessen et al. 2012	62.5	14.7	39	60.8	10.8	23	10.2%	0.13 [-0.39, 0.64]
Vaessen et al. 2013	0.52	0.074	28	0.504	0.055	37	11.1%	0.25 [-0.25, 0.74]
Van Dellen et al. 2012	1.097	0.022	35	1.087	0.018	36	12.1%	0.49 [0.02, 0.97]
Van Diessen et al. 2013	1.051	0.075	35	1.055	0.04	35	12.3%	-0.07 [-0.53, 0.40]
Vlooswijk et al. 2011	1.44	0.083	41	1.39	0.101	23	10.0%	0.55 [0.03, 1.07]
Subtotal (95% CI)			**351**			**277**	**100.0%**	**0.29 [0.12, 0.45]**

Heterogeneity: Tau^2 = 0.00; Chi^2 = 6.66, df = 8 (P = 0.57); I^2 = 0%
Test for overall effect: Z = 3.41 (P = 0.0007)



Meta-analysis of the average path length

The forest plot displays the standardized mean differences of the average path length between focal epilepsy patients and controls with the 95% confidence intervals (CI). No difference is specified with a vertical line at 0. The overall pooled SMD was 0.29 (CI: 0.12 to 0.45, p = 0.0007), that is, a significant higher average path length was observed in whole brain networks of focal epilepsy patients relative to controls.

Average clustering coefficient

Average clustering coefficient summary estimates were based on twelve studies (Bernhardt et al., 2011; Bonilha et al., 2012; Bosma et al., 2009; Horstmann et al., 2010; Liao et al., 2010; Quraan et al., 2013; Raj et al., 2010; Vaessen et al., 2013; Vaessen et al., 2012; van Dellen et al., 2012; van Diessen et al., 2013c; Vlooswijk et al., 2011). The standardized mean difference summary estimate, including 404 networks from focal epilepsy patients and 339 control networks, was 0.35 (CI: 0.05 to 0.65, p = 0.02; Figure 4). Stated differently, the network organization in patients with focal epilepsy was characterized by an increased average clustering coefficient as compared to controls. There was moderate heterogeneity in the data: I^2 = 73%, p <0.0001 (Table 2), indicating that the summary estimate might be unreliable. Results were, similar to the average path length estimate, stable in the sensitivity analysis (see Supplementary Table S4 in File S1).

Meta-regression average path length and average clustering coefficient

No significant relation was found between the standardized mean difference values (for average path length and average clustering coefficient) and the mean age (patients, controls or both) or mean duration of epilepsy.

Subgroup analyses

Modality specific summary estimates for the average path length and average clustering coefficient are provided in Figure 5 and 6, respectively. Both functional and structural focal epilepsy networks were characterized by a significantly increased average path length as compared to control networks: 0.26 for the functional (CI: 0.05 to 0.47, p = 0.02) and 0.30 for the structural networks (CI: 0.01 to 0.60, p = 0.04) (Figure 5). The average clustering coefficient was significantly increased in structural focal epilepsy networks: 0.64 (CI: 0.09 to 1.18, p = 0.02). There was no significant difference in average clustering coefficient for the functional epilepsy networks: 0.20 (CI: -0.14, 0.55, p = 0.25) (Figure 6). Age group specific estimates revealed an increased average path length and average clustering coefficient only for the adults with epilepsy in comparison with controls (see Supplementary Figure S3a and S3b in File S1). No difference in average path length (p = 0.18) and average clustering coefficient (p = 0.13) was found between adults and children with epilepsy. Specific estimates

T2

	Average path length			Average clustering coefficient		
Excluded study	SMD (CI)	I2 (%)	χ2 (p-value)	SMD (CI)	I2 (%)	χ2 (p-value)
None	***0.29 (0.12, 0.45)***	***0***	***0.57***	***0.35 (0.05, 0.65)***	***73***	***<0.0001***
Bernhardt et al., 2011	0.22 (0.04, 0.41)	0	0.68	0.32 (-0.01, 0.66)	73	<0.0001
Bonilha et al., 2012	0.30 (0.13, 0.47)	0	0.55	0.29 (-0.01, 0.59)	71	0.0001
Bosma et al., 2009				0.31 (-0.00, 0.62)	74	<0.0001
Horstmann et al., 2010	0.30 (0.12, 0.47)	0	0.48	0.36 (0.04, 0.69)	75	<0.0001
Liao et al., 2010	0.30 (0.13, 0.47)	0	0.49	0.36 (0.03, 0.69)	75	<0.0001
Quraan et al., 2013				0.37 (0.05, 0.69)	75	<0.0001
Raj et al., 2010				0.29 (-0.01, 0.60)	71	0.0001
Vaessen et al., 2012	0.30 (0.13, 0.48)	0	0.51	0.40 (0.09, 0.72)	73	<0.0001
Vaessen et al., 2013	0.29 (0.12, 0.46)	0	0.47	0.37 (0.04, 0.70)	75	<0.0001
van Dellen et al., 2012	0.26 (0.08 0.43)	0	0.56	0.31 (-0.01, 0.63)	73	<0.0001
van Diessen et al., 2013	0.33 (0.16, 0.51)	0	0.76	0.40 (0.08, 0.72)	73	<0.0001
Vlooswijk et al., 2011	0.26 (0.08, 0.43)	0	0.59	0.44 (0.18, 0.71)	61	0.004

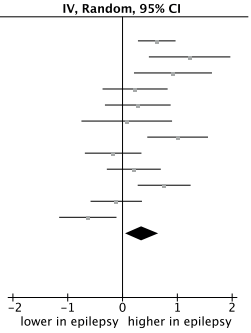
Leave-one-out analysis

The leave-one-out analysis results are shown in this table. The effect of leaving out one study on the overall heterogeneity is presented as the recalculated standardized mean differences (SMD) (after removal of the study in the first column) and the 95 % confidence interval (CI) and corresponding I2 statistic and χ2 test p-value.

F4

Study or Subgroup	Focal epilepsy patients Mean	SD	Total	Controls Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Bernhardt et al. 2011	0.4	0.108	122	0.31	0.21	47	10.3%	0.62 [0.28, 0.97]
Bonilha et al. 2012	0.72	0.016	12	0.7	0.016	26	6.9%	1.22 [0.48, 1.97]
Bosma et al. 2009	2.42	0.64	17	1.886	0.482	17	7.1%	0.92 [0.21, 1.63]
Horstmann et al. 2010	0.693	0.023	21	0.679	0.08	23	8.1%	0.23 [−0.36, 0.82]
Liao et al. 2010	2.221	0.97	18	1.971	0.82	27	8.1%	0.28 [−0.32, 0.88]
Quraan et al. 2013	1.233	0.333	9	1.217	0.017	15	6.3%	0.08 [−0.75, 0.90]
Raj et al. 2010	0.486	0.076	27	0.408	0.077	30	8.5%	1.01 [0.45, 1.56]
Vaessen et al. 2012	1.75	0.35	39	1.81	0.34	23	8.8%	−0.17 [−0.69, 0.35]
Vaessen et al. 2013	2.26	0.339	28	2.2	0.243	37	9.0%	0.21 [−0.29, 0.70]
Van Dellen et al. 2012	1.066	0.014	35	1.056	0.012	36	9.1%	0.76 [0.28, 1.24]
Van Diessen et al. 2013	1.006	0.01	35	1.007	0.007	35	9.2%	−0.11 [−0.58, 0.35]
Vlooswijk et al. 2011	4.48	2.004	41	5.69	1.655	23	8.7%	−0.63 [−1.16, −0.11]
Subtotal (95% CI)			**404**			**339**	**100.0%**	**0.35 [0.05, 0.65]**

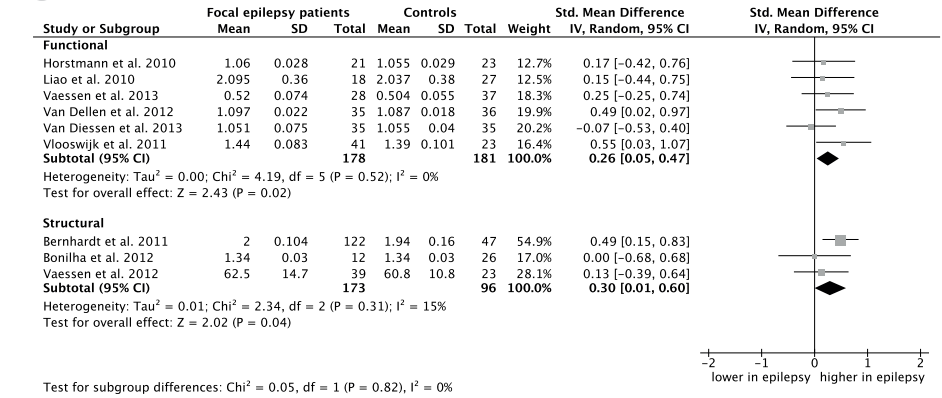
Heterogeneity: Tau² = 0.20; Chi² = 40.59, df = 11 (P < 0.0001); I² = 73%
Test for overall effect: Z = 2.29 (P = 0.02)



Meta-analysis of the average clustering coefficient

The forest plot displays the standardized mean differences (SMD) of the average clustering coefficient between focal epilepsy patients and controls with the 95% confidence intervals (CI). No difference is specified with a vertical line at 0. The overall pooled SMD was 0.35 (CI: 0.05 to 0.65, p = 0.02), that is, a significant higher average clustering coefficient was observed in whole brain networks of focal epilepsy patients relative to controls.

F5



Meta-analysis of the average path length separated by network modality

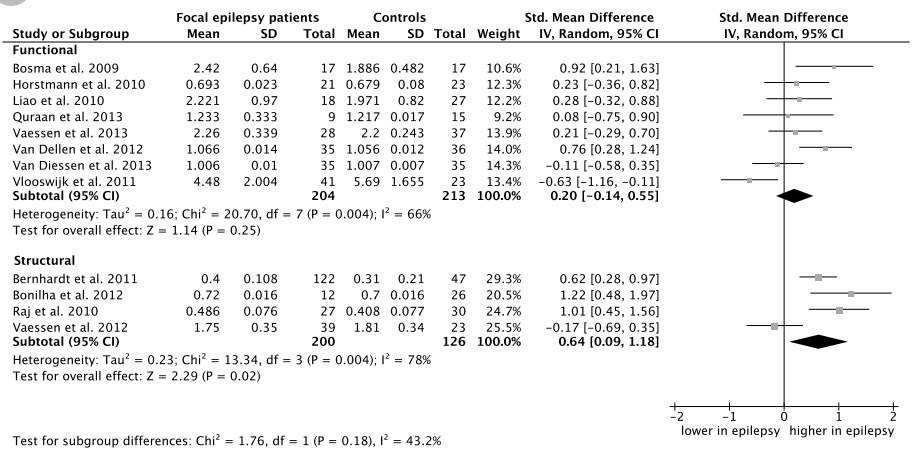
The forest plot displays the standardized mean differences (SMD) between focal epilepsy patients and controls with the 95% confidence intervals (CI) for the functional and structural network studies reporting the average path length. No difference between patients and controls is specified with a vertical line at 0. The overall pooled SMD for the functional average path length was 0.26 (CI: 0.05 to 0.47, p = 0.02) and 0.30 for the structural average path length (CI: 0.01 to 0.60, p = 0.04). The SMDs of these subgroups were not statistically different (p = 0.82, I2 = 0%).

for the subgroup analysis for different types of epilepsy revealed an increased average path length and clustering coefficient for the temporal lobe epilepsy patients only in comparison with controls. There was no difference between the temporal lobe, extratemporal and brain tumor groups in terms of average path length (p = 0.48). The standardized mean differences were significantly different for the average clustering coefficient (p = 0.0002) with an evidently increased segregation for the temporal lobe epilepsy subgroup, but due to a considerable heterogeneity (I^2 = 88.1%), not reliable (see Supplementary Figure S4a and S4b in File S1).

Statistical power

The average statistical power of included studies was (very) low: 20.8 ± 7.6 and 25.5 ± 10.3% for the average path length and average clustering coefficient, respectively (note that the standard recommended statistical power is in the range of 80 to 90 %). Based on the average path length standardized mean difference

F6



Meta-analysis of the average clustering coefficient separated by network modality

The forest plot displays the standardized mean differences (SMD) between focal epilepsy patients and controls with the 95% confidence intervals (CI) for the functional and structural network studies reporting the average clustering coefficient. No difference between patients and controls is specified with a vertical line at 0. The overall pooled SMD for the functional average clustering coefficient was 0.20 (CI: -0.14, 0.55, p = 0.25) and 0.64 for the structural average clustering coefficient (CI: 0.09 to 1.18, p=0.02). The SMDs of these subgroups were not statistically different (p = 0.18, I2 = 43.2%).

of 0.29, at least 188 subjects per group are required to obtain a power of 80%. A group size of 251 is required to reach a power of 90%.

DISCUSSION

In this systematic review and meta-analysis we obtained information on an issue that has not been unequivocal: the network organization in patients with focal epilepsy as compared to healthy individuals. Previous qualitative reviews have identified that case-control studies investigating interictal networks often reveal contradictive results (Kramer and Cash, 2012; van Diessen et al., 2013a). Here, we tried to remove this ambiguity by means of a quantitative estimate of the group differences in average path length, a measure of network integration, and average clustering coefficient, a measure of network segregation. Interictal brain networks in patients with focal epilepsy are characterized by a significant

increase in both average path length and average clustering coefficient, compared to healthy controls. Finally, the statistical power of the included individual network studies was low.

This systematic review adds unique information to previous qualitative reviews (Engel et al., 2013; Kramer and Cash, 2012; Lemieux et al., 2011; Richardson, 2012). First, it clarifies the type of network change in focal epilepsy interictally: whole brain networks of patients are characterized by a less integrated and more segregated organization (that is, a more regular network organization) than healthy controls. Interestingly, a shift towards an even more regular organization is found during the ictal phase, relative to the interictal phase (for review, see (Kramer and Cash, 2012; van Diessen et al., 2013a)). Studies focusing on dynamic network changes in epilepsy – namely before, during and directly after a seizure – have shown that a shift in network organization towards a more regular state increases further during a seizure and eventually returns to pre-ictal values (Kramer et al., 2010; Ponten et al., 2007; Schindler et al., 2008). Possibly, the regular interictal network organization facilitates fast spreading activity in the brain (Ponten et al., 2007; Schindler et al., 2008) making it more susceptible to seizures. Second, our results suggest that (frequent) epileptic seizures may have widespread detrimental effects on the structural and functional integrity of interictal brain networks. Data supporting this hypothesis comes from studies characterizing white matter integrity in focal epilepsy, for overview see (Gross, 2011). A recent systematic review and meta-analysis of clinical studies provided evidence for widespread structural white matter damage distant from the epileptogenic zone (Otte et al., 2012b). Similarly, an animal study revealed that diffuse, widespread damage to the connecting white matter tracts coincides with reduced functional connectivity and network efficiency (Otte et al., 2012a). The pathophysiological mechanism of the remote white matter damage remains unknown. Potential explanations include Wallerian degeneration, axonal swelling caused by frequent recurrent seizures, decreased axonal density, increasing permeability of axonal membranes and demyelination (Concha et al., 2010; Kim et al., 2008). Additionally, it remains unclear, whether epileptic seizures cause the (interictal) network organization to change or if seizures are merely an epiphenomenon of epileptogenic networks (Douw et al., 2010b; van Dellen et al., 2012). Previous research has revealed that (remote) white matter abnormalities coincide with cognitive impairments in focal epilepsy (McDonald

et al., 2008; Riley et al., 2010). More recently, Vaessen and colleagues found distinct structural network changes in cognitively impaired patients with epilepsy, while non-impaired patients did not show network alterations (Vaessen et al., 2012). Interestingly, subgroup analysis in our current study showed that both functional and structural network alterations point in the same direction, although the number of network studies looking at white matter connectivity was too small to draw a firm conclusion. The interplay between structural and functional network alteration is essential to understand the cognitive and behavioral impairments that occur in a considerable part of patients with epilepsy. Future studies, using simultaneous acquisition of functional and structural connectivity, are required to address this issue (Achard et al., 2008; Honey et al., 2009; Zhang et al., 2011). We found no difference between adults and children with epilepsy, however the number of included studies is too small to draw a firm conclusion. More studies on children with epilepsy are needed to clarify if, and to what extent, the developing brain organizes itself differently in comparison with the adult brain when epilepsy manifests. Similarly, subgroup analyses for different types of epilepsy did not reveal significant differences between temporal lobe, extratemporal and brain tumor patients, but heterogeneity in the data might obfuscate a true difference. Increasing the number of studies per subgroup could provide better insights to what extent these subgroups have a different network organization. In addition, we performed meta-regression to explain part of the variation in average path length and average clustering coefficient between the individual studies. We expected differences in network organization to relate to subjects' age and duration of epilepsy (for instance, more damage – and consequently an even less efficient network organization – if a brain is longer exposed to recurrent seizures). However, we found no significant association. Information on other network modifiers such as antiepileptic drug treatment, age at onset, type of epilepsy, presence or absence of a structural lesion, and location of the epileptogenic focus could also be useful in obtaining more details on the relationship between network organization and focal epilepsy, but these patient characteristics were not reported in most of the included studies. Meta-regression was restricted to the mean values reported per study, masking individual subject values. This, or the small number of regression points, could explain the lack of association between patient characteristics and network metrics. A solution to mean regression is individual patient data (IPD) meta-analysis, which is known

to provide more reliable estimates than standard meta-analysis, but requires the collection of the original networks and patient datasets for all studies, which is very time consuming and requires active participation of the corresponding authors of all included studies (Simmonds et al., 2005).

Our power analysis results suggest that future studies that aim to characterize the network organization in terms of average path length and average clustering coefficient in focal epilepsy should consider minimal sample sizes of 188 per group, as the effect sizes of these two specific network metrics were found to be small. Collaborations between academic institutions could help to obtain the required sample sizes. This strategy has been successful in increasing the reliability of study results in the field of genetic epidemiology (Burton et al., 2009). The Human Connectome Project, with over a thousand combined functional MRI scans, is a first successful attempt to boost statistical power in the field of neuroscience and could also serve as an example for future network studies (Smith et al., 2013). Other possibilities to decrease the required sample size are to increase the homogeneity of patients, as was shown for the temporal lobe epilepsy subgroup analysis (Supplementary Figure S4a and S4b in File S1), or to use more advanced network metrics such as the modularity (Vaessen et al., 2013; van Dellen et al., 2012). Using the minimum spanning tree approach, which focuses on the connectivity backbone of the network, might also help to increase study power as these network backbones are less susceptible to noise (Ortega et al., 2008b; van Dellen et al., 2014a; van Diessen et al., 2014b).

Our study has limitations. Despite the important and intuitively appealing network metrics we used in this study, more information can be obtained from the network organization apart from the average path length and average clustering coefficient. In fact, these two metrics are highly correlated, arguing for more and distinct features in characterizing focal epilepsy networks (Li et al., 2011). Also, average path length and average clustering coefficient provide only information on global network topology. Eligible network features are *i*) the long-tailed degree distribution compatible with the existence of super connected nodes, *ii*) the modular community structure, that is, multiple network groups of densely interconnected nodes, *iii*) usage of the minimum spanning tree approach, a robust method that enables comparison of different types of networks (Stam et al., 2014) and *iv*) the rich-club ordering, which is the tendency of nodes with high centrality to form tightly interconnected communities (for reviews see:

(Bullmore and Sporns, 2012; Stam and van Straaten, 2012b; van den Heuvel and Sporns, 2013a)). Furthermore, network analysis in neurophysiological studies is often performed in different frequency bands. In our study, we chose to include only theta frequency derived networks. Most studies have repeatedly identified epilepsy specific changes in this frequency band (van Diessen et al., 2013a). Other frequency bands, however, might contain additional information on epilepsy related network changes that were not considered in our study. Finally, the included studies were diverse in modality, network size and analysis strategy. This heterogeneity relates to the inconsistency between individual study results. Some studies combined different modalities into one study and revealed inconsistent results (Horstmann et al., 2010; Vaessen et al., 2014). For that reason, we performed subanalysis for functional and structural studies. We found a less integrated network organization in patients, both in functional and structural networks, as compared to controls. A more segregated network in patients was found for structural networks only. This might indicate that different mechanisms (or detection sensitivities) are at play for structural and functional network alterations in patients with epilepsy. No differences were found between the epilepsy patients' functional network data and patients' structural network data (Figure 5 and 6). It is therefore not possible to make an unambiguous statement on the differences between functional and structural networks and if these networks interact. Studies investigating functional and structural networks concomitantly are needed to clarify the similarities or discrepancy between modalities (Horstmann et al., 2010; Vaessen et al., 2014).

A difference in the number of network nodes will influence network metrics (van Wijk et al., 2010). Also, different analysis strategies might affect the eventual outcome of network metrics (Constable et al., 2013; van Diessen et al., 2013a). These differences complicate direct comparison of individual study results. However, using the standardized mean difference in our meta-analysis, we intended to correct for scale differences between studies due to modality, connectivity measures and network construction or size. Another important issue, related to our hypothesis, is our implicit assumption that network alterations in focal epilepsies are similar, regardless their etiology. One could argue that this is not the case, as studies have revealed differences in network connectivity and topology for patients with different etiologies (Bettus et al., 2008; van Dellen et al., 2012). Unfortunately, we were not able to

investigate etiology-related differences in network topology due to missing information in most of studies.

In conclusion, this systematic review and meta-analysis revealed that interictal whole brain networks are characterized by a less integrated and more segregated organization in focal epilepsies. A large, sufficiently powered study is required to confirm our findings. Future work may focus on *i*) pinpointing the exact cause of changes in brain-wide network organization, *ii*) identification of relevant effect modifiers such as antiepileptic therapy, duration of epilepsy or type of epilepsy and *iii*) characterizing network differences in specific subgroups of focal epilepsy using a range of features beyond measures that quantify network integration and segregation. When interested in functional connectivity, we encourage using more than one connectivity measure to investigate the robustness of the possible network alterations found in patients with epilepsy. Finally, when attempting to unravel causality in the relation between epileptic seizures and network alterations in patients with epilepsy, we suggest the use of longitudinal studies and a systematic evaluation and interpretation of the data according to guidelines how to draw a conclusion on causality (Asano et al., 2013).

SUPPORTING INFORMATION

S1

Pubmed (NCBI) Search

((TS=(epilepsy OR seizure OR convulsion) AND TS=(DTI OR "diffusion tensor imaging" OR "diffusion magnetic resonance imaging" OR "diffusion weighted imaging" OR EEG OR electroencephalogram OR MEG OR magnetoencephalogram OR fMRI OR "functional MRI" OR "functional magnetic resonance imaging" OR sMRI OR "structural MRI" OR "structural magnetic resonance imaging" OR "cortical thickness") AND TS=(Network OR "network analysis" OR "network theoretical analysis" OR "network-theoretical analysis" OR "graph analysis" OR "graph theoretical analysis" OR "graph-theoretical analysis" OR "clustering coefficient" OR "path length" OR "shortest path" OR "small-world index" OR "small world index" OR "small-worldness" OR "small worldness" OR "local efficiency" OR "global efficiency" OR segregation OR integration OR topology))) AND Language=(English)

Databases=SCI-EXPANDED, CPCI-S Timespan=All Years

ISI Web of Science (Thomson Reuters) search

((TS=(epilepsy OR seizure OR convulsion) AND TS=(DTI OR "diffusion tensor imaging" OR "diffusion magnetic resonance imaging" OR "diffusion weighted imaging" OR EEG OR electroencephalogram OR MEG OR magnetoencephalogram OR fMRI OR "functional MRI" OR "functional magnetic resonance imaging" OR sMRI OR "structural MRI" OR "structural magnetic resonance imaging" OR "cortical thickness") AND TS=(Network OR "network analysis" OR "network theoretical analysis" OR "network-theoretical analysis" OR "graph analysis" OR "graph theoretical analysis" OR "graph-theoretical analysis" OR "clustering coefficient" OR "path length" OR "shortest path" OR "small-world index" OR "small world index" OR "small-worldness" OR "small worldness" OR "local efficiency" OR "global efficiency" OR segregation OR integration OR topology))) AND Language=(English)

Refined by: Web of Science Categories=(CLINICAL NEUROLOGY OR NEUROIMAGING)

Timespan=All Years. Databases=SCI-EXPANDED, CPCI-S

Embase (Excerpta Medica Database) search

((TS=(epilepsy OR seizure OR convulsion) AND TS=(DTI OR "diffusion tensor imaging" OR "diffusion magnetic resonance imaging" OR "diffusion weighted imaging" OR EEG OR electroencephalogram OR MEG OR magnetoencephalogram OR fMRI OR "functional MRI" OR "functional magnetic resonance imaging" OR sMRI OR "structural MRI" OR "structural magnetic resonance imaging" OR "cortical thickness") AND TS=(Network OR "network analysis" OR "network theoretical analysis" OR "network-theoretical analysis" OR "graph analysis" OR "graph theoretical analysis" OR "graph-theoretical analysis" OR "clustering coefficient" OR "path length" OR "shortest path" OR "small-world index" OR "small world index" OR "small-worldness" OR "small worldness" OR "local efficiency" OR "global efficiency" OR segregation OR integration OR topology))) AND Language=(English)

Refined by: Research Areas=(NEUROSCIENCES NEUROLOGY)

Databases=SCI-EXPANDED, CPCI-S Timespan=All Years

Search strategy

For the online databases Pubmed (NCBI), ISI Web of Science (Thomson Reuters) and Embase (Excerpta Medica Database).

$T2

Study	Modality	Recording characteristics	Condition	Reference	Artifact management
Bartolomei et al., 2006	MEG	SF: 312.5 Hz Bandpass filter of 0.25-125 Hz	Awake, no-task, EC	n.a.	Selection of artifact free epochs by visual analysis
Bosma et al., 2009	MEG	SF: 312.5 Hz Bandpass filter of 0.25-125 Hz	Awake, no-task, EC	n.a.	Selection of artifact free epochs by visual analysis
Horstmann et al., 2010	EEG	SF: 254.31 Hz Bandpass filter of 0-50 Hz	Awake, no-task,15 min EO, 15 min EC Acoustical instruction to open/ close eyes	Right mastoid and average common reference	EOG to record ocular artifacts Wavelet-based correction scheme to minimize influence of technical (e.g., amplifier resets) and physiologic artifacts (e.g., eye movements/blinks or head movements)
Liao et al., 2010	fMRI	FS: 1.5 T Echoplanar imaging sequence	Awake, no-task, EC	T1-weighted image for anatomic reference	First 10 images discarded to ensure magnetization equilibrium Other images: acquisition time-delay and head motion correction, translation or rotation > +1 mm or +10 = excluded
Quraan et al., 2013	EEG	SF: 500 Hz Bandpass filter of 2-20 Hz	Awake, no-task, 3 min EO, 3 min EC	Average common reference	EOG to record ocular artifacts Manual removal remaining artifacts
Vaessen et al., 2013	fMRI	FS: 3.0 T Echoplanar imaging sequence	n.r.	T1-weigthed 3D fast field echo for anatomic reference	Correction for motion artifacts and high frequency noise

Table S2. Continued

Study	Modality	Recording characteristics	Condition	Reference	Artifact management
Van Dellen et al., 2012	MEG	SF: 625 Hz Bandpass filter of 0.25-125 Hz	Awake, no-task, EC	n.a.	Selection of artifact free epochs by visual analysis
Van Diessen et al., 2013	EEG	SF: 512 Hz Bandpass filter of 0.16-70 Hz	Awake, no-task, EC	Average reference	Removal of Fp1, Fp2, A1, and A2 electrodes to minimize eye-movement artifacts Selection of artifact free epochs by visual analysis
Vlooswijk et al., 2011	fMRI	FS: 3.0 T Echoplanar imaging sequence	Awake, 6 times 30s word-paradigm, 6 times 30s no-task resting state	T1-weigthed 3D fast field echo for anatomic reference	Acquisition time-delay and head motion correction

Additional methodological information on the encephalographic and functional MRI studies

SF = sample frequency, FS = field strength, Hz = hertz, T = tesla, EC = eyes closed, EO = eyes open, EOG = electrooculography, n.a. = not applicable, n.r. = not reported, Fp = frontopolar, A = auricular.

ST3

Study	Quality indicators from the Newcastle-Ottawa Scale (Wells)							
	Selection		Comparability		Exposure			
	1	2	3	4	5A	5B	6	Total score
Bartolomei et al., 2006	*		*	*		*	*	5
Bernhardt et al., 2011	*	*	*	*	*	*	*	7
Bonilha et al., 2012	*	*	*	*	*	*	*	7
Bosma et al., 2009	*	*	*	*	*	*	*	7
Horstmann et al., 2010	*	*	*	*		*	*	6
Liao et al., 2010	*	*	*	*		*	*	6
Quraan et al., 2013	*	*	*	*			*	5
Raj et al., 2010	*	*	*		*	*	*	6
Vaessen et al., 2012	*	*	*	*		*	*	6
Vaessen et al., 2013	*	*	*	*	*		*	6
van Dellen et al., 2012	*	*	*	*		*	*	6
van Diessen et al., 2013	*	*	*	*	*	*	*	7
Vlooswijk et al., 2011	*	*	*	*			*	5

Study quality assessment

The table presents the Newcastle-Ottawa Scale quality assessment categories. These categories are included: 1 = cases independently validated; 2 = cases are representative of the population; 3 = community controls; 4 = controls have no history of focal epilepsy; 5A = study controls for age; 5B = study controls for additional factors; 6 = ascertainment of exposure by blinded interview or secure record.

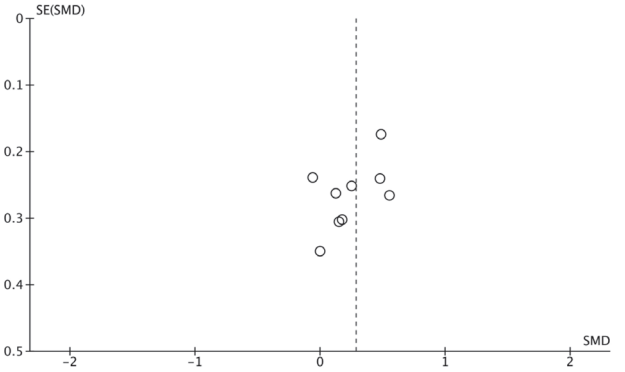
ST4

	Average path length				Average clustering coefficient			
	I^2 (%)	p-value	SMD (CI)	p-value	I^2 (%)	p-value	SMD (CI)	p-value
None (= weighted; high sparsity level)	0	0.56	0.26 (0.10, 0.42)	0.001	73	<0.0001	0.35 (0.05, 0.65)	0.02
Network direction:								
Binary instead of weighted[i]	8	0.37	0.22 (0.05, 0.38)	0.009	75	<0.00001	0.32 (0.00, 0.63)	0.05
Sparsity level[ii]:								
Low	0	0.60	0.24 (0.08, 0.40)	0.003	72	<0.0001	0.36 (0.06, 0.65)	0.02
Middle	0	0.65	0.23 (0.08, 0.39)	0.003	73	<0.0001	0.34 (0.03, 0.64)	0.03

Sensitivity analysis

The robustness of the summary estimates is presented as recalculations of the summary estimates at different network sparsity levels and for binary or weighted networks. [i] In the analysis of network directionality, we used the binary instead of weighted data from the average clustering coefficient and average path length provided by (Horstmann et al., 2010) and (Vaessen et al., 2013); [ii] In the sparsity level analyses we used data from low and middle instead of high sparsity levels provided by (Vlooswijk et al., 2011), (Liao et al., 2010), and (Vaessen et al., 2013). SMD = standardized mean difference, CI = 95 % confidence interval.

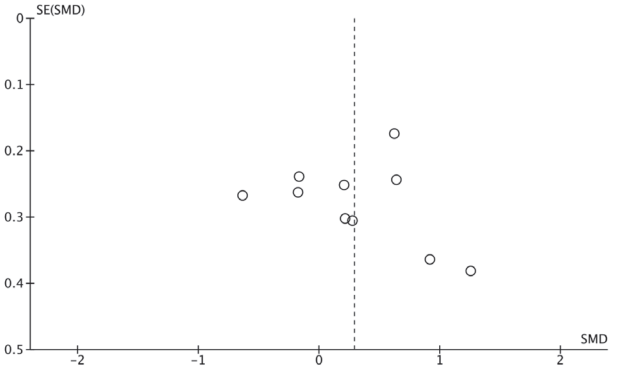
SF1



Funnel plot of average path length for individual studies

A funnel plot for the standardized mean difference (SMD) average path length estimates from individual studies against the standard error (SE) of the SMD as a measure of the study's precision. The dashed vertical line corresponds to the overall summary SMD estimate.

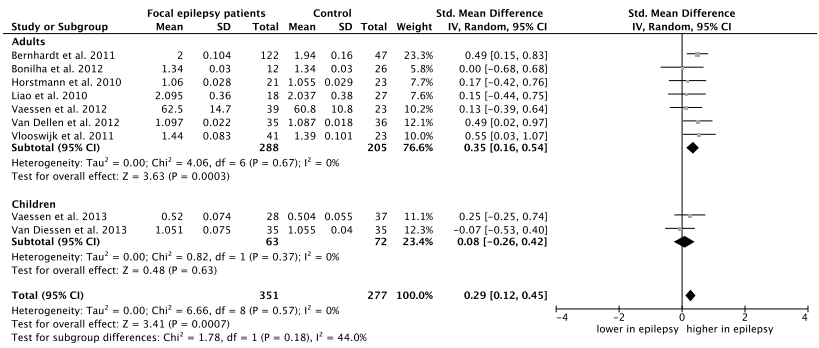
SF2



Funnel plot of average clustering coefficient for individual studies

A funnel plot for the standardized mean difference (SMD) average clustering coefficient estimates from individual studies against the standard error (SE) of the SMD as a measure of the study's precision. The dashed vertical line corresponds to the overall summary SMD estimate

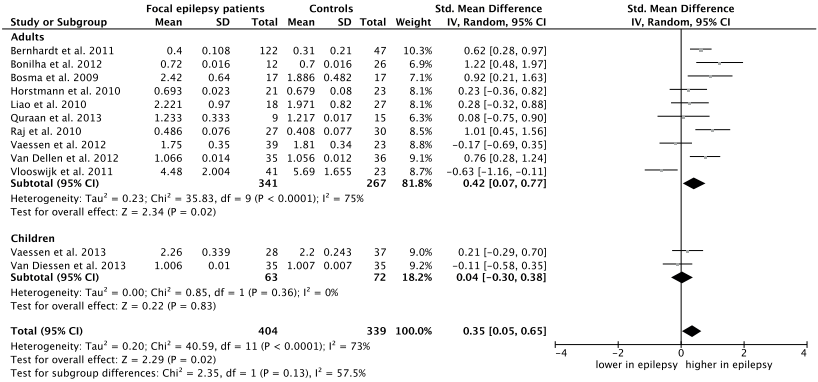
SF3



A) Meta-analysis of the average path length according to age

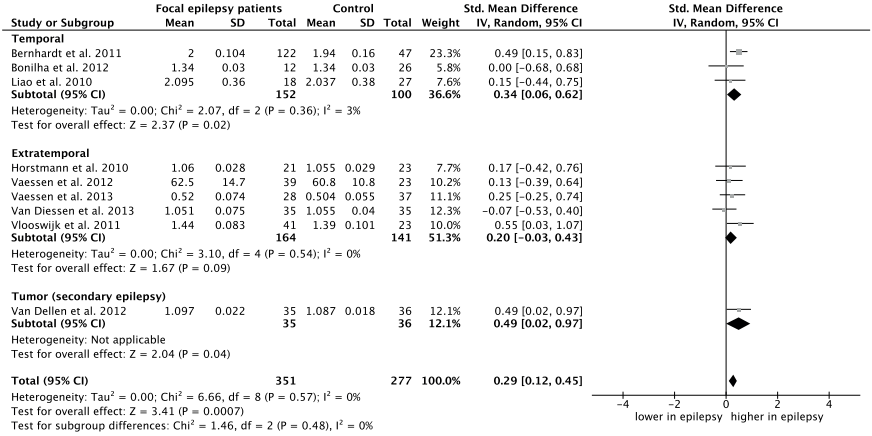
The forest plot displays the standardized mean differences (SMD) between focal epilepsy patients and controls with the 95% confidence intervals (CI) for the network studies reporting the average path length. Studies are divided in two categories: studies on children and studies on adults. No difference between patients and controls is specified with a vertical line at 0. The overall pooled SMD for the average path length of adults was 0.35 (CI: 0.16 to 0.54, p = 0.0003) and 0.08 (CI: -0.26 to 0.42, p = 0.63) for the path length of children. The SMDs of these subgroups were not statistically different (p = 0.18, I2 = 44.0%).

SF3



B) Meta-analysis of the average path length according to age

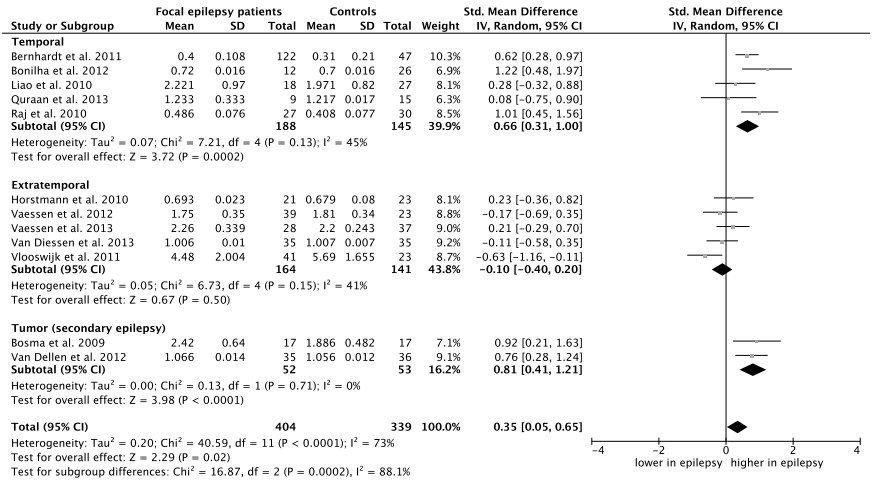
The forest plot displays the standardized mean differences (SMD) between focal epilepsy patients and controls with the 95% confidence intervals (CI) for network studies reporting the average clustering coefficient. Studies are divided in two categories: studies on children and studies on adults. No difference between patients and controls is specified with a vertical line at 0. The overall pooled SMD for the clustering coefficient of adults was 0.42 (CI: 0.07 to 0.77, p < 0.0001) and 0.04 (CI: -0.30 to 0.38, p = 0.83) for the average path length of children. The SMDs of these subgroups were not statistically different (p = 0.13, I^2 = 57.5%).



A) Meta-analysis of the average path length according to type of epilepsy

The forest plot displays the standardized mean differences (SMD) between focal epilepsy patients and controls with the 95% confidence intervals (CI) for the network studies reporting the average path length. Results are separated for studies that included only TLE patients and studies that included different focal epilepsy patients. No difference between patients and controls is specified with a vertical line at 0. The overall pooled SMD for the average path length of studies that included TLE patients was 0.34 (CI: 0.06 to 0.62, p = 0.02) and 0.25 (CI: 0.05 to 0.46, p = 0.02) for the average path length of studies including different focal epilepsy patients. The SMDs of these subgroups were not statistically different (p = 0.48, I^2 = 0%).

sF4



B) Meta-analysis of the average clustering coefficient according to type of epilepsy

The forest plot displays the standardized mean differences (SMD) between focal epilepsy patients and controls with the 95% confidence intervals (CI) for the network studies reporting the average clustering coefficient. Results are separated for studies that included only TLE patients and studies that included different focal epilepsy patients. No difference between patients and controls is specified with a vertical line at 0. The overall pooled SMD for the average clustering coefficient of studies that included TLE patients was 0.66 (CI: 0.31 to 1.00, p = 0.0002) and 0.15 (CI: -0.23 to 0.54, p = 0.44) for the clustering average coefficient of studies including different focal epilepsy patients. The SMDs of these subgroups were statistically different (p = 0.0002), but due to a considerable heterogeneity (I^2 =88.1%), not reliable.

CHAPTER 5

ARE HIGH FREQUENCY OSCILLATIONS ASSOCIATED WITH ALTERED NETWORK TOPOLOGY IN FOCAL EPILEPSY?

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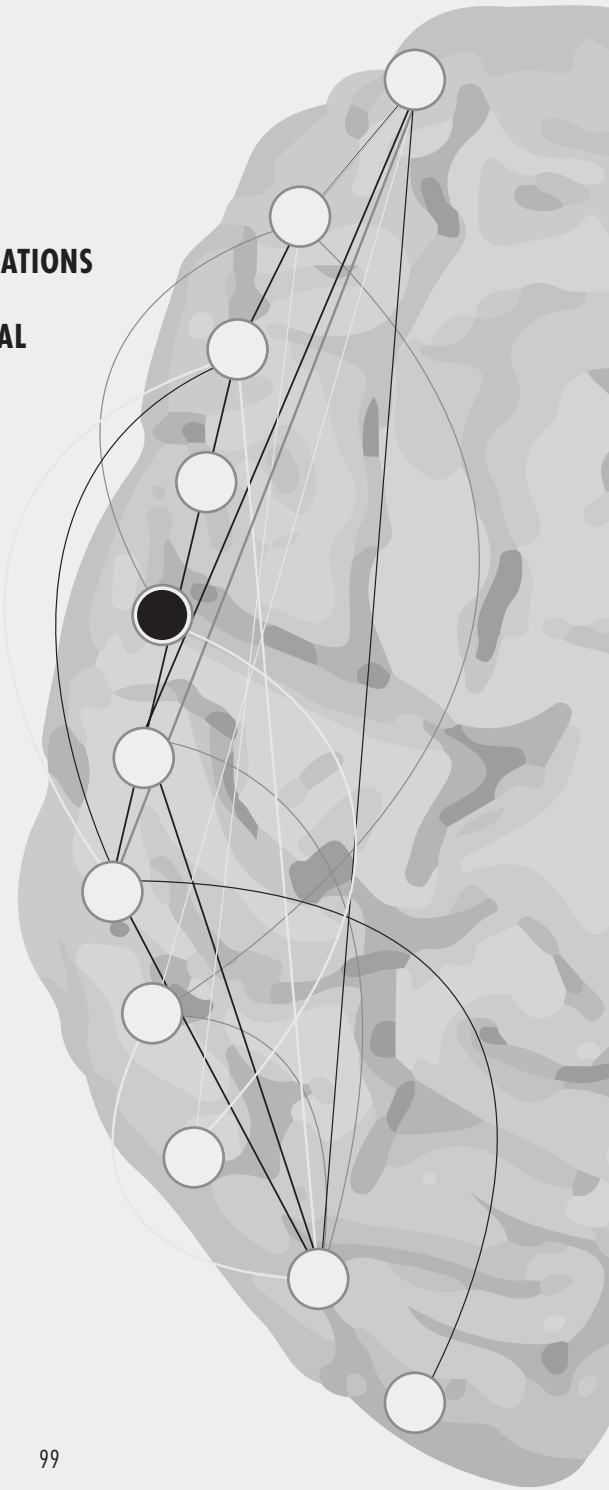


ABSTRACT

Objective: neurophysiological studies have reported functional network alterations in epilepsy, most consistently in the theta frequency band. Highly interconnected brain regions (so-called 'hubs') seem to be important in these epileptic networks. High frequency oscillations (HFOs) in intracranial EEG recordings are recently discovered biomarkers that can identify the epileptogenic area and are thought to result from altered neuronal interactions. We studied whether the epileptogenic zone (identified by HFOs and seizure onset zone) is associated with pathological hubs.

Methods: bilateral depth electrode recordings from the hippocampus and amygdala were available from twelve patients suspected of temporal lobe epilepsy. HFOs, classified as ripples (80-250Hz) and fast ripples (250-500Hz), and epileptiform spikes were marked for all patients in a five-minute epoch of slow-wave sleep. For each channel, we computed hub-measures from a period without epileptiform spikes.

Results: we found that the epileptogenic zone was associated with a decreased hub-value in the theta frequency band. The amount of HFOs, especially fast ripples, was negatively correlated with the hub-value per channel. Results from post-hoc analysis of other frequency bands, particularly the broad- and gamma frequency band, pointed in the same direction as the results for the theta frequency band.

Significance: these findings suggest a pathological functional 'isolation' of the epileptogenic zone in the interictal state.

INTRODUCTION

Partial epilepsy is increasingly seen as a 'network disorder' despite its focal character (Engel et al., 2013; Kramer and Cash, 2012; Richardson, 2012). Temporal lobe epilepsy (TLE) is the most common form of partial epilepsy and often associated with mesial temporal sclerosis (MTS) (Engel, 1996). MTS is characterized by a progressive hippocampal cell loss and, consequently, reorganization of adjacent mesial structures (Bernasconi et al., 2005; Kalviainen et al., 1997) and (extra-) temporal structures (Gross et al., 2006; Mueller et al., 2006; Weber et al., 2007). This reorganization is likely to contribute to epileptogenicity (Heinemann, 2004) with emerging pathological networks (Blumcke et al., 2012).

Studies have used connectivity measures to relate structural abnormalities in TLE with brain functioning. Functional imaging (Bettus et al., 2009; Laufs et al., 2007; Mankinen et al., 2011; Pittau et al., 2012; Voets et al., 2012) and intracranial EEG recordings (Bettus et al., 2008; van Dellen et al., 2009) revealed an altered functional connectivity in the pathological temporal lobe regions and distant, functionally related, regions. A method used to characterize the overall organization of structural and functional networks is network analysis (Bullmore and Sporns, 2009; Reijneveld et al., 2007; Rubinov and Sporns, 2010; Stam and van Straaten, 2012c). Network analysis provides a theoretical framework to model complex systems, such as the brain, as a collection of nodes (i.e. brain regions) and edges (i.e. connections between brain regions). Healthy brain networks have a so-called 'small-world' topology (Bullmore and Sporns, 2009; Stam, 2010): an adequate balance between local segregation (high clustering coefficient) and global integration (short average path length). In partial epilepsy, a disturbed local segregation and/or global integration is present, leading to a less efficient network topology (Horstmann et al., 2010; Liao et al., 2010; van Dellen et al., 2009; Vlooswijk et al., 2011). These changes appear most prominent in the theta frequency band (4-8 Hz) (Bettus et al., 2008; Douw et al., 2010a; Douw et al., 2010b; van Dellen et al., 2012). Highly interconnected nodes within epileptic networks or so-called 'hubs' are thought to reflect pathological tissue that may have a central role in the altered network organization (Wilke et al., 2011) and may be responsible for the fast spreading epileptic activity within a network in the ictal state (Bonifazi et al.,

2009; Morgan and Soltesz, 2008; Ortega et al., 2008a). It is unknown to what extent the epileptogenic zone itself is reflected by local changes in the interictal functional network.

The seizure onset zone (SOZ) and interictal epileptiform spikes (the irritative zone) represent the potential epileptogenic area in clinical practice. Recently, high frequency oscillations (HFOs) have been proposed as a more reliable marker of the epileptogenic zone (Bragin et al., 1999b; Jacobs et al., 2008; Staba et al., 2007; Urrestarazu et al., 2006; Worrell et al., 2004). The surgical removal of regions generating HFOs turned out to be a better predictor of seizure freedom than spikes or the SOZ (Akiyama et al., 2011; Jacobs et al., 2010; Wu et al., 2010). HFOs are divided into ripples (80-250 Hz) and fast ripples (250-500 Hz) and are thought to arise from distinct neurophysiological mechanisms: ripples are associated with the rhythmic firing of interneurons, whereas fast ripples represent co-firing of pathological interconnected principle neurons (Bragin et al., 1999a; Bragin et al., 2002). Another theory suggests that fast ripples result from the out-of-phase firing of neurons (Ibarz et al., 2010). It has been a topic of debate whether HFOs, especially fast ripples, themselves induce a pathological epileptic network or are simply a by-product of epileptogenic tissue. The spatial relation between HFOs and networks organization and the presence of pathological hubs can help to solve this pathophysiological puzzle.

We use a network analytical approach to investigate how the epileptogenic zone is reflected in focal network alterations in patients suspected of TLE. Both HFOs and the SOZ are used to define the epileptogenic zone. We hypothesize that brain tissue associated with the epileptogenic zone, identified by HFOs and the SOZ, is differently connected within the functional network compared to brain regions not associated with the epileptogenic zone.

METHODS

Patient selection

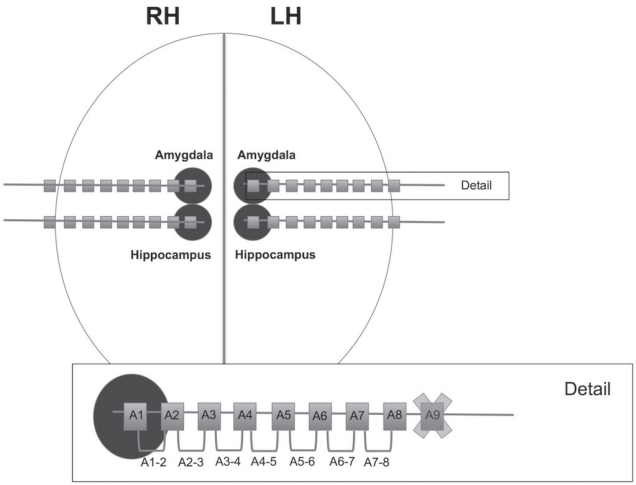
Between 2004 and 2008, 60 patients underwent depth electrode placement as pre-surgical work-up for selective surgery at the Montreal Neurological Institute. The electrophysiological recording was performed for clinical reasons. From this group, patient inclusion criteria for this study were: (1) the availability of bitemporal depth EEG recordings from both the amygdala and hippocampus, and (2) the availability of conclusive clinical intracranial EEG reports. In this

study we chose to focus on the amygdala and hippocampus electrodes only to enable group wise comparison. The Montreal Neurological Institute and the Hospital Research Ethics Committee approved this study. All patients signed an informed consent.

Depth electrode recordings

Between 6 and 10 depth-electrodes were placed stereotactically in each patient, using an image-guidance system (SNN Neuronavigation System, Mississauga, Canada). For this study, we only included the depth-electrodes aimed at the amygdalae and hippocampi in the eventual analysis. The electrodes were manufactured onsite (9 contacts per electrode, contact surface of 0.8mm2) as described earlier (Jacobs et al., 2008; Urrestarazu et al., 2006). The electrode tips were placed in the amygdala and the hippocampus in both hemispheres. A reference electrode was placed intracranially on the parietal lobe. The sample

F1



Schematic representation of selected intracranial depth electrodes

Two depth electrodes that were placed in the right and two in the left amygdala and hippocampus were selected for analyses. Each electrode had nine contacts, of which one (contact 9) was mostly outside the brain and left out of the analysis, because of artifacts. The analyses were performed using bipolar montages rendering seven bipolar channels from eight contacts (see detail). RH=right hemisphere, LH=left hemisphere, A=amygdala. A1=most inner contact. A9=most outer contact.

frequency was 2000 Hz with an amplifier band-pass filter at 0.5-500 Hz. All signals were recorded and viewed using Harmonie (Stellate, Montreal, Canada). For each electrode, seven consecutive bipolar channels were analyzed. The outer channel (bipolar recording 8-9) was excluded from the analysis, because of the presence of artifacts in most patients. Fourteen bipolar channels in each hemisphere were available for analysis for all included patients (figure 1).

Marking events and defining SOZ

Five minutes of slow-wave sleep, interictal depth electrode EEG-recordings at least four hours away from any seizure activity were selected. Fast ripple, ripple and spike events were manually marked. We selected the events from slow wave sleep, as the rates of HFOs and spikes are highest during slow-wave sleep (Bagshaw et al., 2009). To accomplish consensus about the markings, two reviewers (J.J. and M.Z.) separately marked the first minute of each EEG-recording. An inter-observer agreement was calculated using Cohen's kappa coefficient. A Cohen's kappa coefficient of >0.5 was interpreted as a sufficient agreement. We identified HFOs by displaying data with the maximum time resolution (0.6 s, 1200 Hz) and splitting the computer display vertically as previously described (Jacobs et al., 2008; Jacobs et al., 2009). On the left display, an 80 Hz high-pass filter was used, an on the right display a 250 Hz high-pass filter. An event visible on the left display, and not on the right display, was marked as a ripple. An event visible on the right display was considered a fast ripple. We marked events as HFOs if at least four consecutive oscillations were present. Two events were considered separate, when at least two non-HFO oscillations were seen in between. Considering the variability between patients in the number of HFOs, we expressed the number of HFOs per channel in a percentage to optimize group wise comparison. In this normalization the number of HFOs per channel was divided by the number of HFOs per patient. The SOZ was identified in the EEG-channels showing the first ictal activity during EEG-recording by a clinical neurophysiologist (F.D), who was blinded for the selection of HFOs and spikes.

Functional connectivity

We selected four epochs of 8.19 seconds each (4096 samples) per patient during awake, eyes closed and rest conditions to assure stable EEG dynamics

for the calculation of functional connectivity (Douw et al., 2010a; van Dellen et al., 2012; van Diessen et al., 2013c). The depth EEG was recorded at 2000 Hz and down sampled to 500 Hz. In the selected epochs during wakefulness with eyes closed, some HFOs occurred in several patients, especially in the deeper hippocampal channels, because their rate of occurrence often exceeded 1 per second. We focussed on selecting epochs without epileptiform spikes, free of motion-induced artifacts and if possible with a low number of HFOs, especially those that occurred in only one channel at a time. Next, epochs were converted to ASCII files for further analysis with Brainwave software (available at http://home.kpn.nl/stam7883/brainwave.html). Functional connectivity was computed from each selected epoch by means of the Phase Lag Index (PLI) (Stam et al., 2007b). A PLI value per channel was obtained from each epoch and the average PLI (over four epochs) was used for further analysis. The PLI measures the phase coupling between two signals, while precluding volume conduction as a confounding factor by disregarding a phase difference of zero. As a result, PLI is less sensitive to confounding by common sources than other functional connectivity measures (Stam et al., 2007b). The PLI ranges from 0 to 1. A PLI of 0 indicates no phase coupling between signals, or coupling with a phase difference centered on 0 ± π radians. A PLI of more than 0 indicates a certain phase coupling. An index of the asymmetry of the phase difference distribution can be obtained from a time series of phase differences $\Delta\Phi(t_k)$ in the following way: $PLI = \left|\left\langle sign\left[\Delta\Phi(t_k)\right]\right\rangle\right|$ in which <...> denotes the average over time *t*. A detailed description of the calculation and specifications of PLI can be found elsewhere (Stam and Reijneveld, 2007). The strength of the PLI was calculated for each possible pair of channels, resulting in a 14 by 14 matrix for each hemisphere. Interhemispheric connectivity was not considered. Functional connectivity measures were calculated and filtered for six different frequency bands: broad (0.5-50 Hz), delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz) and gamma (30-50 Hz).

Network analysis

Based on the functional connectivity between each pair of channels, we constructed weighted networks. In these networks, the channels are represented as nodes (or vertices) and the PLI between two electrodes as edges. From these networks, global and local characteristics can be inferred (Bullmore and Sporns,

2009; Reijneveld et al., 2007; Rubinov and Sporns, 2010; Stam and van Straaten, 2012c). We focused on the hub-measures of each node in the network to investigate how HFOs are related to alterations in local network topology. Several network hub-measures exist to calculate the 'node centrality' (Joyce et al., 2010; Zuo et al., 2012). The most straightforward is called 'strength'. In a weighted network, it sums the weights of edges connected to each node. A high strength means that the node has strong connections to many other nodes in the network. A more advanced method to estimate the centrality of a node is eigenvector centrality (EC). EC is increasingly used in neurosciences due to a more accurate estimation of node centrality in combination with low computational costs (de Haan et al., 2012; Hardmeier et al., 2012; Lohmann et al., 2010). Like strength, EC determines the importance of a node on the basis of the number and weight of edges to other nodes. In addition, EC takes into account how those other nodes are connected. EC is thus a more accurate measure of node centrality as it takes into account the node centrality of immediate neighbouring nodes. Mathematical calculations for network construction, strength and EC are presented in the next section.

Mathematical calculations for network construction, strength and EC

Network construction: for each patient, we constructed a weighted undirected network per hemisphere, described by the graph *G = (N, W)*, where N is the set of all intracranial EEG channels per hemisphere (14 channels) and $W = \{w_{ij}\}$ is the *N x N* symmetric weight matrix, where $w_{ii} = 0$ and w_{ij} the PLI determined between channel *i* and *j*.

Strength: sums the weights of edges connected to each node. The strength for node *i* was defined as: $d_i^w = \sum_{j \neq i}^{n} w_{ij}$

Eigenvector centrality: is based on the greatest eigenvector of the weight matrix W (Newman, 2007). The eigenvectors of the weighted matrix indicate the nodes that show many connections with most other nodes in the network. It specifically favors nodes that are connected to nodes that are themselves central within the network (Bonacich, 1987). If λ is the largest eigenvalue and ec^w the corresponding eigenvector, then $W \times ec^w = \lambda \times ec^w$ or similar $ec^w = \frac{1}{\lambda} W \times ec^w$ and $ec^w = \mu \sum_{j}^{n} w_{ij} ec_j^w$, where $\mu \frac{1}{\lambda}$ is the proportionality factor so that ec_i^w is proportional to the sum of connectivity scores of all nodes connected to it.

Statistical analysis

First, we explored the relation between differences in node centrality (by means of strength and EC) and the number of HFOs and spikes. Second, we investigated the differences in strength and EC, between EEG channels associated with SOZ (SOZ-channels) versus EEG channels not associated with the SOZ. In all analyses, we used a linear mixed model (LMM) with 'patients' and 'channels' as random factors to correct for interpatient correlations. In the analysis where we explored the relation between differences in node centrality and the number of HFOs and spikes, we used 'fast ripples', 'ripples' and 'spikes' as fixed factors (for each fixed factor, we ran an independent analysis). Similarly, we used 'SOZ' as fixed factor for exploring the relation between node centrality and the SOZ. All statistical analyses were performed using R (R package *nlme*). Analyses were primarily performed in the theta frequency band, as epilepsy studies repeatedly identified alterations in network topology in the theta frequency band. Consecutively, we explored other frequency bands. A p-value below 0.05 was considered significant.

RESULTS

Patient characteristics

Twelve patients met our inclusion criteria (mean age 38.4 years, 8 men and 4 women). Eventually, nine patients received a diagnosis of TLE, one patient had multifocal epilepsy and two patients had both TLE and frontal lobe epilepsy. The clinical information from the last visit of the patient to the clinic was used for classifying the patient's outcome after surgery according to Engel's classification (Engel, 1993). Two patients had outcome class 1, which was defined as 'seizure free'; three patients had outcome class 2, with only occasionally disabling seizures; three patients had outcome class 3, meaning that a worthwhile improvement was reached and one patient did not have any improvement after surgery (class 4). In two patients, no surgery was performed. Clinical details are presented in table 1.

HFOs/spikes and network measures

In all the patients, the inter-observer agreement for marking the HFOs and spikes resulted in a Cohen's kappa coefficient of >0.5 and was considered a sufficient agreement. The patterns of fast ripples, ripples and spikes across channels are shown in figure 2. No differences were found between channels in strength or EC (figure 3). Significant negative associations were found between EC in the theta frequency band and the HFOs (ripples: $p<0.05$, $t=-2.00$; fast ripples $p<0.02$, $t=-2.38$) (figure 4). No significant associations were found between EC in the theta frequency band and spikes ($p=0.21$, $t=-1.26$). To illustrate the relation between EC and HFOs we schematically plotted both variables in a network representation in figure 5. For strength in the theta frequency band, no significant associations were found with HFOs or spikes. Post-hoc analysis in other frequency bands revealed significant negative correlations between hub-measures in the broad and gamma frequency bands and the HFOs/spikes. A significant negative correlation was found between fast ripples, and strength in the alpha frequency band (details in table 2).

SOZ and network measures

Channels associated with the SOZ showed a significantly decreased strength and EC in theta-frequency band compared to channels not associated with the SOZ (strength and SOZ: $t=-3.76$, $p<0.01$; EC and SOZ: $t=-2.71$, $p<0.01$). A schematic illustration is provided in figure 6. In other frequency bands, significant differences were found for strength in broadband frequency and for EC in the beta- and gamma frequency band (table 3).

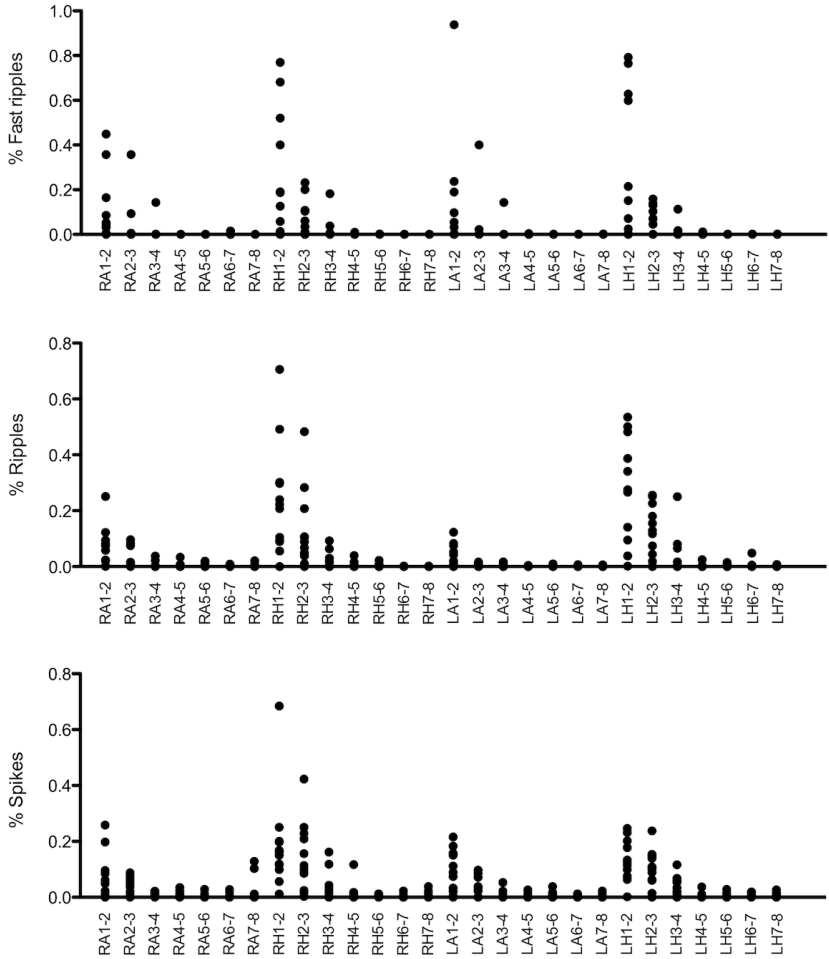
T1

Patient	Gender	Age (yrs)	Type of epilepsy	MRI findings	SOZ (per included channel)	Resected area
1	Male	46	bilateral TLE	Bilateral hippocampal atrophy	LA1-2, LH1-3, RA1-3, RH1-3	RSAH, RantT
2	Female	30	bilateral TLE and FLE	Left temporal atrophy	LA1-3, LH1-3	RSAH
3	Female	45	bilateral TLE	No structural abnormalities	LA1-2, LH1-3, RA1-2, RH1-3	LSAH
4	Male	29	bilateral TLE	Right hippocampal atrophy	LA1-2, LH1-2, RA1-8, RH1-4	RSAH
5	Male	18	bilateral TLE	Left hippocampal atrophy	LA1-2, LH1-3, RA1-3, RH1-3	RSAH
6	Female	48	bilateral TLE	Right hippocampal and amygdala atrophy	LA1-3, LH1-2, RA1-2, LH1-3	No surgery
7	Male	46	bilateral TLE	Right hippocampal malrotation	RA1-8, RH1-3	RSAH
8	Male	33	left FLE and TLE	Abnormalities in right superior frontal gyrus, left orbito-frontal cortex and temporal lobe	LA4-8	LantT
9	Female	42	multifocal	Right temporal atrophy and atypical subcortical white matter anomalies	LA1-2, LH1-3, RA1-2, RH1-2	RSAH
10	Male	44	bilateral TLE	Left temporal dysplasia	LA1-3, LH1-3	LSAH
11	Male	25	bilateral TLE	Bilateral periventricular nodular heterotopias	LA1-2, LH1-4, RH1-3, RA1-3	RSAH
12	Female	55	bilateral TLE	Left temporal atrophy	LA1-3, LH1-3	No surgery

Patient characteristics

TLE=temporal lobe epilepsy; FLE=frontal lobe epilepsy; SOZ=seizure onset zone; LA=left amygdala; RA=right amygdala; LH=left hippocampus; RH=right hippocampus. Channel numbers in the SOZ column corresponds to anatomical location of electrode contacts (see figure 1). For example: RA1-2 = innermost bipolar channel of right amygdale rod. RSAH=right selective amygdalo-hippocampectomy, LSAH=left selective amygdalo-hippocampectomy, RantT=right anterior temporal resection, LantT=left anterior temporal resection. N.a.=not applicable

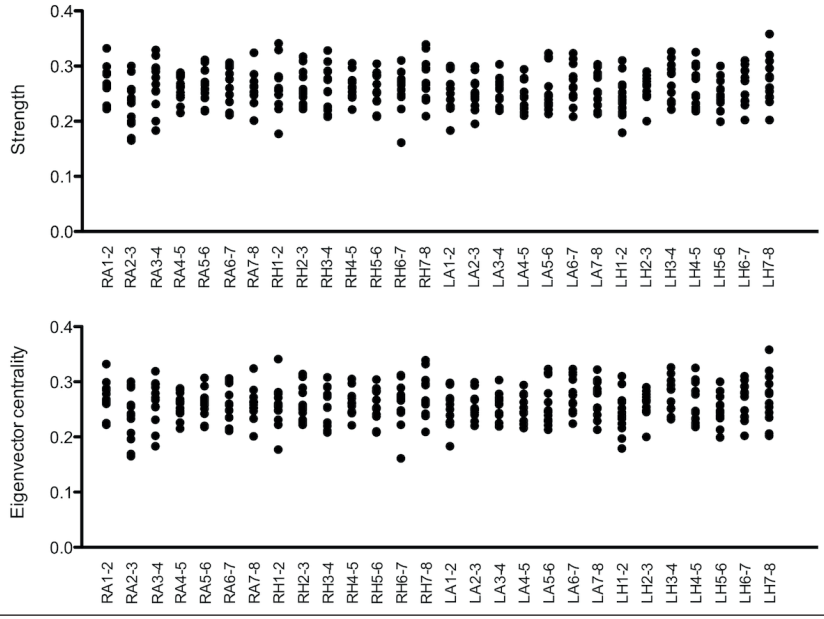
F2



HFOs and spikes per channel

Percentage of fast ripples, ripples and spikes per channel for the included twelve patients. Each dot represents the percentage of events in a channel per patient, so there are 12 dots per channel name. RA=right amygdala; RH=right hippocampus; LA=left amygdala; LH=left hippocampus. Channel number corresponds to anatomical location (see figure 1). For example: RA1-2=innermost bipolar channel of right amygdala electrode.

F3



Network measures per channel

Strength (top) and eigenvector centrality (bottom) per channel in theta frequency band. Each dot represents the centrality value per patient. Channel number corresponds to anatomical location (see figure 1). For example: RA1-2=innermost bipolar channel of right amygdala electrode. No channel bound distribution exist for either strength or eigenvector centrality.

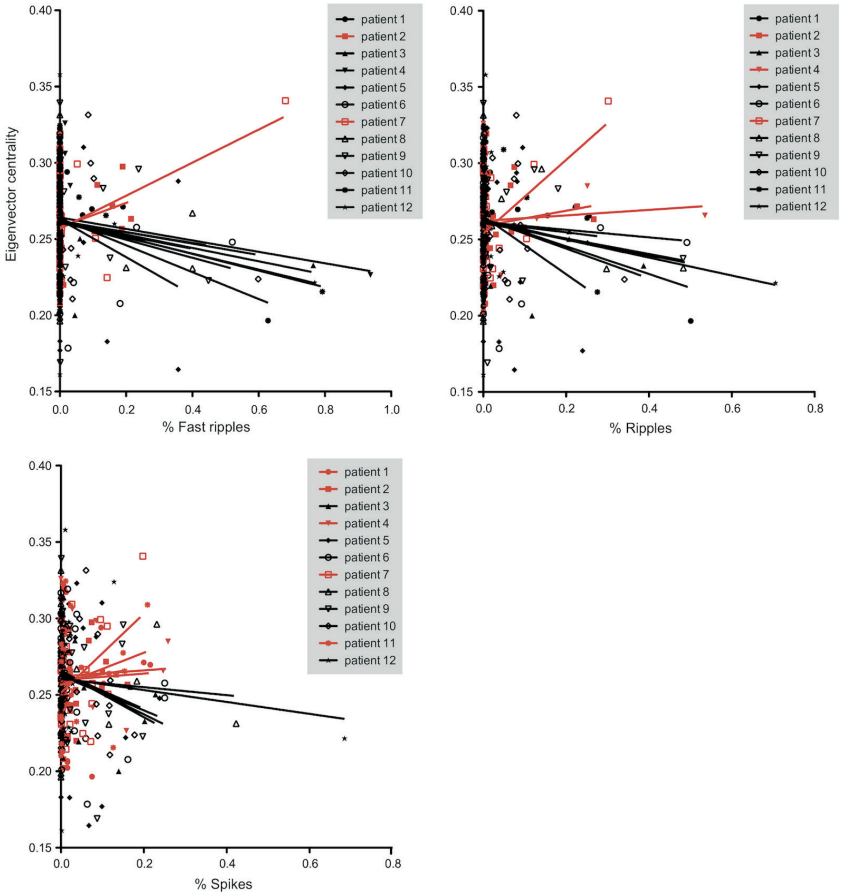
T3

Frequency band	Strength		Eigenvector centrality	
	t-value	p-value	t-value	p-value
Broad band	**-2.55**	**0.011**	-1.15	0.253
Delta band	0.001	0.999	-0.17	0.866
Theta band	**-3.76**	**0.001**	**-2.71**	**0.007**
Alpha band	-0.64	0.517	-0.59	0.554
Beta band	-1.33	0.185	**-2.15**	**0.033**
Gamma band	-0.80	0.424	**-2.83**	**0.005**

Associations between SOZ network measures

(strength and eigenvector centrality). (*) Significant ($p<0.05$). (**) Significant after correcting for multiple post-hoc comparisons (false discovery rate test).

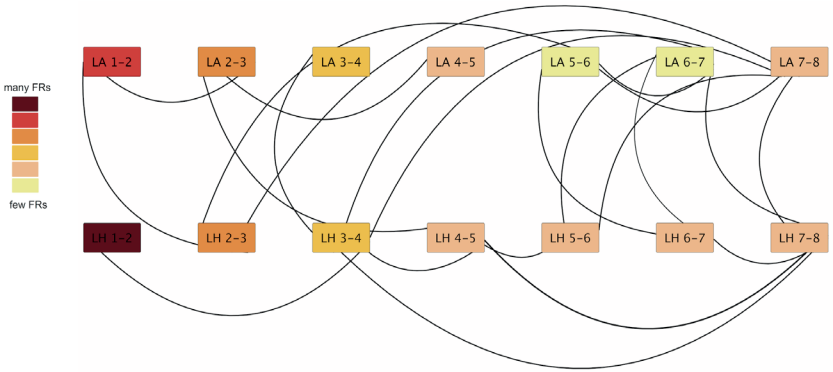
F4



Association HFOs/spikes and eigenvector centrality

Associations between eigenvector centrality in theta frequency band and percentage of fast ripples (left graph), ripples (middle graph) and spikes (right graph) per patient. Red lines indicate positive associations, black lines indicate negative associations.

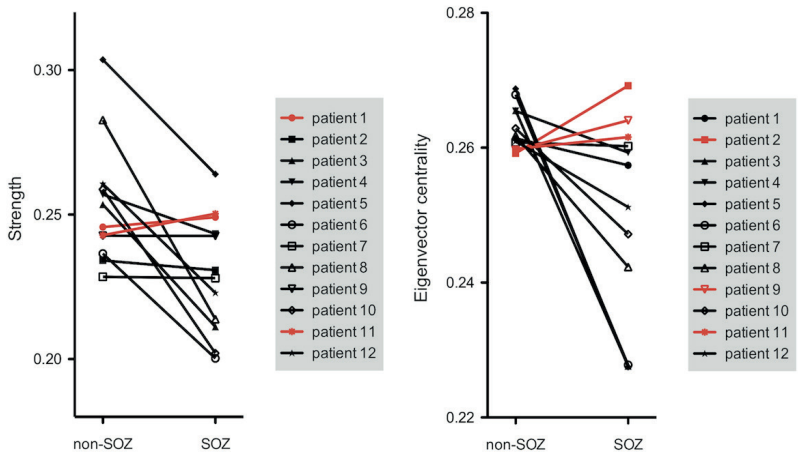
F5



Functional network based on intracranial recordings

Schematic illustration of constructed functional network ((average values (n=12), left hemisphere, theta frequency band)). Color of electrode corresponds to the normalized number of fast ripples (FRs). Lines represent functional connections between two electrodes (threshold=PLI >0.25). What can be seen is that some electrodes, like LA1-2 and LH1-2 have only few connections, while the amount of fast ripples is high, while other electrodes, like LA5-6 and LH7-8 have many strong connections. Even more, these channels are connected to channels that are themselves connected to many other channels. LA=left amygdala, LH=left hippocampus.

F6



Association centrality measures and SOZ

Relation between centrality measures and channels associated with SOZ versus channels not associated with SOZ (non-SOZ). Left figure: strength. Right figure: eigenvector centrality. Each line represents one patient. Red lines indicate positive associations, black lines indicate negative associations.

DISCUSSION

In this explorative network study we analysed the relation between the epileptogenic zone (identified by HFOs and SOZ) and local network alterations in patients suspected of TLE. We found a negative association between the number of HFOs and EC in the theta frequency band that was the clearest from fast ripples. We did not find this association for spikes. We found no relation between strength and HFOs or spikes in the theta frequency band. Both strength and EC were significantly decreased in the theta band in the channels associated with the SOZ. Significant results for other frequency bands pointed in the same direction as the results for the theta frequency band. Based on previous research we primarily expected to find associations in the theta frequency band, as it has repeatedly been associated with network alterations in epilepsy". Nevertheless, the association between fast ripples and EC in the theta frequency band was only nearly significant after correcting for multiple post-hoc comparisons ($p=0.054$). Together, these findings suggest that the epileptogenic zone is spatially distinct, indicating a functional isolation and that the level of isolation further increases when more HFOs, especially fast ripples, are present. Interestingly, previous research shows that seizure-generating areas are less connected to other brain regions suggesting a similar relative isolation of the epileptogenic zone (Warren et al., 2010).

What does this functional isolation tell us about the pathophysiology of HFOs and the differences between fast ripples, ripples and spikes? As mentioned earlier, ripples and fast ripples are thought to arise from distinct neurophysiological mechanisms and it has been a subject of debate whether fast ripples result from hypersynchronous activity or out-of-phase firing (Bragin et al., 1999a; Bragin et al., 2002; Ibarz et al., 2010). Furthermore, in vitro studies have shown distinct features of ripples and fast ripples: healthy neurons are capable of generating HFOs at ripple frequency under epileptic conditions (like increasing extracellular potassium), but not at fast ripple frequency (D'Antuono et al., 2005; Dzhala and Staley, 2004; Jiruska et al., 2010). The presence of fast ripples probably reflects true epileptogenic reorganization, while ripples (and spikes) rather result from abnormal firing within a possibly normal functional network. Our results underline this theory, as the functional isolation of the epileptogenic zone was where fast ripples were most consistently found. Converging evidence points to the existence of more than one type of ripple frequency oscillations (Engel et al., 2009; Jefferys

et al., 2012; Nagasawa et al., 2012). A recent study showed that ripples associated with the presence of interictal epileptiform discharges in patients with neocortical epilepsies are more specific markers for the epileptogenic zone than ripples not associated with interictal epileptiform discharges (Wang et al., 2013). This could explain the similar, but weaker, negative association between the number of ripples and EC. Future research should be directed at finding whether ripple frequency oscillations associated with the presence of interictal epileptiform discharges reflect similar neuronal mechanisms as fast ripples.

As in previous network studies (Bettus et al., 2008; Douw et al., 2010a; Douw et al., 2010b; van Dellen et al., 2012), we found alterations in functional network properties in the theta frequency band. Our study thus contributes to the idea that alterations in functional networks in epilepsy might be particularly present in the theta frequency band. Further investigations, however, revealed similar relations between node centrality and HFOs, spikes or SOZ in other frequency bands, especially in relation to broad- and gamma frequency band. It is unclear how the results in other frequency bands should be interpreted. Although previous studies have shown that gamma band oscillations are also associated with the epileptogenic zone (Allen et al., 1992; Jirsch et al., 2006; Traub et al., 2001; Worrell et al., 2004), only one study reported network alterations in the gamma frequency band in epilepsy (Wilke et al., 2011). The gamma frequency band is closest to the ripple band and, as a result, the presence of ripples might influence the network alterations in the gamma frequency band. Furthermore, the relation between network alterations and the gamma frequency band could be underreported in epilepsy as many neurophysiological studies have used surface recordings for network construction. Muscle activity is known to contaminate neurophysiological surface recordings in higher frequencies (Whitham et al., 2007). Consequently, network analysis in higher frequency bands based on surface recordings is less reliable. More research is therefore needed to investigate whether the gamma frequency band is equally important for epilepsy related network changes as the theta frequency band. Significant correlations in the broad frequency band are likely to reflect the aforementioned bands and are thus less specific. Although we repeatedly found significant negative correlations at the group level, figures 4 and 6 illustrate that a few patients showed a positive association between the epileptogenic zone and network analytical centrality-measures. For example, patient 2 showed a positive association between the eigenvector centrality in the

theta frequency band and the number of fast ripples, ripples and spikes. A similar association was found for the eigenvector centrality and the SOZ. Possibly, an epileptogenic zone outside the investigated area could be accountable for this finding as patient 2 was diagnosed with both TLE and FLE. Patient 11 showed a positive correlation between the seizure onset zone and the eigenvector-centrality. This patient had bilateral periventricular heterotopias and a poor outcome (Engel 4), so the primary seizure onset zone might have been missed. Patient 9 showed a positive correlation between the seizure onset zone and the eigenvector centrality as well. This patient was suspected of having multifocal epilepsy. Patient 7 showed a positive correlation between HFOs (fast ripples and ripples) and hub-measures, but no positive correlation between SOZ and hub-measures. This patient was suspected of having pure MTLE and neither a dual pathology nor a poor surgical outcome could account for the positive association. These explanations will remain speculative until they are investigated in larger cohorts.

Only few studies have addressed local functional network alterations in relation to the epileptogenic zone in graph theoretical terms. Nonetheless, one study is available that investigated network properties during ictal activity in patients with intractable, neocortical-onset epilepsy by computing centrality measures from intracranial EEG grids (Wilke et al., 2011). Time-varying centrality values were available for each channel before, during and directly after a seizure. Directly after seizure onset, centrality measures of 'active channels' (associated with SOZ) were decreased for all frequency bands and gradually increased after seizure cessation. In the pre-ictal and post-ictal state, these active channels showed increased centrality measures compared to 'inactive channels'. Interestingly, the increased node centrality in the epileptogenic zone found by Wilke and colleagues tend to contradict our results. Possibly, differences are related to patient population (neocortical epilepsy versus mainly TLE patients), the chosen 'network of interest' (intracranial grids versus depth electrodes), and methodological differences (other functional connectivity and node centrality measures). Also, functional networks in epilepsy seem to have different properties in the interictal state compared to the pre- and post-ictal state (Ponten et al., 2007).

A potential limitation of our study is that network construction was derived from bipolar montages. As a consequence, most electrodes were sampled twice, while the outermost contacts are sampled once. Theoretically this could influence the connectivity measures. However, by using PLI, a connectivity measure that is less

sensitive to confounding by common sources (Stam et al., 2007b), we minimized this problem. Furthermore, it remains unclear if the decreased node centrality in channels with HFOs is causally related to the actual presence of HFOs or to the anatomical region. Most of the patients suffered from bilateral TLE and have HFOs/spikes in both hemispheres and were primarily identified in the deepest EEG-channels (located in either the amygdala or hippocampus). We did not, however, find that specific channels had higher or lower strength or EC values (see figure 3): an anatomical bias for the node centrality thus seems not likely. Furthermore, the negative association between the number of HFOs and centrality advocates a true relation. Larger studies are needed to verify these findings.

Although it remains difficult to make any assumptions on the causal relation between the presence of HFOs and the functional isolation that we found in our study, a possible explanation could be that the functional isolation in itself is the cause of HFOs. Previous research has shown that HFOs could be seen as a marker of diseased tissue with a different 'eigenfrequency' compared to healthy tissue (Buzsaki and Draguhn, 2004). Possibly, the functional isolation causes this eigenfrequency to change. The functional isolation of a relatively small number of neurons in the epileptogenic zone could be responsible for the maintenance of the oscillation at a high frequency (this in contrast to an ictal state, which normally starts with rhythmic discharges at a high frequency and gradually decrease as a larger area gets involved). This explanation is speculative and needs further investigation with neuronal modeling studies.

Accurate localization of the epileptogenic zone forms a crucial part in epilepsy surgery to gain seizure freedom. Over the last five years, much research has been dedicated to HFOs as an improved biomarker for the epileptogenic zone. Although many clinical studies have retrospectively shown the added value of HFOs in epilepsy surgery (Jacobs et al., 2009; Ochi et al., 2007; Wu et al., 2010), little was known about the relation of HFOs and topological properties of functional networks. This study establishes a link between HFOs and an altered network topology in epilepsy, phenomena that have been studied separately. Network measures might be useful to delineate the epileptogenic tissue and predict seizure freedom after resection (Wilke et al., 2011). The combination of both approaches could become useful in the pre-surgical evaluation to determine the resected area in patients with intractable epilepsy. More studies are needed to investigate this potential and to clarify the relation between HFOs and alterations in network properties.

CHAPTER 6

DOES SLEEP DEPRIVATION ALTER FUNCTIONAL ELECTROENCEPHALOGRAPHY NETWORKS IN CHILDREN WITH FOCAL EPILEPSY?

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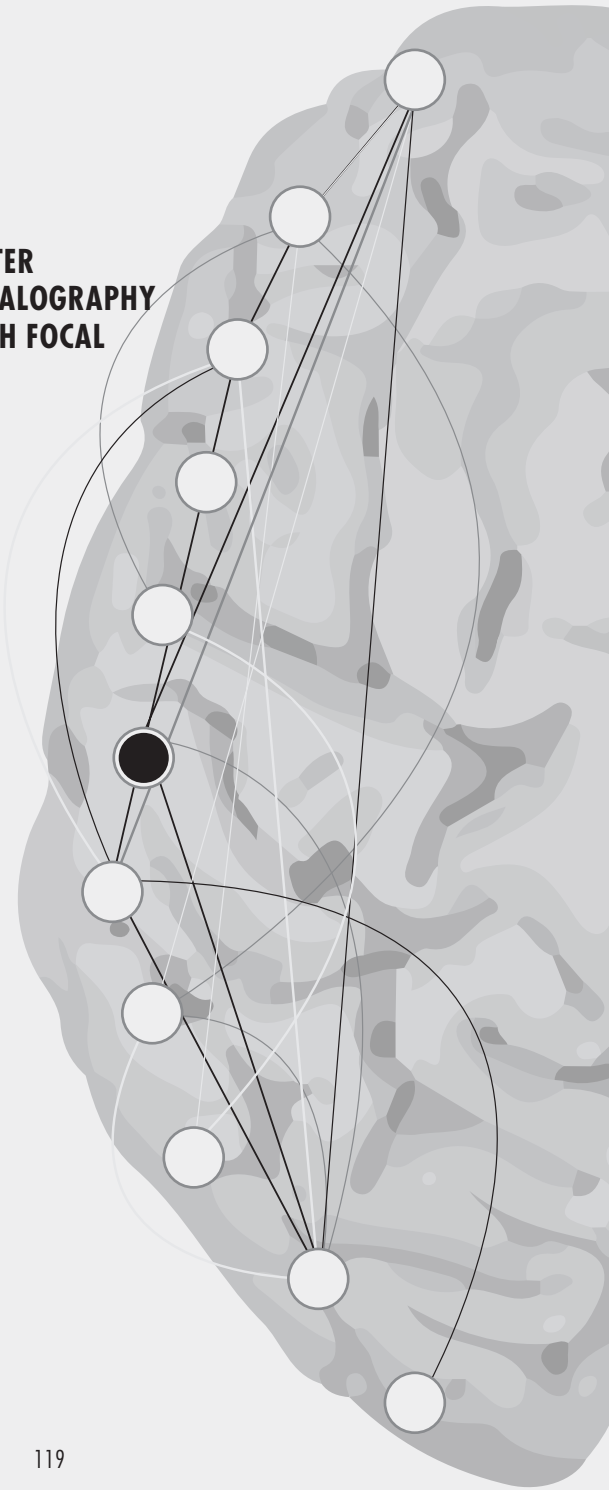


ABSTRACT

Objective: electroencephalography (EEG) recordings after sleep deprivation increase the diagnostic yield in patients suspected of epilepsy if the routine EEG remains inconclusive. Sleep deprivation is associated with increased interictal EEG abnormalities in patients with epilepsy, but the exact mechanism is unknown. We aimed to characterize the effect of sleep deprivation on the interictal functional network organization using a unique dataset of paired routine and sleep deprivation recordings in patients and controls.

Methods: we included children referred to the first seizure clinic of our center with suspected new onset partial epilepsy in whom a routine interictal and a sleep deprivation EEG (SD-EEG) were performed. For both time points weighted functional networks were constructed based on interictal artifact free time-series. Routine and sleep deprivation networks were characterized at different frequency bands using minimum spanning tree (MST) measures (leaf number and diameter) and classical measures of integration (path length) and segregation (clustering coefficient).

Results: twenty-one children with partial epilepsy were included. Seventeen children, in whom the diagnosis of epilepsy was excluded, served as controls. A significant interaction was found for leaf number and diameter between patients and controls after sleep deprivation: patients showed a shift towards a more path-like MST network whereas controls showed a shift towards a more star-like MST network.

Significance: a shift towards a more path-like MST network organization after sleep deprivation in patients is in accordance with previous studies showing a more regular network organization in the ictal state. Furthermore, this change in network organization might relate to the increased epileptiform abnormalities found in patients after sleep deprivation. Finally, MST measures were more sensitive in detecting network changes as compared to the classical measures of integration and segregation.

INTRODUCTION

An interictal electroencephalogram (EEG) is routinely acquired in patients suspected of epilepsy to support the clinical diagnosis. Nevertheless, the interictal EEG recording often lacks epileptiform abnormalities or is insufficient to determine classification of the epilepsy syndrome. EEG recordings after sleep deprivation (SD-EEG) improve the diagnostic yield of interictal EEG recordings (Malow, 2004; Smith, 2005; Wirrell, 2010). In the past, debate has centered on the question whether it is the recording of sleep itself or sleep deprivation that promotes the increased presence of epileptiform abnormalities and thereby its sensitivity. Converging evidence exists that SD-EEG recordings improve the detection of epileptiform abnormalities and help to determine classification of the epilepsy syndrome, independently of the presence of sleep during the EEG recording (Ellingson et al., 1984; Fountain et al., 1998).

The mechanism for the increased presence of epileptiform abnormalities after sleep deprivation, however, remains unclear. This clinical phenomenon might originate from an altered functional network organization after sleep deprivation (Koenis et al., 2013). Brain functioning is increasingly perceived as a complex network of interacting brain regions (Bassett and Bullmore, 2009; Bullmore and Sporns, 2009; Stam and van Straaten, 2012b), which can be studied using, among others, EEG recordings. Quantification of connections between brain regions enables characterization of functional network organization by means of network analysis. Network analysis reduces complex systems, such as the brain, to a collection of nodes (brain regions) and edges (connections between brain areas). Previous research in epilepsy has focused on two network measures to characterize global changes and efficiency in network organization: the clustering coefficient (a measure of segregation) and path length (a measure of integration). A less efficient interictal network organization was repeatedly identified in patients with epilepsy, due to a disturbed local segregation or global integration (Bernhardt et al., 2011; Douw et al., 2010b; Horstmann et al., 2010; Liao et al., 2010; Vaessen et al., 2012; van Dellen et al., 2012; van Diessen et al., 2013c; Vlooswijk et al., 2011).

Network analytical studies require various choices and assumptions that influence the outcome (van Wijk et al., 2010). For example, it remains unclear how differences in network density (i.e. number of connections) should be

taken into account when comparing networks between groups. A solution for this problem might be offered by the so-called minimum spanning tree (MST) approach. The MST approach creates a unique network based on the weighted connections between nodes. Furthermore, it connects all the nodes in the network without forming cycles and thereby reducing the connection costs. In this way, networks are obtained with an identical number of connections and thereby facilitating comparison between groups by taking only the most important connections into account. An increasing number of neuropsychiatric studies have already successfully used this approach to characterize networks and to improve group comparisons (Boersma et al., 2013; Lee et al., 2006; Lee et al., 2010; Ortega et al., 2008a; Schoen et al., 2011).

In this study, we investigated whether alterations in network organization after sleep deprivation were different between patients and controls. Considering the increased presence of epileptiform abnormalities after sleep deprivation, we hypothesized to find a deviant network organization in patients compared to controls after sleep deprivation. In addition, to investigate an added value of the MST approach, we compared the results of standard weighted network analysis (clustering coefficient and path length) with the MST approach.

MATERIALS AND METHODS

Patients

Children who visited the outpatient First Seizure Clinic of the University Medical Center Utrecht, the Netherlands, between January 2006 and December 2012 were eligible for our study. We included children in whom both a routine EEG and SD-EEG recording was performed for clinical reasons. We accepted a maximum interval of nine months between routine EEG and SD-EEG since aging is associated with alterations in functional network organization (Boersma et al., 2011). Children in whom the diagnosis of epilepsy was definite – as judged by the occurrence of multiple seizures, their semiology, and supported by neurophysiological recordings or neuroimaging – were compared to a control group. The control group consisted of children who were referred with clinical events suspected of seizures but in whom the diagnosis of epilepsy was eventually excluded, based on expert opinion, on clinical follow-up, and on the results of ancillary investigations, including routine and SD-EEG recordings. For both groups, we excluded children with neurological or psychiatric co-

morbidities, including the presence of developmental delay of unknown origin. The institutional ethical committee approved the study and concluded that the Dutch Medical Research Involving Human Subjects Act did not apply, and written informed consent was not required.

Data acquisition and selection

The EEG was recorded at 21 scalp electrodes (Fp1, Fp2, F8, F4, Fz, F3, F7, A2, T8, C4, Cz, C3, T7, A1, P8, P4, Pz, P3, P7, O1 and O2), according to the international 10-20 system (SystemPlus Evolution, Micromed), against G2 as a reference electrode and referenced to an average montage for further analysis. Impedance of each electrode was kept below 5 kΩ. Data was high- and low-pass filtered at 0.5 and 70 Hz, respectively. The sampling frequency was 512 Hz.

Four epochs of 8.19 seconds (that is, 4096 samples) were selected during awake and eyes-closed condition and visually inspected by [EvD]. In order to investigate the actual contribution of sleep deprivation on functional networks, the epochs from SD-EEG recordings were selected before sleep and drowsiness, when an occipital alpha rhythm was still present and slow eye-movements were absent. Epochs were chosen free of epileptiform abnormalities, abnormal slowing and electrocardiographic or motion-induced artifacts, for which two frontoparietal and basal temporal electrodes (Fp1, Fp2, A1, and A2) were excluded. The epochs were independently re-inspected by a clinical epileptologist [FEJ] on artifacts and epileptiform abnormalities. Finally, the selected EEG epochs were converted to ASCII files for further analysis.

Evaluation of added clinical value sleep deprivation EEG

EEG recordings after sleep deprivation were performed to improve the diagnostic yield. We considered an SD-EEG of added clinical value when the SD-EEG recording provided: (1) new information regarding localization of the epileptogenic focus and (2) additional information regarding spreading of epileptiform activity. A confirmation of the clinical findings of the first EEG recording was not considered as added value.

Functional connectivity

Functional connectivity between electrodes was computed from each selected epoch by means of the Phase Lag Index (PLI) (Stam et al., 2007b) (Brainwave software; http://home.kpn.nl/stam7883/brainwave.html version 0.9.116 authored by [CJS]). A PLI value for each pair of electrodes was computed for each epoch and the average PLI (over four epochs) was used for further analysis. The PLI was calculated separately for the following frequency bands: delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-13 Hz) and beta (13-30 Hz). PLI is a quantitative measure for the phase coupling between two signals and ranges from 0 to 1. During the calculation the influence of volume conduction is diminished by disregarding phase differences of zero. PLI is less sensitive for confounding by common sources than other functional connectivity measures (Stam et al., 2007b). A PLI value of 0 indicates no phase coupling between signals, or coupling with a phase difference centered on $0 \pm \pi$ radians. A PLI > 0 indicates the presence of phase coupling. An index of the asymmetry of the phase difference distribution can be obtained from a time series of phase differences $\Delta\Phi(t_k)$ in the following way: $PLI = \left|\left\langle sign\left[\Delta\Phi(t_k)\right]\right\rangle\right|$ where $\Delta\Phi$ is the difference between instantaneous phases for two time-series $[-\pi, \pi]$, t_k are discrete steps, and in which < > denotes the average over time *t*. A more detailed description of the calculation and specifications of PLI can be found elsewhere (Stam and Reijneveld, 2007).

Network analytical measures

Network construction: for each dataset, we constructed a weighted undirected network, described by the graph *G = (N, W)*, where N is the set of all 17 EEG electrodes and $W = \{w_{ij}\}$ is the $N \times N$ symmetric weight matrix, where $w_{ii} = 0$ and w_{ij} the PLI determined between node *i* and *j*. Global network properties were quantified via weighted clustering coefficient and path length. We repeated the construction of functional networks and analysis for different frequency ranges.

Weighted clustering coefficient: the clustering coefficient is a measure of degree to which nodes in a graph tend to cluster together. We used the weighted clustering coefficient C as described in (Stam et al., 2009). To calculate C from weighted networks, the weights between node *i* and other nodes *j* should be symmetrical ($w_{ij} = w_{ji}$) and $0 \leq w_{ij} \leq 1$. These conditions are fulfilled when using PLI as weight definition. The (weighted) C of node *i* is then defined as

$$C_i^w = \frac{\sum_{k\neq i}\sum_{\substack{l\neq i \\ l\neq k}} w_{ik}w_{il}w_{kl}}{\sum_{k\neq i}\sum_{\substack{l\neq i \\ l\neq k}} w_{ik}w_{il}}$$

in the sums of this formula $i = k$, $i = l$ and $k = l$ are not included. In the case in which w_{ij} equals either 0 or 1 the definition was equivalent to the definition for unweighted graphs (Watts and Strogatz, 1998). The mean weighted clustering coefficient was defined as

$$C_{mean}^w = \frac{1}{N}\sum_{i=1}^{N} C_i^w$$

Weighted path length: for a given node *i* in the graph, the path algorithm finds the path with lowest cost (i.e. the shortest path length) between that node and every other node. For the weighted path length, the path between two nodes *i* and *j* is found by minimizing the sum of weights assigned to the edges on their path. The average path length (L) for node *i* to all other nodes is defined as $\Delta\Phi(t_k)$

Here, $\min\{l_{ij}^w\}$ is the weighted path length between node *i* and *j*, calculated using Dijkstra's algorithm (Dijkstra, 1959). We considered high values of the PLI as close functional distance and low values of the synchronization index as large functional distance (i.e.). In our dataset no disconnected nodes were present. The mean weighted path was defined as

$$L_{mean}^w = \frac{1}{N}\sum_{i=1}^{N} l_i^w$$

Normalization: for each functional dataset, path length and clustering coefficient were normalized using 50 surrogate networks. This number was sufficient to result in stable surrogate network properties. Surrogates networks were constructed using the random rewiring procedure described elsewhere (Maslov and Sneppen, 2002). Normalized weighted path length was defined as $C / \langle C_{surrogate} \rangle$ and normalized clustering coefficient was defined as $C / \langle C_{surrogate} \rangle$.

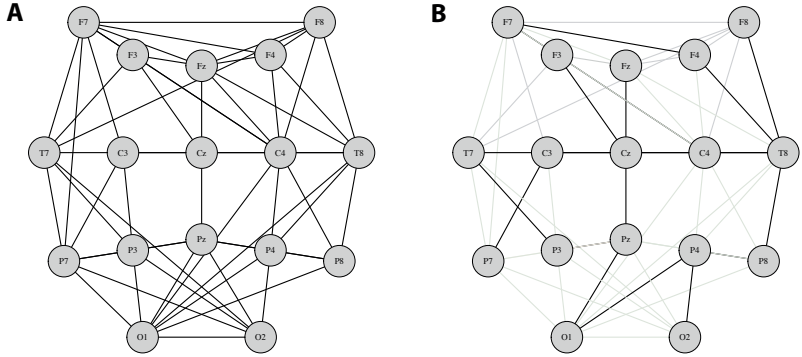
Minimum spanning tree

The MST creates a unique network based on the weighted connections between nodes. Furthermore, it connects all the nodes in the network without forming cycles and thereby reduces the connection costs. To compute the MST we used Kruskal's algorithm (Kruskal, 1956). This algorithm orders the weight of all edges in an ascending way, and subsequently starts the construction of a minimum spanning tree performing the following step as many times as possible: adding the edge with the highest PLI until all nodes N are connected in a loopless network consisting of $N - 1$ edges. Thus, if adding an edge results in formation of a cycle within the network, this edge will be skipped. Given the number of electrodes included in our analysis, trees contained 17 nodes and 16 edges. From these trees, several measures can be quantified (Boersma et al., 2013). For our study, we focused on the two most straightforward MST measures: leaf number and diameter that give information on topological features of trees (figure 2). The leaf number is defined by the number of nodes in the tree with degree = 1 (i.e. these nodes are connected by only one edge to the network) and has a lower bound of 2 and an upper bound of m = $N - 1$. The leaf number presents an upper bound to the diameter of the spanning tree that is the largest distance between any possible pair of nodes of the tree. The upper limit of the diameter of the tree is defined as diameter = m – leaf number + 2, implying that the largest possible diameter will decrease with increasing leaf number (Boersma et al., 2013). Both leaf number and diameter were normalized between 0 and 1. In summary, by excluding less important connections in the network, the MST network is built from the most efficient connections and enables a direct comparison between two networks since the number of nodes and connections are similar (figure 1).

Statistical analysis

First, we explored the effect of sleep deprivation in each group separately by comparing relative power spectra, network and MST measures from routine EEG and SD-EEG recordings for each frequency band with a paired t-test. To investigate whether a different network alteration was observed after sleep deprivation in patients compared with controls, we used a repeated measures analysis of variance (ANOVA) with type of EEG (EEG versus SD-EEG) as within factor and group (patients versus controls) as between factor and type of EEG × group as interaction for each frequency band. Differences between groups

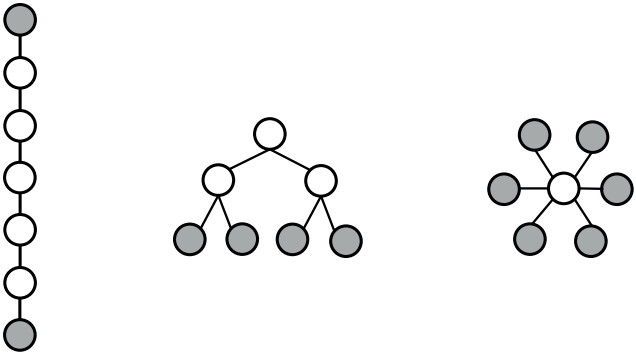
F1



Standard and MST networks

Two schematic illustrations of networks: (A) a standard network and (B) a MST network wherein all nodes are connected only once resulting in a loopless network.

F2



Three network topologies based on MST network

On the left a path-like topology with few leafs and long diameter; on the right a star-like topology with many leaves and a moderate diameter. In the middle an intermediate form combining the qualities of a line-like and star-like topology. Notify that all networks have the same number of nodes and connections. Leafs colored in green.

T1

Patient	Gender	Age (years)	EEG report	SD-EEG report	SD-EEG has added value	MRI findings
1	F	5.1	Single SWC in right central region	Several SWCs in the right central region	Yes	No abnormalities
2	F	6.0	Aspecific activity in the left frontal region	Several SWCs in the midparietal region. Increase of Spikes during NREM sleep	Yes	No abnormalities
3	M	7.1	No abnormalities	Several SWCs in the left frontal region while awake and during sleep	Yes	Increased signal intensities basal ganglia & cerebellum
4	M	7.4	Single spike midparietal	Several spikes in the midparietal region while awake and during sleep	Yes	Not performed
5	M	8.8	Active focus generating SWCs in the right centro-temporal region	Active focus generating SWCs in the right centro-temporal region while awake and during sleep	No	No abnormalities
6	M	8.8	Spike activity in the left central region	Spike activity in the left central region	No	No abnormalities
7	M	9.1	No abnormalities	Several spikes in the left central region	Yes	Small aspecific lesions cerebellum
8	F	9.2	Slow wave activity in the left temporal region and several times paroxysmal activity	One single paroxysmal activity	No	No abnormalities
9	M	9.4	No abnormalities	Epileptic activity originating from midline region	Yes	No abnormalities
10	M	9.5	No abnormalities	Several spikes and sharp waves in the right hemisphere increasingly present during sleep	Yes	Not performed
11	F	9.9	Possible spikes in the left fronto-temporal region	Several SWCs in the left temporal region spreading towards frontal region	Yes	No abnormalities
12	M	10.2	Slow wave activity in the right parietal-central region	Several SWCs while awake and during sleep	Yes	Tuberous sclerosis
13	F	10.6	No abnormalities	One single sharp wave in the right hemisphere	No	No abnormalities
14	M	12.2	No abnormalities	Ictal activity in the left temporo-occipital region during sleep	Yes	Old ischemic lesion left parietal

Table 1. Continued

Patient	Gender	Age (years)	EEG report	SD-EEG report	SD-EEG has added value	MRI findings
15	M	12.9	Aspecific abnormalities in the frontal region and isolated sharp wave complexes	During sleep increase of aspecific multifocal abnormalities	No	No abnormalities
16	M	13.1	Aspecific abnormalities in the left fronto-temporal region and a single SWC in right temporal region	Several SWCs in the right central region while awake and during sleep	Yes	Not performed
17	M	13.6	No abnormalities	Several spikes in the temporal region	Yes	Small lesion fronto-basal
18	M	13.8	Several SWCs in the left frontal region	Several SWCs in the left frontal region	No	No abnormalities
19	M	15.0	Aspecific abnormalities in the right and left temporal region	Aspecific abnormalities one single SWC	No	No abnormalities
20	M	15.1	Sharp wave acitivity in the right hemisphere	Sharp wave acitivity and SWCs in the right frontal region	Yes	No abnormalities
21	M	16.5	Aspecific abnormalities in the left frontal region	Several spikes and SWCs in the left frontal region; during sleep generalized activity	Yes	No abnormalities

Patient characteristics and clinical details

Patient characteristics and clinical details. F=female, M=male. SWC = Spike Wave Complex, NREM sleep = non Rapid Eye-Movement sleep

in age and gender were explored using independent t-test and chi-square test respectively. All analyses were performed in SPSS. A p-value below 0.05 was considered significant.

RESULTS

Patient characteristics

In total, 21 children with a definitive diagnosis of partial epilepsy were included in this study (5 girls, mean age 10.7 ± 3.1 years). Clinical details are summarized in table 1. Average time between EEG and SD-EEG recording was 2.4 ± 2.6 months. The control group consisted of seventeen children (7 girls, mean age 10.5 ± 2.5 years). Average time between their EEG and SD-EEG recording was 1.9 ± 1.1 months. No significant differences were found for age ($t = -0.11$, $p = 0.9$) and gender ($\chi^2 = 1.3$, $p = 0.3$) between patient and control groups. In three patients, anti-epileptic drug treatment was initiated after routine EEG recording and before SD-EEG recording. In 15 out of 21 patients (71%) the SD-EEG provided new information regarding localization of the epileptic focus or on the spreading of epileptiform activity and therefore considered of additional clinical value (table 1). No epileptiform activity was found in controls during SD-EEG recording.

Differences between regular and SD-EEG in relative power spectra per group

Paired t-tests were performed for relative power spectra per frequency band for patients and controls separately. In patients, no significant differences between spectra obtained from standard EEGs and SD-EEGs were found (table 2). In controls, however, an increase of the relative spectrum in the beta frequency band was found after sleep deprivation ($p = 0.012$).

Differences between regular and SD-EEG in network and MST measures per group

Paired t-tests were performed for each network and MST characteristic per frequency band for patients and controls separately (table 3a and 3b respectively). In patients, a significant decrease in leaf number ($p = 0.037$) after sleep deprivation was found only in the alpha frequency band. In the other frequency bands, no significant differences in network measures were found after sleep deprivation. In controls, the clustering coefficient ($p = 0.021$) and leaf number ($p = 0.014$) in the delta frequency band increased after sleep deprivation and the diameter decreased ($p = 0.037$).

T2

	Patients					Controls				
	EEG-power	sd	SD-EEG power	sd	p-value	EEG-power	sd	SD-EEG power	sd	p-value
Delta band	0.421	0.075	0.428	0.104	0.672	0.449	0.071	0.424	0.102	0.080
Theta band	0.217	0.078	0.222	0.079	0.594	0.216	0.074	0.207	0.077	0.460
Alpha1 band	0.167	0.089	0.160	0.095	0.585	0.154	0.081	0.157	0.107	0.791
Alpha2 band	0.105	0.086	0.939	0.086	0.134	0.088	0.053	0.106	0.072	0.089
Beta band	0.781	0.029	0.851	0.035	0.117	0.078	0.031	0.089	0.038	0.012*

Paired t-tests between EEG and SD-EEG recordings

For relative power spectra in five frequency bands. Alpha band is separated in a lower and upper frequency band. sd = standard deviation. * Significant ($p < 0.05$)

T3

	Delta band		Theta band		Alpha band		Beta Band	
	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value
Path length	0.774	0.448	0.150	0.882	1.492	0.151	1.059	0.302
Clustering coefficient	0.317	0.755	-1.840	0.081	-1.803	0.086	1.304	0.207
Diameter	0.383	0.705	0.447	0.660	1.366	0.187	-0.923	0.367
Leaf number	1.072	0.297	-0.103	0.916	-2.189	0.041*	1.769	0.092

A) Network measures for patients

Paired t-test between EEG and SD-EEG recordings of patients for network measures (path length and clustering coefficient) and MST measures (diameter and leaf number). A positive t-value indicates an increase of network/MST measure after sleep deprivation. A negative t-value indicates a decrease of network/MST measure after sleep deprivation * Significant ($p < 0.05$)

T3

	Delta band		Theta band		Alpha band		Beta Band	
	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value
Path length	1.104	0.286	-1.283	0.218	1.043	0.313	-0.353	0.729
Clustering coefficient	2.561	0.021*	-0.449	0.659	0.770	0.452	-0.687	0.502
Diameter	-2.269	0.037*	0.438	0.668	-1.409	0.178	-0.682	0.505
Leaf number	-2.759	0.014*	-0.502	0.623	1.007	0.329	-0.054	0.958

B) Network measures for controls

Paired t-test between EEG and SD-EEG recordings of controls for network measures (path length and clustering coefficient) and MST measures (diameter and leaf number). A positive t-value indicates an increase of network/MST measure after sleep deprivation. A negative t-value indicates a decrease of network/MST measure after sleep deprivation * Significant ($p < 0.05$)

Interaction in network and MST measures

Repeated measures ANOVA were performed for each network and MST characteristic separately for each frequency band. Significant interaction was only found for the leaf number ($p = 0.03$) and a trend for diameter ($p = 0.061$) in the alpha frequency band (table 4). The interaction for leaf number and diameter increased when including only patients in whom SD-EEG recording was considered to be of additional diagnostic value ($p = 0.009$ and $p = 0.03$ respectively) (figure 3).

F3

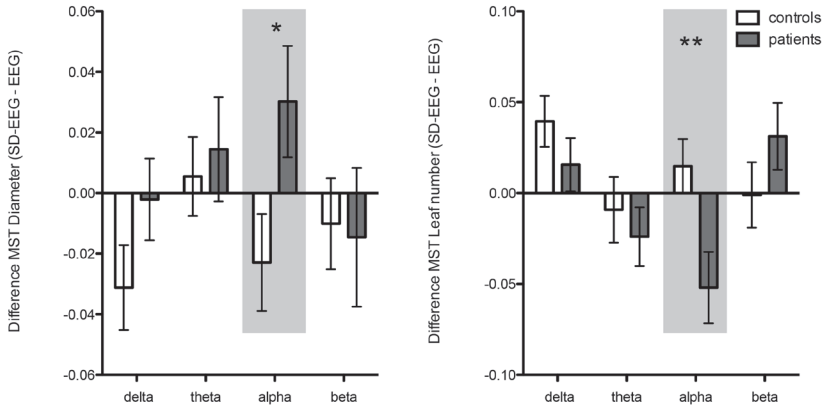


Illustration of interaction effects from MST measures diameter (left graph) and leaf number (right graph) per frequency band as revealed with a repeated measures ANOVA (mean values and standard error of the mean bars). In this analysis we included only patients in whom the SD-EEG was of added value (n=15), and all controls (n=17) (table 4). There was a significant interaction for diameter in the alpha band; the diameter increased in patients whereas an opposite effect was found for controls. For leaf number, a significant interaction was found in the alpha band; the leaf number decreased in patients whereas an opposite effect was found for controls. Together, these results in the alpha band suggest a shift towards a path-like topology for patients after sleep deprivation and a shift towards a star-like topology for controls. * Significant ($p < 0.05$)

T4

	Interaction (all patients, n=21) Type of EEG*Group		Interaction (added value SD-EEG, n=15) Type of EEG*Group	
	F-value	p-value	F-value	p-value
Delta-band				
Path length	0.115	0.737	0.008	0.928
Clustering coefficient	2.123	0.154	0.456	0.505
Diameter	3.976	0.054	2.256	0.144
Leaf number	2.144	0.152	1.359	0.253
Theta-band				
Path length	1.018	0.320	1.395	0.247
Clustering coefficient	0.394	0.534	0.321	0.575
Diameter	1.558	0.220	0.183	0.672
Leaf number	0.103	0.750	0.361	0.552
Alpha-band				
Path length	0.075	0.785	0.059	0.809
Clustering coefficient	2.881	0.098	2.038	0.164
Diameter	3.735	0.061	4.724	0.038*
Leaf number	5.101	0.030*	7.680	0.009*
Beta-band				
Path length	0.977	0.330	0.216	0.645
Clustering coefficient	1.852	0.182	0.077	0.783
Diameter	0.058	0.812	0.030	0.864
Leaf number	1.360	0.251	1.539	0.224

Interaction repeated measures ANOVA

Interactions from repeated measures ANOVA for each network and MST measures. In the left column, the interactions when including all patients (n=21). In the right column, the interactions when including only those patients in whom the SD-EEG was of added clinical value (n=15). * Significant ($p < 0.05$).

DISCUSSION

An EEG after sleep deprivation is often performed in patients suspected of epilepsy when the standard EEG recording is inconclusive. Little is known, however, about the mechanism behind the increased sensitivity of SD-EEG recordings in epilepsy. In this study we investigated whether a network analytical approach could clarify this phenomenon. A significant interaction was found in our repeated measures analysis for leaf number and diameter between patients and controls after sleep deprivation: patients showed a shift towards a more path-like MST network whereas controls showed a shift towards a more star-like MST network (figure 2). This interaction became even more significant when including only the patients in whom the SD-EEG recording was of additional clinical value (table 4). Together with the increased presence of epileptiform abnormalities after sleep deprivation in patients, the network shift towards a more path-like MST network could reflect an inadequate compensatory mechanism of the epileptic brain.

Previous research has shown that functional networks change during seizure generation and propagation into a more regular network organization (i.e. highly segregated and poorly integrated network) (Kramer et al., 2010; Kramer et al., 2008; Ponten et al., 2007; Ponten et al., 2009; Schindler et al., 2007; Schindler et al., 2008). This shift towards a regular topology could depend on the decreased centrality of so-called 'hub-nodes' in the network or an altered synchronizability during the ictal state (van Diessen et al., 2013a). Furthermore, long-term continuous evaluation of functional networks derived from intracranial recordings, revealed large fluctuations in clustering coefficient and path length during the day (Kuhnert et al., 2010). These fluctuations over time, largely attributed to daily rhythms, showed an increased regularization of functional networks during night-time in patients with partial epilepsy (Kuhnert et al., 2010) whereas a shift towards a more segregated and integrated network during sleep was observed in healthy subjects (Ferri et al., 2008). Possibly, this shift towards a more regular network during sleep in patients with epilepsy explains why the epileptic brain is more susceptible to both epileptiform discharges and seizures during sleep. Although we did not investigate network alterations during sleep, a similar mechanism could explain the increased presence of epileptiform discharges after sleep deprivation. This study revealed a shift towards a more path-like topology in patients (i.e. increased diameter and decreased leaf number). Considering the path-like network as a network wherein

nodes are less centrally connected and share basic characteristics with a regular network, it is possible that the mechanisms underlying sleep deprivation-induced network alterations mimic the changes of a functional network during the ictal state or during sleep. Interestingly, these changes in network organization could coincide with an increase of cortical excitability that has previously been described as a pro-convulsive effect, accountable for the induction of epileptogenic activity after sleep deprivation (Badawy et al., 2006; Manganotti et al., 2006). Furthermore, a significant interaction was only found in the alpha frequency band. Previous studies have suggested that each frequency band is associated with distinct networks and cognitive processes (Basar et al., 2001; von Stein and Sarnthein, 2000). For example, spontaneous alpha activity is typically found when eyes are closed whereas task-related alpha activity is typically found during sensory, motor and primarily top-down cognitive processes (von Stein and Sarnthein, 2000). Although it remains uncertain how our results relate to changes in the cognitive domain, it suggests an involvement of the alpha frequency band in normal sleep physiology.

The MST approach has been suggested as an appropriate method to overcome certain limitations of network studies, particularly in relation to differences in network densities between groups (van Wijk et al., 2010). Nevertheless, few studies have compared standard network measures, such as clustering coefficient and path length, to MST measures (Boersma et al., 2013). We only found significant differences in network alteration between controls and patients for the MST measures leaf number and diameter, suggesting that these measures are more sensitive to network alterations in patients with epilepsy. Larger studies investigating and comparing both approaches are needed to verify this. In addition, like clustering coefficient and path length, leaf number and diameter are highly correlated, arguing for more and distinct MST features to characterize networks.

To date, only one study has investigated the influence of sleep deprivation on functional networks (Koenis et al., 2013), but only in healthy subjects. Koenis and others reported network alterations in the alpha frequency band during an eyes-closed condition, namely a shift towards a more random network. In our control group, we found a significant increase in clustering coefficient, a decreased diameter and increased leaf number in the delta frequency band. Together, these results suggest a more star-like MST network in controls after sleep deprivation. Interestingly, a star-like MST network shares basic characteristics with that of a random network organization (i.e. a poorly segregated and highly integrated

network). Although we found a similar shift in network organization as Koenis and others, it is challenging to explain the differences in frequency bands between both studies (alpha versus delta). Possibly, a different patient population (age) and methodology (different EEG recordings) are accountable for these differences. (Smit et al., 2012; van Wijk et al., 2010). Besides network measures, we also measured the relative power spectrum. In accordance with previous literature, we found an increase in beta power spectrum after sleep deprivation in healthy subjects (Gast et al., 2011) whereas we did not find this effect in patients. Possibly, the lack of increased beta power in patients after sleep deprivation reflects an inadequate compensation of the epileptic brain to maintain beta frequency band specific functions, such as integration of multi-sensory information (von Stein and Sarnthein, 2000).

Although our study provides further insight in the mechanism of increased sensitivity of EEG recordings after sleep deprivation, several limitations should be mentioned. First, our patient sample is limited (21 children) and included only children with partial epilepsy. One could therefore argue that our results might be restricted to this age range of patients. Nevertheless, the increased sensitivity of SD-EEG recordings is particularly manifested in children suspected of partial epilepsy (Shinnar et al., 1994) and our population is therefore ideal to study this phenomenon. Furthermore, we used a repeated measures design to explore the influence of sleep deprivation in controls and patients, and not a crossover design as our data was collected retrospectively. Both patients and controls underwent first a routine EEG and than a SD-EEG recording. Though unlikely, this could cause a bias in our results. Finally, in three patients anti-epileptic drugs were started before the SD-EEG recording. Considering the potential influence of anti-epileptic drugs on functional networks (van Diessen et al., 2013a), this could have deviated our results. In all three patients, however, treatment started only a few days before SD-EEG recording and was therefore still at a very low dosage. An additional analysis, after excluding these patients, revealed similar results.

In conclusion, this study provides insights into the mechanisms behind the increased presence of epileptiform abnormalities after sleep deprivation in children with partial epilepsy. We suggest that an inadequate compensatory shift of the epileptic network towards a more path-like topology after sleep deprivation is accountable for the increased epileptiform abnormalities often found in patients with epilepsy.

CHAPTER 7

ELECTROENCEPHALOGRAPHY FUNCTIONAL NETWORKS IN NEWLY DIAGNOSED EPILEPSIES

Submitted

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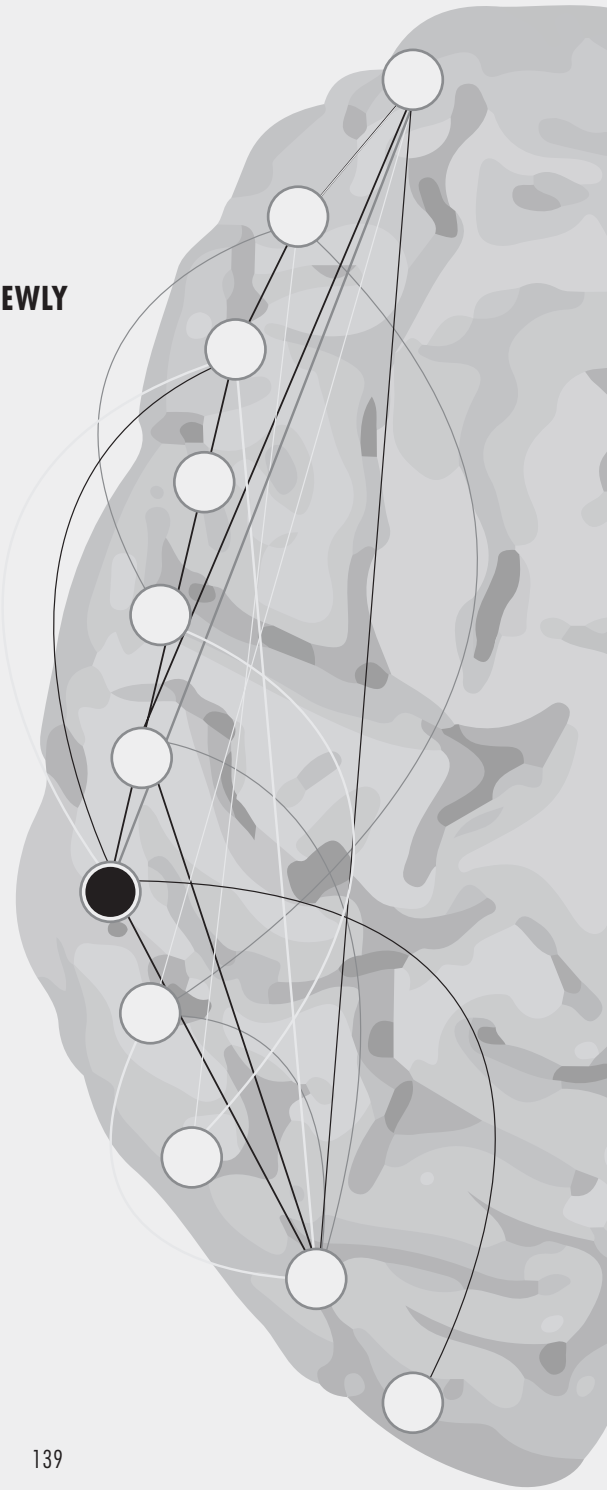


ABSTRACT

Objective: previous research has shown that brain network organization is disrupted in chronic epilepsies, which may partly explain the associated cognitive and behavioral deficits. It remains unclear, however, to what extent these brain networks are altered at an early stage of the disease. This information may be important to improve our understanding on the course of network alterations and their association with recurrent seizures and cognitive deficits.

Methods: 89 drug-naïve children with newly diagnosed epilepsy (62 focal and 27 generalized epilepsy) were included. The control group comprised 179 children. Functional brain networks were based on interictal electroencephalography recordings obtained at first consultation. Both conventional network metrics and minimum spanning tree (MST) metrics were computed to characterize topological network differences in distinct frequency bands. In addition to global network measures of integration and segregation, the maximum betweenness centrality was computed to investigate if highly interconnected nodes were altered in newly diagnosed epilepsies.

Results: network alterations between groups were only identified by MST metrics. Differences were most pronounced in the delta band, in which a loss of network integration and a significant lower maximum betweenness centrality was found in children with focal epilepsies compared to healthy controls. A reversed group difference was found in the upper alpha band. The network topology in generalized epilepsies was relatively spared.

Significance: interictal network alterations are already present in focal epilepsy at an early stage of the disease. Since network alterations were only identified with MST metrics, we argue that these alterations are subtle at the early stage and aggravate at later stages as a result of persisting seizures. Finally, we speculate that functional network differences between focal and generalized epilepsies could provide a new perspective to find a scientific base for the new classification of epilepsies.

INTRODUCTION

There is increasing evidence that brain functioning is emerging from a complex interplay of different brain areas, which are organized in a highly efficient manner (Bullmore and Sporns, 2012). A balance between network integration and segregation in combination with the existence of highly interconnected brain areas (so-called 'hubs') are needed to support this normal brain functioning (Stam, 2014).

This balanced network organization of the brain is disturbed in epilepsy (Engel et al., 2013; Kramer and Cash, 2012; Richardson, 2012; van Diessen et al., 2013a). Studies have previously shown that network alterations in epilepsy may aggravate with a longer duration of the disease (Liao et al., 2010; van Dellen et al., 2009), eventually resulting in a loss of network integrity that could – at least – partly explain the associated behavioral and cognitive deficits in patients (Vaessen et al., 2012; Vlooswijk et al., 2011). As these studies have typically focused on patients with chronic epilepsies, it remains unclear how these networks are altered at an early stage of the disease. This information may be important to improve our understanding on the course of network alterations in epilepsy and their association with recurrent seizures and cognitive deficits.

In this study we investigated to what extent functional network alterations already exist at an early stage of the disease. To avoid possible confounding effects of antiepileptic drugs or recurrent seizures on the construction of functional networks – derived from interictal electroencephalography (EEG) recordings – we focused on a patient cohort of newly diagnosed childhood epilepsies. A second aim of the study was to investigate how focal and generalized epilepsies differ from each other in term of functional network organization. The tendency to generate focal or (primary) generalized seizures may reflect a distinct underlying mechanism (Berg and Scheffer, 2011; Stefan and Lopes da Silva, 2013). Whereas generalized seizures are characterized as fast and diffuse spreading activity in bilateral distributed networks (Meeren et al., 2005), focal seizures are associated with unilateral networks that spread beyond the isolated epileptogenic region (Gotman, 2008).

Conventional network metrics include average (shortest) path length, a measure of integration, and average clustering coefficient, a measure of segregation. These were computed to characterize global network organization (Rubinov and Sporns,

2010). Considering age-related changes in network density (Smit et al., 2012) and its possible influence on the computation of conventional network metrics (Fornito et al., 2013; van Wijk et al., 2010), we also constructed the minimum spanning tree (MST) of the network (Stam et al., 2014). The MST represents a stable network core of the original network and allows an unbiased comparison of networks with a different density. In addition to global network properties, we were interested if the connectivity of highly interconnected nodes were altered in newly diagnosed epilepsies, as was previously revealed in chronic epilepsies (van Diessen et al., 2013b; Varotto et al., 2012; Wilke et al., 2011).

METHODS

Patient selection

We included children who visited the outpatient First Seizure Clinic of the University Medical Center Utrecht between January 2008 and May 2013 after one or more suspected epileptic event(s) and had an EEG recording of sufficient quality for the construction of networks (see section 'data acquisition and selection'). Definitive diagnosis of epilepsy was based on ancillary investigations and clinical follow-up. We selected three groups: 1) children who were diagnosed with focal epilepsy; 2) children who were diagnosed with generalized epilepsy and 3) a control group of children in whom the diagnosis of epilepsy was excluded, based on clinical history, EEG report, and at least one year of uneventful follow up. None of the children were taking antiepileptic medication at time of EEG recording. The institutional ethical committee approved the study and concluded that the Dutch Medical Research Involving Human Subjects Act did not apply, and written informed consent was not required.

Data acquisition and selection

The EEG was recorded at 21 scalp electrodes according to the international 10-20 system (SystemPlus Evolution, Micromed). Impedance of each electrode was kept below 5 kΩ. Data was high- and low-pass filtered at 0.5 and 70 Hz, respectively. The sampling frequency was 512 Hz. Frontoparietal and basal temporal electrodes were excluded (Fp1, Fp2, A1, and A2) from further analyses. For each patient, the first four artifact-free epochs of 4096 samples (8 seconds) were selected by on the authors [EvD] and independently re-inspected by a clinical epileptologist [FEJ] on artifacts and epileptiform abnormalities. Before calculating functional connectivity

and network metrics, epochs were band-pass filtered into the commonly used frequency bands delta (0.5–4 Hz), theta (4–8 Hz), lower alpha (8–10 Hz), upper alpha (10–13 Hz) and beta (13–30 Hz). The relative spectral power was computed and averaged over all channels, epochs and subjects separately for each group. This was obtained by converting the raw EEG recordings from the time domain into the frequency domain with a fast Fourier transformation with a frequency resolution of 1/8 s = 0.125 Hz. All further analyses were performed for these bands separately. Data acquisition and analyses followed a recent published practical guideline (van Diessen et al., 2014a) and were performed in BrainWave [version 0.9.116, authored by CJS, http://home.kpn.nl/stam7883/brainwave.html]

Functional connectivity

Functional networks were constructed per epoch, based on functional connectivity between each electrode time series' pair. Functional connectivity was quantified between 0 and 1 with the phase lag index (PLI). PLI is a measure of the asymmetry of the distribution of instantaneous phase differences between two time-series. A PLI of 0 indicates no phase coupling between time-series, or coupling with a phase difference centered on $0 \pm \pi$ radians. A PLI > 0 indicates the presence of phase coupling. A more mathematical description of computing the PLI can be found elsewhere (Stam et al., 2007b).

Network analytical approach

Network construction: for each epoch, we constructed a weighted undirected network, described by the graph *G = (N, W)*, where N is the set of all 17 EEG electrodes and $W = \{w_{ij}\}$ is the *N x N* symmetric weight matrix, where $w_{ii} = 0$ and w_{ij} the PLI determined between node *i* and *j*.

Conventional network metrics: two metrics were used to describe global network properties, namely the average clustering coefficient and the average shortest path length. The average clustering coefficient is a global measure of network segregation and reflects the clustered connections around individual network nodes. We computed the weighted clustering coefficient similar to (Stam et al., 2009), and included the PLI as weight for the coupling strength between nodes. The shortest path length is the minimal number of edges that must be traversed to travel from one network node to another. The average path length, a global measure of

network integration, is this average minimal number of edges of all possible node connections. The weighted average path length is found by minimizing the sum of weights (that is, PLI coupling strength) assigned to the edges on their path, using Dijkstra's algorithm (Dijkstra, 1959). To reduce the effect of network density, both conventional network metrics were normalized using 50 surrogate networks. This number was sufficient to result in stable surrogate network properties.

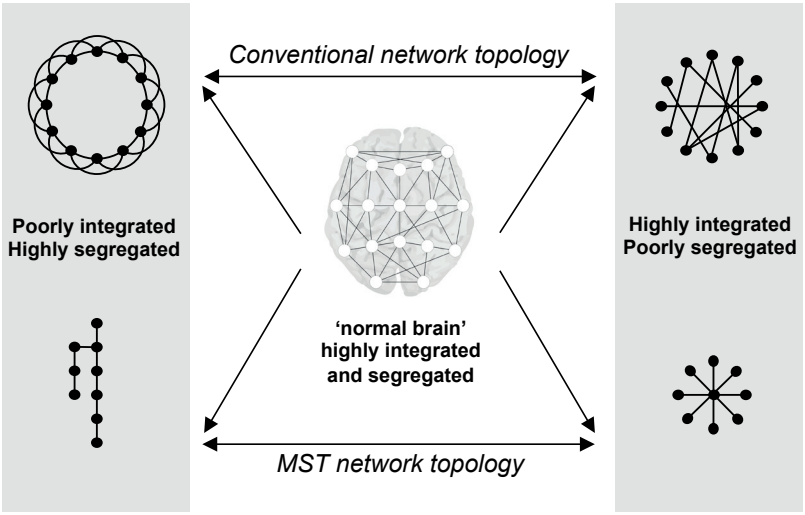
Minimum spanning tree (MST)

The MST is a unique acyclic subnetwork that contains most of the strongest connections of the original undirected, weighted network, for review and computational properties see (Stam et al., 2014). We used Kruskal's algorithm to compute the MST (Kruskal, 1956), which orders the weight of all edges, and subsequently starts the construction of a MST performing the following step as many times as possible: adding the edge with the highest PLI until all nodes N are connected in a loopless network consisting of N – 1 edges. Thus, if adding an edge results in formation of a cycle within the network, this edge will be skipped. Given the number of electrodes included in the analysis, trees contained 17 nodes and 16 edges. From these trees, several measures can be quantified (Tewarie et al., 2014b). The MST leaf number and diameter are the two most straightforward MST metrics that give global information on the MST topology, in particular on the level of integration of the network. The leaf number L is defined by the number of nodes in the tree with degree = 1 (i.e. these nodes are connected by only one edge to the network) and has an upper bound of $m = N - 1$. The leaf number presents an upper bound to the diameter of the spanning tree that is the largest distance between any possible pair of nodes of the tree. The upper limit of the diameter of the tree is defined as diameter = $m - L + 2$, implying that the largest possible diameter will decrease with increasing leaf number. Figure 1 illustrates how these global MST metrics relate to the conventional network metrics of clustering coefficient and path length.

Besides global MST metrics leaf number and diameter, we also computed the betweenness centrality (BC) for each tree node, which is a network hub measure (Rubinov and Sporns, 2010). BC values are determined as the number of shortest paths between any pair of nodes i and j that are running through node u, divided by the total number of paths between i and j. BC values range between 0 and 1. Leaf nodes have a betweenness centrality of 0, while network nodes where all

shortest paths run through have a BC of 1. We summarized the MST subgraph by taking the maximum betweenness centrality (BC_{max}). For a graphical representation of MST network topologies and metrics, see Figures 1 and 2.

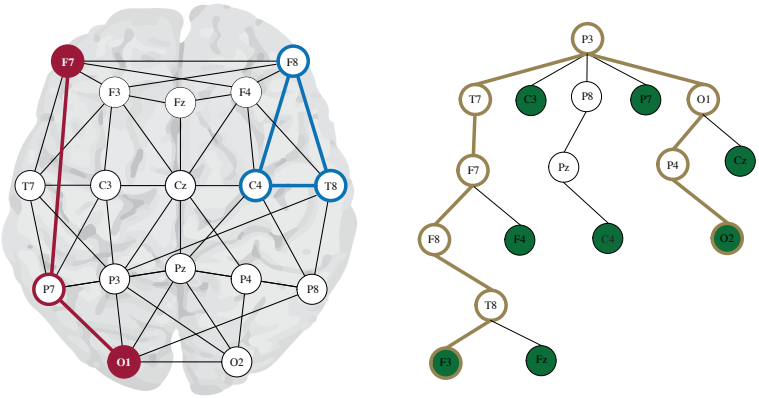
F1



Schematic illustration of conventional network and MST topology

The upper two graphs represent fully connected, conventional networks. Global network properties to describe these conventional networks are clustering coefficient, a measure of network segregation, and path length, a measure of network integration. The upper left figure has a *regular* network topology, with a high average clustering coefficient and a long average path length. The upper right figure has a *random* network topology, with a low average clustering coefficient and a short average path length. The *line-like* MST network topology (left, below), characterized by a long MST diameter and a low MST leaf number, shares the basic characteristics of a regular network topology. The *star-like* MST network topology (right, below), characterized by a short MST diameter and a high MST leaf number, shares the basic network characteristics of a random network topology. All these topologies deviate from a normal brain organization (centre figure), which combines the properties of a highly integrated and segregated network.

F2



Traditional network and MST network

Based on the data derived from focal epilepsy group (n=62). For illustrative purposes we binarized the weighted network and showed only the strongest connections in the left schematic figure (that is, edges are removed wit lower PLI values). The clustering coefficient based on triangles (one triangle shown in blue) is equivalent to the fraction of the node's neighbours that are also neighbours of each other. The path length in a binary network is the minimal number of edges that must be traversed to travel from one node to another. In red an example path is given which contains the minimal number of edges (i.e. two) between the two uniform red nodes. Right figure represents the minimum spanning tree (MST) subgraph of the original weighted network. The green nodes represent leafs of the network and the brown line indicates the longest shortest path length in the MST subgraph (MST diameter). The upper node in the MST subgraph (P3) is the node with the highest betweenness centrality and thus represents the BC max of the MST subgraph.

Statistical analysis

Spectral power and network metrics were averaged across the epochs per subject prior statistical analysis. We compared topological network differences between children with focal epilepsy, generalized epilepsy and healthy controls by means of an analysis of variance for the relative power spectrum, conventional and MST network metrics. This was repeated for each frequency band separately. Sex (Boersma et al., 2011) and age (Smit et al., 2012) influence functional network topologies. We therefore included both as covariates in the analysis to correct for potential sex and age-related group effects. Post-hoc tests were performed if an overall significant group difference were found. A p-value below 0.05 was considered significant. Statistical analyses were performed in SPSS.

RESULTS

Demographics

In total, 62 children with a definitive diagnosis of focal epilepsy (42 boys, mean age 9.1 ± 3.4 years) and 27 children with a definitive diagnosis of generalized epilepsy were included in this study (15 boys, mean age 8.8 ± 4.1 years). As a control group, we included 179 children (92 boys, mean age 8.5 ± 4.2 years). Clinical characteristics and etiological classification according to the newly proposed classification by the International League Against Epilepsy (Berg et al., 2010), of the children with focal and generalized epilepsy separately, is provided in Table 1.

Spectral power

No significant differences in spectral power were found between children with epilepsy and controls for any of the frequency bands (Table 2).

Differences between groups in conventional and MST network metrics

Figure 2 shows a conventional network and a MST subnetwork based on the original data. All results from the statistical analyses are presented in Table 3 and Figure 3. No significant differences in PLI and the conventional network metrics – average clustering coefficient and average path length – were found between groups.

In the delta and upper alpha frequency band, the MST diameter revealed a significant overall group effect ($p = 0.002$ and $p = 0.002$, respectively). Post-hoc analysis revealed a significant increase in MST diameter in children with focal epilepsy compared to controls ($p = 0.001$) and a trend towards an increased MST diameter in focal compared to generalized epilepsies ($p = 0.055$) in the delta band. In the upper alpha band, however, post-hoc analysis revealed a significant decrease of MST diameter in children with focal epilepsy compared to controls ($p = 0.001$).

In the delta and upper alpha band, the MST leaf number revealed a significant overall group effect ($p = 0.016$ and $p = 0.006$, respectively). Post-hoc analysis revealed a significant decrease in MST leaf number in children with focal epilepsy compared to controls in the delta band ($p = 0.007$). In the upper alpha band, however, the MST leaf number was significantly increased in children with focal epilepsy compared to controls ($p = 0.002$).

T1

	Generalized epilepsies (n=27)	Focal epilepsies (n=62)
Age of seizure onset (years)	9.3±4.3	8.5±3.7
Development		
- Normal (n)	21	48
- Delayed (n)	6	14
Structural abnormalities		
- Yes (n)	0	27
- No (n)	14	24
- Not available (n)	13	11
Etiological diagnosis		
- Presumed genetic* (n)	16	18
- Structural-metabolic (n)	11	42
- Unknown (n)	none	2

Clinical characteristics and definitive diagnosis after follow-up for both groups

Developmental status was based on schooling level of the child and intelligence quotient (IQ) score, if available, and scored as 'delayed' when children attended special schooling or had an IQ < 70. Structural abnormalities scores were based on the available neuroimaging results. ILAE classification of etiology is according to the newly proposed classification in 2010. *The presumed genetic group included for children with generalized epilepsies: myoclonic epilepsy of infancy or juvenile myoclonic epilepsy (n = 5), and childhood or juvenile absence epilepsy (n = 11). For children with focal epilepsies the presumed genetic group included: benign epilepsy with centrotemporal spikes (n = 16), or benign epilepsy of childhood with occipital paroxysms (n = 2)

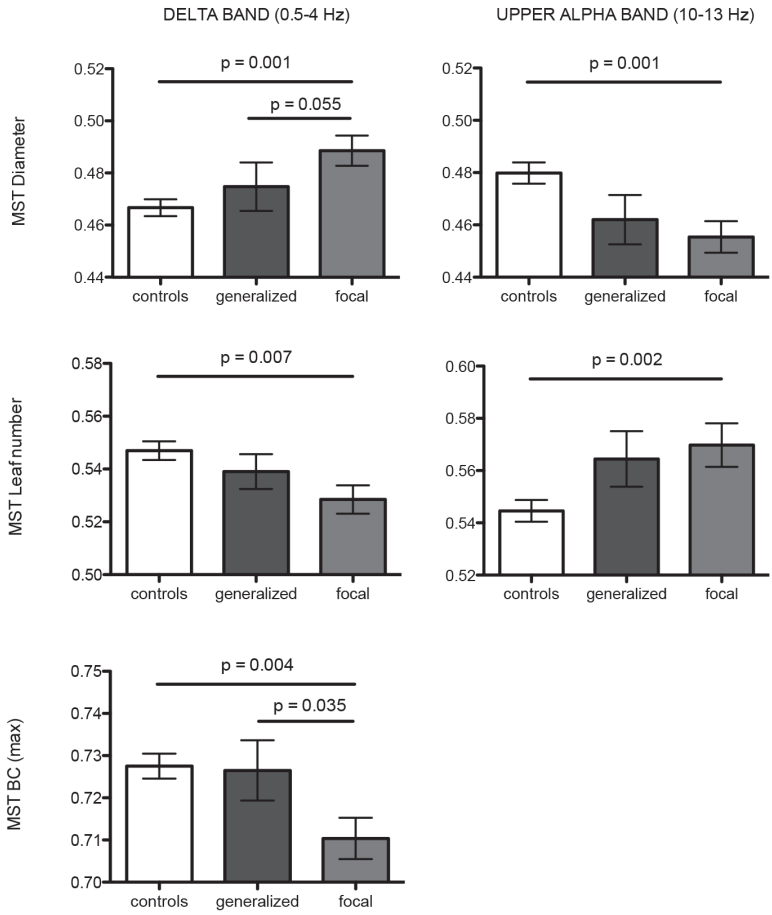
T2

Frequency band	Controls mean (se)	Generalized epilepsy mean (se)	Focal epilepsy mean (se)	F-value	P-value
Delta band	0.503 (0.007)	0.484 (0.017)	0.514 (0.012)	1.050	0.352
Theta band	0.213 (0.005)	0.231 (0.013)	0.203 (0.009)	1.535	0.217
Lower alpha band	0.125 (0.006)	0.133 (0.014)	0.131 (0.010)	0.240	0.786
Upper alpha band	0.068 (0.004)	0.062 (0.010)	0.069 (0.007)	0.176	0.838
Beta band	0.072 (0.002)	0.072 (0.006)	0.065 (0.004)	1.168	0.313
Delta band	0.503 (0.007)	0.484 (0.017)	0.514 (0.012)	1.050	0.352

Analysis of variance for power spectra separately for each frequency band

se = standard error of the mean

F3



Post-hoc comparisons between groups for MST metrics

Left graphs represent MST metrics in delta band and right column represents MST metrics in upper alpha band. Upper row group differences for MST diameter, middle row group differences for MST leaf number and lower row MST maximum betweenness centrality (MST BCmax, only for delta band as no significant analysis of variance was found for other frequency bands)

T3

Frequency band	Controls mean (se)	Generalized epilepsy mean (se)	Focal epilepsy mean (se)	F-value	P-value
Delta band					
PLI	0.151 (0.001)	0.150 (0.003)	0.149 (0.002)	0.113	0.893
Path length	0.908 (0.001)	0.912 (0.002)	0.908 (0.002)	1.343	0.263
Clustering coefficient	1.006 (0.002)	1.006 (0.005)	1.000 (0.003)	1.704	0.184
MST diameter	0.466 (0.003)	0.474 (0.009)	0.490 (0.006)	6.145	0.002*
MST leaf number	0.547 (0.003)	0.539 (0.009)	0.528 (0.006)	4.195	0.016*
MST BC (max.)	0.727 (0.003)	0.727 (0.008)	0.710 (0.005)	4.398	0.013*
Theta band					
PLI	0.139 (0.002)	0.139 (0.004)	0.136 (0.003)	0.430	0.651
Path length	0.908 (0.001)	0.909 (0.002)	0.908 (0.002)	0.157	0.855
Clustering coefficient	1.008 (0.002)	1.005 (0.005)	1.008 (0.003)	0.181	0.835
MST diameter	0.473 (0.003)	0.471 (0.009)	0.471 (0.006)	0.088	0.916
MST leaf number	0.548 (0.004)	0.545 (0.009)	0.547 (0.006)	0.053	0.948
MST BC (max.)	0.717 (0.003)	0.722 (0.008)	0.721 (0.005)	0.386	0.680
Lower alpha band					
PLI	0.216 (0.004)	0.219 (0.010)	0.217 (0.007)	0.059	0.942
Path length	0.913 (0.001)	0.908 (0.003)	0.913 (0.021)	1.041	0.355
Clustering coefficient	1.017 (0.002)	1.009 (0.006)	1.021 (0.004)	1.304	0.273
MST diameter	0.458 (0.004)	0.459 (0.010)	0.454 (0.007)	0.134	0.875
MST leaf number	0.569 (0.004)	0.578 (0.011)	0.576 (0.008)	0.590	0.555
MST BC (max.)	0.726 (0.003)	0.731 (0.009)	0.728 (0.006)	0.153	0.858
Upper alpha band					
PLI	0.164 (0.003)	0.163 (0.008)	0.176 (0.005)	1.827	0.163
Path length	0.907 (0.001)	0.908 (0.003)	0.909 (0.002)	0.344	0.709
Clustering coefficient	1.005 (0.002)	1.006 (0.006)	1.009 (0.004)	0.415	0.661
MST diameter	0.479 (0.004)	0.464 (0.009)	0.456 (0.006)	6.133	0.002*
MST leaf number	0.545 (0.004)	0.562 (0.010)	0.569 (0.007)	5.197	0.006*
MST BC (max.)	0.716 (0.003)	0.716 (0.008)	0.730 (0.005)	2.817	0.062

Table 3. Continued

Frequency band	Controls mean (se)	Generalized epilepsy mean (se)	Focal epilepsy mean (se)	F-value	P-value
Beta band					
PLI	0.074 (0.001)	0.075 (0.002)	0.075 (0.002)	0.199	0.820
Path length	0.903 (0.001)	0.907 (0.002)	0.901 (0.001)	2.570	0.078
Clustering coefficient	0.991 (0.002)	0.996 (0.005)	0.987 (0.003)	1.381	0.253
MST diameter	0.494 (0.003)	0.502 (0.008)	0.494 (0.005)	0.477	0.621
MST leaf number	0.523 (0.003)	0.516 (0.009)	0.516 (0.006)	0.661	0.517
MST BC (max.)	0.705 (0.002)	0.699 (0.006)	0.704 (0.004)	0.485	0.616

Analysis of variance for network metrics separately for each frequency band

*Significant ($p<0.05$) after correcting for multiple post-hoc comparisons (false discovery rate test), se = standard error of the mean.

The MST BC_{max} showed a significant effect in the delta band only ($p = 0.013$). Post-hoc analysis revealed a significant decrease in MST BC_{max} in children with focal epilepsy compared to controls ($p = 0.002$) and to children with generalized epilepsy ($p = 0.035$) in the delta band.

Finally, subdividing the epilepsy groups according to their developmental status, presence of structural abnormalities and etiological diagnosis did not reveal significant differences (data not shown).

DISCUSSION

This study compared network topology of focal and (primary) generalized newly onset childhood epilepsies with that of controls, by means of EEG-based functional networks in drug-naïve children at initial clinical presentation. The differences were most pronounced in the delta band, in which MST diameter was increased, MST leaf number was decreased and a significantly lower BC_{max} was found for focal epilepsies compared to controls and to children with generalized epilepsies. In contrast to generalized epilepsies, where the network topology was relatively spared, these results suggest that network integrity is disrupted in focal epilepsy at an early stage of the disease.

The network alterations in focal epilepsy suggest a more line-like MST network configuration, which indicates a less integrated network topology (Figure 1). This is in accordance with a recently published meta-analysis where we reported a less integrated and more segregated network topology in patients with chronic focal epilepsy (van Diessen et al., 2014c). In contrast to the studies reviewed in that meta-analysis, our study only revealed a loss of network integrity when investigated with MST metrics, and not when looking at conventional network metrics. This could be related to computational differences between both approaches. The MST focuses on the connectivity backbone of the network, thereby disregarding weaker – and more noisy – connections (Stam et al., 2014). In addition, the MST is an unbiased method for the comparison of networks with a different density and thus resulting in a more robust network characterization (Tewarie et al., 2014b). This allows the MST to better detect subtle network alterations that are present at an early stage of the disease, but cannot be detected yet with the more conventional network metrics average clustering coefficient and average path length. We hypothesize that these alterations will aggravate at later stages as a result of persisting seizures (Liao et al., 2010; van Dellen et al., 2009). This is in accordance with the observation that drug naïve and previously healthy rats with neocortical, non-lesional, focal epilepsy develop similar network alterations during the course of their seizure disorder (Otte et al., 2012a). Furthermore, the reduced MST BC_{max} suggests that the most crucial nodes of the network, with a high node centrality, become less important in focal epilepsy. Together, these results indicate a failure of the normal hierarchical network, which has recently been suggested as a 'final common pathway' for several brain diseases, including epilepsy (Stam, 2014).

In contrast, newly onset generalized epilepsies were not significantly associated with functional network alterations, and were more comparable with healthy controls in terms of network configuration. This observation partly contradicts a recent study in which children with newly diagnosed either focal or generalized epilepsy were found to have reduced network integrity (Bonilha et al., 2014). This discrepancy could be related to differences between structural and functional networks. Whereas our study is based on EEG-based functional networks, Bonilha and colleagues constructed structural networks from regional volumetric properties. This may suggest a decoupling of structural and functional network changes in generalized epilepsies (Zhang et al., 2011). The study further revealed that structural network alterations, studied with conventional network metrics such as

clustering coefficient and path length, disappear at higher density thresholds in generalized epilepsies and not in focal epilepsies, suggesting that differences between focal and generalized epilepsy might also exist when focusing on the strongest structural connections (Bonilha et al., 2014).

Differences in functional network topology between focal and generalized epilepsies may reflect the ability of the brain to generate either focal or generalized seizures. Considering that generalized seizures are conceptualized as rapidly engaging bilaterally distributed networks (Meeren et al., 2005), a functional network topology wherein highly interconnected network nodes are spared could be essential to produce primarily generalized seizures. This proposition is partly supported by previous modelling work, showing that a relatively undamaged network is able to produce generalized seizures (Terry et al., 2012). Interestingly, this dichotomy in terms of functional network topology between focal and generalized seizures might provide a new perspective to find a scientific base for the new classification of epilepsies (Berg et al., 2010).

In agreement with previous studies on EEG-based functional brain networks in focal epilepsies our main effects were found in the lower frequency bands (Bartolomei et al., 2006; Bettus et al., 2008; Douw et al., 2010a; Douw et al., 2010b; Horstmann et al., 2010; van Dellen et al., 2012; van Diessen et al., 2013b). Nevertheless, we also found significant differences in the upper alpha band. This band showed a reversed effect in terms of network changes when compared to the delta band. A decreased MST diameter and an increased MST leaf number were found for focal epilepsies compared to controls. Since no other study on epilepsy has previously shown changes in the upper alpha frequency, we can only speculate on their significance. One might hypothesize that changes in different frequency bands reflect different aspects of an (inadequate) compensatory mechanism of the diseased brain. Possibly, the reason why this effect has not been reported is related to the patient characteristics. Most of the previous studies have focussed on adults with chronic focal epilepsy, who were on antiepileptic drug treatment. Instead we have only included drug-naïve children with newly onset epilepsy. Use of antiepileptic medication is known to selectively effect higher frequencies (Arzy et al., 2010), which in turn could have confounded the results in higher frequency bands of earlier EEG network studies.

This study has several limitations. First, etiologies in both patient groups were heterogeneous. Although our aim was to compare focal with generalized

epilepsies, the diversity of etiology made it difficult to further explain global network differences. Subgroups defined by etiology were too small to analyse. Furthermore, we were interested to see how global network alterations were related to network differences on a node level. Again, the variety of localisation of epileptogenic foci did not allow sufficiently powered statistical analyses. As a result, we were not able to relate previous findings of a functionally isolated epileptogenic focus (Burns et al., 2014; van Diessen et al., 2013b; Warren et al., 2010), to a loss of integration and a lower BC_{max} on a whole-brain network level. Third, for the inclusion of patients and controls we were restricted to children who consulted the outpatient clinic only. As a result, the control group included children initially suspected of having had a possible epileptic seizure. Although epilepsy was excluded after thorough follow-up, this might have introduced a bias in our results. Further, patients with an epileptic encephalopathy, or a high seizure load necessitating admission, were excluded. Finally, despite the fact the MST approach has been successfully applied in epilepsy research (Ortega et al., 2008b; van Dellen et al., 2014a; van Diessen et al., 2014b), more studies are needed to see how reliable these findings are.

In conclusion, this study illustrates that network alterations already exist at an early stage in children with focal epilepsy whereas no significant alterations were found for generalized epilepsies. Since network alterations were only found with the MST approach, we conclude that these changes are subtle at the beginning of the disease and aggravate at later stages as a result of persisting seizures. The network differences between focal and generalized epilepsies suggest that functional dynamics are different, which could proof useful in providing a scientific rationale for the classification of epilepsies. More studies relating functional network changes to clinical profiles are needed.

CHAPTER 8

IMPROVED DIAGNOSIS IN CHILDREN WITH FOCAL EPILEPSY USING A MULTIVARIABLE PREDICTION MODEL BASED ON ELECTROENCEPHALOGRAPHY NETWORK CHARACTERISTICS

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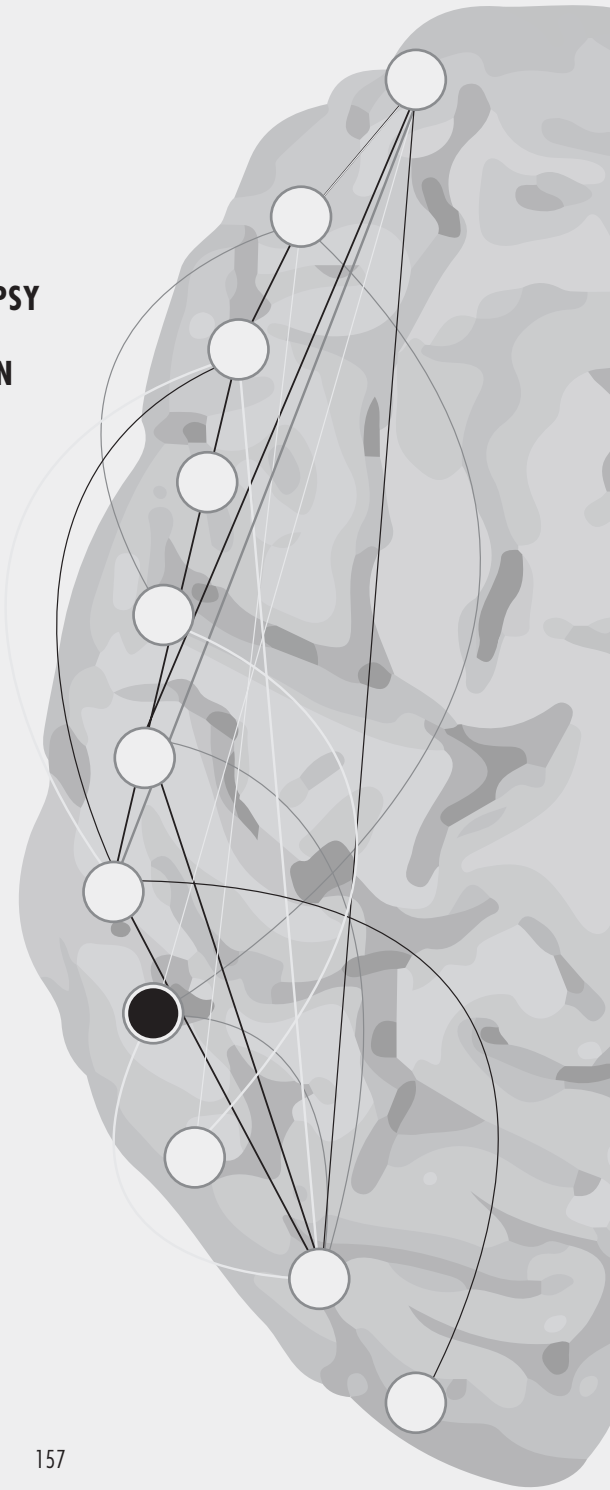


ABSTRACT

Objective: electroencephalogram (EEG) acquisition is routinely performed to support an epileptic origin of paroxysmal events in patients referred with a possible diagnosis of epilepsy. However, in children with partial epilepsies the interictal EEGs are often normal. We aimed to develop a multivariable diagnostic prediction model based on EEG functional network characteristics.

Methods: routinely performed interictal EEG recordings at first presentation of 35 children diagnosed with partial epilepsies, and of 35 children in whom the diagnosis epilepsy was excluded (control group), were used to develop the prediction model. Children with partial epilepsy were individually matched on age and gender with children from the control group. Periods of resting-state EEG, free of abnormal slowing or epileptiform activity, were selected to construct functional networks of correlated activity. We calculated multiple network characteristics previously used in functional network epilepsy studies and used these measures to build a robust, decision tree based, prediction model.

Results: based on epileptiform EEG activity only, EEG results supported the diagnosis of with a sensitivity and specificity of 0.77 and 0.91 respectively. In contrast, the prediction model had a sensitivity of 0.96 [95% confidence interval: 0.78–1.00] and specificity of 0.95 [95% confidence interval: 0.76–1.00] in correctly differentiating patients from controls. The overall discriminative power, quantified as the area under the receiver operating characteristic curve, was 0.89, defined as an excellent model performance. The need of a multivariable network analysis to improve diagnostic accuracy was emphasized by the lack of discriminatory power using single network characteristics or EEG's power spectral density.

Significance: diagnostic accuracy in children with partial epilepsy is substantially improved with a model combining functional network characteristics derived from multi-channel electroencephalogram recordings. Early and accurate diagnosis is important to start necessary treatment as soon as possible and inform patients and parents on possible risks and psychosocial aspects in relation to the diagnosis.

INTRODUCTION

Epilepsy is a common neurological disorder, yet, accurate diagnosis and classification at an early stage still poses a challenge to the clinician. The diagnosis of epilepsy is primarily based on the clinical history and may be supported by information provided by interictal EEG recording. For proper classification of the epilepsy syndrome the EEG is indispensable. Additional neuroimaging or sleep deprivation EEG is often used when the initial clinical diagnosis is not conclusive or when more information is needed for classification of the epilepsy syndrome and assessment of prognosis. Clinical diagnosis has a high interobserver variation. One study found that 25 percent of patients were incorrectly diagnosed as having had a seizure at the initial presentation (Hamiwka et al., 2007). In addition, evaluation of EEG abnormalities may be subjective (Niedermeyer and Lopes da Silva, 1987) and is not very sensitive. For example, epileptiform activity is demonstrated only in 29 to 55 percent of patients on routinely performed EEG recordings (Goodin et al., 1990). To a lesser extent, EEG abnormalities may also be found in healthy controls, especially in children (Smith, 2005). Together, interictal EEG recordings are supportive but often not conclusive in the initial clinical diagnosis of epilepsy. Developing EEG measurements with increased sensitivity and specificity would be highly valuable in the early clinical diagnosis of epilepsy. Accurate diagnosis at an early stage may allow more rapid optimization of treatment and improve counselling with regard to lowering risks with necessary life rules and restrictions. Particularly children with partial epilepsy could benefit from early and accurate diagnosis, since interictal epileptiform EEG activity is often absent (Smith, 2005), possibly causing a delay in diagnosis and decision-making.

In this study we used the expanding knowledge on functional neural network organization in normal and diseased brain (Bassett and Bullmore, 2009). Brain network organization is typically summarized using multiple network characteristics such as global efficiency, local clustering, power-law degree distribution and centrality measures (Rubinov and Sporns, 2010). Recently, it has been shown that interictal functional network characteristics differ between controls and patients with chronic partial epilepsy (Bettus et al., 2009; Chavez et al., 2010; Horstmann et al., 2010; Liao et al., 2010; Vlooswijk et al., 2011).

We aimed to explore the clinical value of functional network characteristics by investigating network characteristics separately and combined in a multivariable diagnostic prediction model. We hypothesized that functional network characteristics enhance sensitivity and specificity of diagnosis of partial epilepsy in children at initial assessment.

MATERIALS AND METHODS

Patients

Children referred, between January 2006 and December 2010, to the outpatient department of pediatric neurology, University Medical Center Utrecht, The Netherlands after one or more suspected epileptic event(s) were eligible for our study. We included children who were eventually diagnosed with new onset partial epilepsy. Children with neurological or psychiatric co-morbidities, including developmental delay, were excluded. The clinical diagnosis of epilepsy was defined by at least two unprovoked seizures within one year, judged by two neurologists to be of epileptic origin. The clinical diagnosis was supported in a subset of patients by epileptiform abnormalities (interictal epileptiform discharges (IEDs) such as sharp waves, (poly) spikes or (poly) spike-wave complexes or abnormal slowing), on routinely performed EEG. In patients clinically diagnosed with epilepsy but with a normal routine EEG recording, the diagnosis was confirmed by subsequent sleep deprivation EEG recordings, neuroimaging or clinical follow-up with history of more highly suspected events. An MRI was performed in all children diagnosed with epilepsy, not classified as idiopathic focal epilepsy. Epilepsy was excluded in the control group, based on clinical history, EEG results, and at least one year of uneventful follow up. This control group was individually matched with the patient group on gender and age. Neither patients nor controls had a history of febrile seizures, generalized epilepsy, or were on (chronic) anticonvulsive medication.

The institutional ethical committee approved the study and concluded that the Dutch Medical Research Involving Human Subjects Act did not apply, and written informed consent was not required.

Data acquisition and selection

Routinely performed interictal EEG recording was available for each child. Interictal EEGs were recorded according the international 10-20 system (SystemPlus Evolution, Micromed) in awake and eyes closed (resting-state)

condition against an average reference electrode. Impedance of each electrode was kept below 5 kΩ. Data was high- and low-pass filtered at 0.16 and 70 Hz, respectively. Sampling frequency was 512 Hz.

All EEG recordings contained 21 scalp electrodes (F8, F4, Fz, F3, F7, T8, C4, Cz, C3, T7, P8, P4, Pz, P3, P7, O1, O2, Fp1, Fp2, A1, and A2) and were visually inspected (EvD). To assure stable EEG brain dynamics for the calculation of network characteristics, for each subject we selected four epochs (eight seconds each) at the beginning of the interictal EEG recording (Boersma et al., 2011; Douw et al., 2010a). All epochs were free of IEDs, abnormal slowing, and electrocardiographic or motion-induced artifacts. Two frontoparietal and basal temporal electrodes (Fp1, Fp2, A1, and A2) were excluded to minimize eye-movement artifacts. The epochs were independently re-inspected by a clinical epileptologist (FEJ) on artifacts and IEDs. Finally, all selected EEG epochs were converted to ASCII files to enable functional network analysis. All data were additionally filtered to obtain standard broadband frequencies in the range of 0.5 to 45 Hz.

To check whether subjects with partial epilepsy could be distinguished from controls by means of spectral analysis, we computed the relative and absolute power spectral densities averages over epochs and subjects (Hamilton, 1994).

Computation of functional network characteristics

Individual functional EEG networks were constructed for each subject using their broadband filtered data. Functional network organization is based on the relatively new concept of functional connectivity. The statistical interdependencies for each pair of EEG electrode time series are considered as functional connectivity and used to construct a functional network per subject for each of the four epochs and were averaged per subject. Multiple complementary methods exist to estimate the statistical interdependency between two time series (Pereda et al., 2005). We based our functional network construction on a functional connectivity index that was previously applied in epilepsy studies, namely the synchronization likelihood (SL) (Altenburg et al., 2003; Douw et al., 2010a; Ferri et al., 2004; Ponten et al., 2007). SL detects both linear and nonlinear dependencies between the time series and is considered to be a measure of generalized synchronization (Stam and Van Dijk, 2002). The SL expresses the functional connectivity as a value between 0 and 1, and allows the construction of weighted functional networks

(i.e., the connectivity strength between two electrodes is preserved) (Stam and Reijneveld, 2007). Weighted SL networks were constructed using BrainWave software (http://home.kpn.nl/stam7883/brainwave.html).

We have chosen to include network characteristics used to study functional networks in epilepsy and published recently in neuroscience papers (for review see (Rubinov and Sporns, 2010)). For each weighted network, we calculated the following characteristics: weighted degree centrality (strength), weighted shortest path length, weighted clustering coefficient, weighted betweenness centrality, weighted closeness centrality and weighted eigenvector centrality. An additional characteristic was the powerlaw distribution index (Clauset et al., 2007; Newman, 2005). Apart from mean values of each network characteristic across all network nodes, we calculated both minimal and maximal values to increase the information used for building a multivariable diagnostic prediction model. The mathematical forms of the network characteristics are provided in the next paragraph.

Mathematical forms of the network characteristics

Network construction: for each of the 70 subjects we constructed a weighted undirected network, described by the graph $G = (N, W)$, where N is the set all 17 EEG electrodes and $W = \{w_{ij}\}$ is the $N \times N$ symmetric weight matrix, where $w_{ii} = 0$ and w_{ij} the synchronization likelihood index determined between electrode i and j (Stam and Van Dijk, 2002).

Weighted degree centrality (strength): four network characteristics of edge centrality were included in the model, which determined the relative importance of a node within the graph (Opsahl et al., 2010). The weighted degree centrality, or strength for node i was defined as

$$d_i^w = \sum_{j \neq i}^{n} w_{ij}$$

The minimal, mean and maximal strength were defined as

$$D_{\min}^w = \min\{d_i^w\}$$

$$D_{mean}^w = \frac{1}{n}\sum_{i=1}^{n} d_i^w$$

$$D_{\max}^w = \max\{d_i^w\}$$

Weighted shortest path length: for a given node *i* in the graph, the shortest path algorithm finds the path with lowest cost (i.e. the shortest path length) between that node and every other node. For the weighted shortest path length, the path between two nodes *i* and *j* is find by minimizing the sum of weights assigned to the edges on their path. The average shortest path length for node *i* to all other nodes is defined as

$$l_i^w = \frac{1}{n-1}\sum_{i \neq j} \min\{l_{ij}^w\}$$

Here, $\min\{l_{ij}^w\}$ is the weighted shortest path length between node *i* and *j*. We considered high values of the synchronization index as close functional distance and low values of the synchronization index as large functional distance (i.e. $l_{ij}^w = 1/w_{ij}$). In our dataset no disconnected nodes were present. The minimal, mean and maximal weighted shortest path were defined as

$$L_{\min}^w = \min\{l_i^w\}$$

$$L_{mean}^w = \frac{1}{n}\sum_{i=1}^{n} l_i^w$$

$$L_{\max}^w = \max\{l_i^w\}$$

Weighted closeness centrality: edges that have short distances to other edges have high closeness; this principle is used in the calculation of the weighted closeness centrality. Formally

$$cc_i^w = \left[\sum_{j \neq i}^{n} l_{ij}^w\right]^{-1}$$

The minimal, mean and maximal weighted closeness centrality were defined as

$$CC_{\min}^w = \min\{cc_i^w\}$$

$$CC_{mean}^w = \frac{1}{n}\sum_{i=1}^{n} cc_i^w$$

$$CC_{\max}^w = \max\{cc_i^w\}$$

Weighted betweenness centrality: the weighted betweenness centrality relies on the identification of the number of weighted shortest paths that pass through a node. The more passages the higher the betweenness centrality. The weighted betweenness centrality is defined as

$$bc_i^w = \frac{1}{(n-1)(n-2)} \sum_{j \neq k, k \neq i, j \neq i}^{n} \frac{g_{jk}^w(i)}{g_{jk}^w}$$

where g_{jk}^w is the shortest path between two nodes and $g_{jk}^w(i)$ is the number of those nodes that pass through node *i*. The mean and maximal weighted betweenness centrality were defined as

$$BC_{mean}^w = \frac{1}{n} \sum_{i=1}^{n} bc_i^w$$

$$BC_{\max}^w = \max\{bc_i^w\}$$

Eigenvector centrality: eigenvector centrality is based on the greatest eigenvector of the weight matrix W (Newman, 2007). The eigenvectors of the weight matrix indicate the nodes that show high connections with most other nodes. In contrast to the weighted degree centrality, it specifically favors nodes that are connected to nodes that are themselves central within the network (Bonacich, 1987). If λ is the largest eigenvalue and ec^w the corresponding eigenvector, then $W \times ec^w = \lambda \times ec^w$

or similar $ec^w = \frac{1}{\lambda} W \times ec^w$ and $ec_i^w = \mu \sum_{j}^{n} w_{ij} ec_j^w$, where $\mu = \frac{1}{\lambda}$ is the proportionality

factor so that ec_i^w is proportional to the sum of connectivity scores of all nodes connected to it. We used the minimal, mean and maximal values of the eigenvector in our model, defined as

$$EC_{\min}^w = \min\{ec_i^w\}$$

$$EC_{mean}^w = \frac{1}{n} \sum_{i=1}^{n} ec_i^w$$

$$EC_{\max}^w = \max\{ec_i^w\}$$

Weighted clustering coefficient: the clustering coefficient is a measure of degree to which nodes in a graph tend to cluster together. We used the weighted clustering coefficient (Onnela et al., 2005; Saramäki et al., 2007). The weighted clustering coefficient for each node *i* was defined as

$$c_i^w = \frac{\sum_{j \neq i} \sum_{h \neq (i,j)} w_{ij}^{\frac{1}{3}} w_{ih}^{\frac{1}{3}} w_{jh}^{\frac{1}{3}}}{d_i^w (d_i^w - 1)}$$

and takes into account the weights of all edges in a triangle, excluding weights not participating in any triangle. The minimal, mean and maximal weighted clustering coefficient were defined as

$$C_{\min}^w = \min\{c_i^w\}$$

$$C_{mean}^w = \frac{1}{n} \sum_{i=1}^{n} c_i^w$$

$$C_{\max}^w = \max\{c_i^w\}$$

Power law scaling index: the collection of (weighted) degree centrality measures d_i^w in a functional brain network often forms a distribution, *p(d)*, that decays as a power law (Sporns et al., 2004). This power law decay ranges from a minimal value $d_{\min}$ to the maximal value, and scales with the index α as $p(d) = (\alpha - 1) d_{\min}^{\alpha - 1} d^{-\alpha}$. The power law scaling index α was one of the network characteristics included in the prediction model. The calculation of $d_{\min}$ and α was done by a robust maximum likelihood method (Newman, 2005).

Model development

The diagnostic prediction model was built with available software packages in the open-source R environment (Kung, 2007; Ripley, 2001). The predictive diagnostic model has been based on a robust ensemble algorithm, namely the random forest classifier (Breiman, 2001) using a free implementation provided as the random forest package (Liaw and Wiener, 2002) The core of the random forest classifier is the binary decision tree, a data type that stores elements hierarchically in nodes. Each decision tree is grown on different bootstrapped sample collections (i.e., randomly drawn instances with replacement from the original dataset) on

a randomly selected subset of all available predictors. The random selection of predictors increases the generalizability of the individual decision trees, whereas the collection of multiple decision trees in one forest increases model performance (Breiman, 2001).

For our data, a subset of 5 random predictors for each decision tree was found to give highest accuracy. After building the random forest diagnostic prediction model, we assessed the ability to differentiate between subjects with and without partial epilepsy using the area under the Receiver Operating Characteristic (ROC) curve (AUC) (Zou et al., 2003). An AUC higher than 0.8 reflects excellent discrimination (Hanley and McNeil, 1982). However, the AUC is typically considered to be too optimistic when the diagnostic model is tested on the same data that is used to build the model. Internal validation methods correct for this. We used the regular bootstrapping approach for internal validation, which is the preferred method when dealing with relative small datasets (Harrell Jr et al., 1984; Steyerberg et al., 2003). With this method, the model is rebuild on multiple random samples drawn with replacement from the full dataset. In this study we only report the bootstrap corrected results.

Statistical analysis

Group differences on all network characteristics were individually assessed with the independent Student T-test. Each diagnostic prediction model was fit on 1000-bootstrapped realization of the original dataset. We calculated a bootstrap corrected average ROC curve and corresponding average AUC, sensitivity, specificity, positive predictive and negative predictive values. All statistical analyses were performed in R using the pROC (Robin et al., 2011) package (Ripley, 2001).

RESULTS

Patient characteristics

A total of 419 children visited the outpatient department of pediatric neurology, between January 2006 and December 2010, after a recent event of possible epileptic origin. In total, 75 children were diagnosed with generalized epilepsies, 69 with partial epilepsy and 38 with febrile seizures. In 52 cases, diagnosis remained undetermined but clinical follow-up revealed no event(s). Of the 69 patients who were eventually diagnosed with partial epilepsy, only 35 patients both met our strict inclusion criteria and had an EEG recording of sufficient

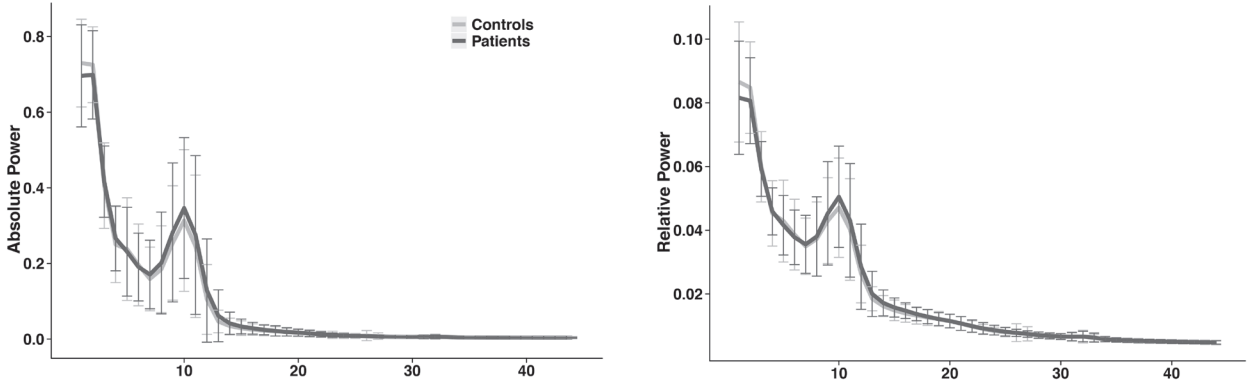
quality for functional network analysis (11 girls and 24 boys, mean age 10.1 ± 3.4 years). In 185 children, epilepsy was excluded and an alternative clinical diagnosis was made for the paroxysmal event(s). From this group, 35 controls were selected; individually match on age and gender with the patient group (mean age 9.9 ± 3.1 years). Detailed clinical characteristics are provided as supplemental information (for patients *Table S1* and for controls *Table S2*). None of the children had paroxysmal event in the days prior or post EEG recording, thereby excluding pre- or postictal changes of the EEG signal (Ponten et al., 2007). Epileptiform interictal EEG activity was present in 77% (27 out of 35) of patients and supported clinical diagnosis. Subsequent sleep deprivation EEG was performed in 5 patients showing epileptiform activity in all cases. In the remaining 3 patients, the diagnosis of epilepsy was reconfirmed by clinical follow-up. The average number of seizures prior to presentation at the outpatient department was 5 (range: 1–36). The events of the children in the control group were eventually diagnosed as syncope (11 cases), behavioural/ psychogenic events, including tic and stereotypy (11 cases), staring/non-attentiveness without epileptic origin (7 cases), pavor nocturnus (3 cases), arrhythmia (1 case), segmental non-epileptic myoclonic jerks (1 case) and breath holding spells (1 case). In the control group, aspecific EEG abnormalities were present in 9% (3 out of 35).

Power spectrum and network characteristics

Power spectral density revealed no differences between patients and controls (Figure 1). None of the individual network characteristics was significantly different between groups (Figure 2). The lack of discriminatory power in the frequency analysis and individual network characteristics emphasized the need for a multivariable model. The network characteristics used in the multivariable model contained unique information, as shown in our correlation matrix (Figure 3).

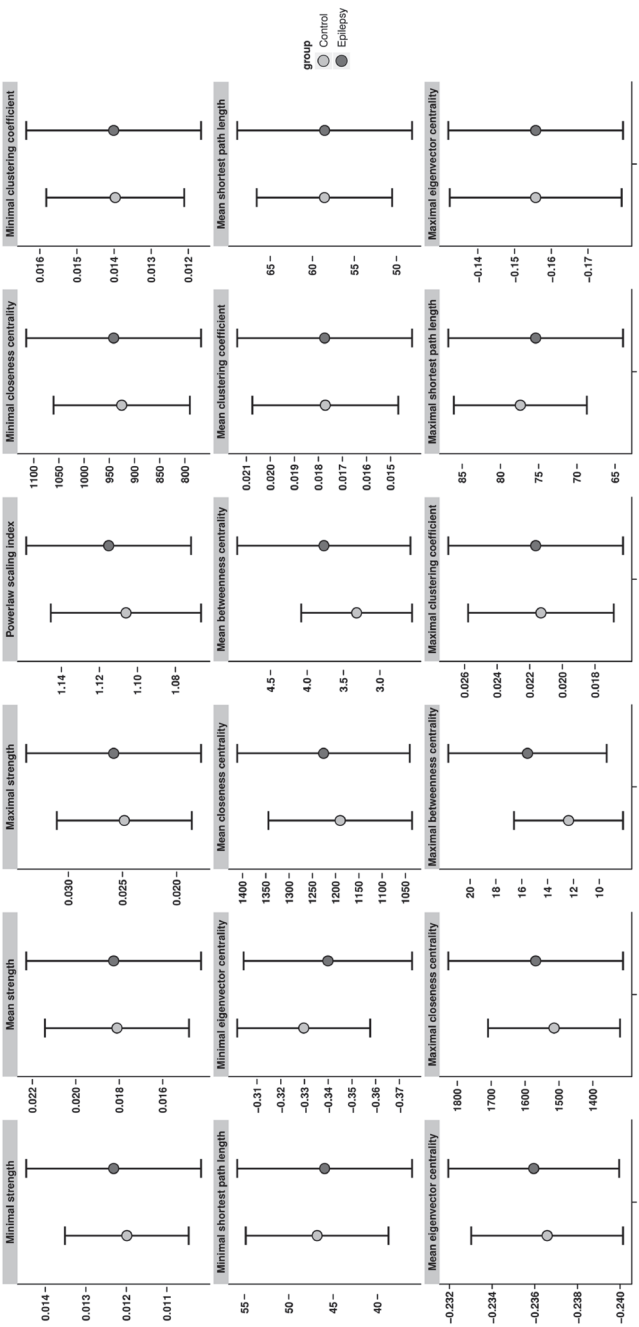
Diagnostic model

The ROC curve for the predictive model using broadband network characteristics is shown in Figure 4. The model had a mean sensitivity of 0.96 [95% confidence interval (CI): 0.78 – 1.00], mean specificity of 0.95 [CI: 0.76 – 1.00], mean positive predictive value of 0.96 [CI: 0.82 – 1.00] and a mean negative predictive value of 0.96 [CI: 0.81 – 1.00]. The model performance was excellent, with an AUC of 0.89 [CI: 0.80 – 0.95]. We found similar results in terms of model



Power spectral density plots of patients and controls

Mean absolute and relative power spectral densities between 0 and 45 Hz averaged over epochs and subjects per group. Variation, defined as the standard deviation, is indicated by cross bars. Spectral densities between groups largely overlap.

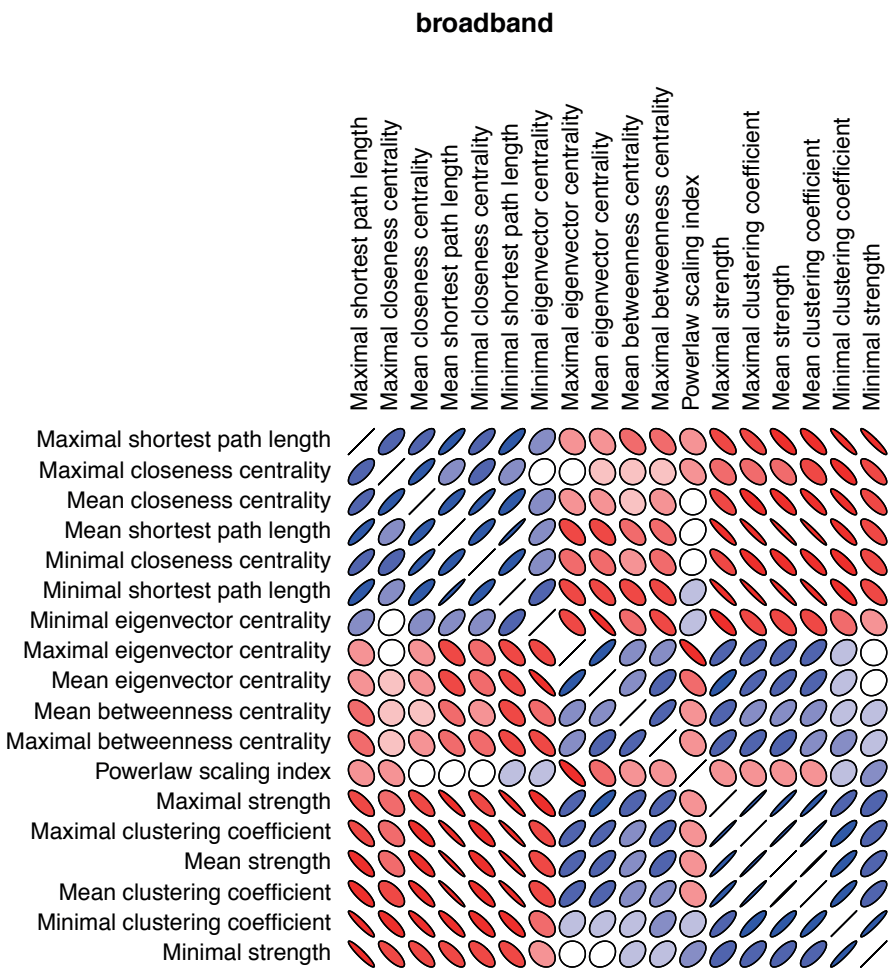


F2

Individual network characteristics of patients and controls

Mean and standard deviation of all network characteristics, used in the predictive model, calculated for the broadband frequency. No significant differences were found for each characteristic between groups.

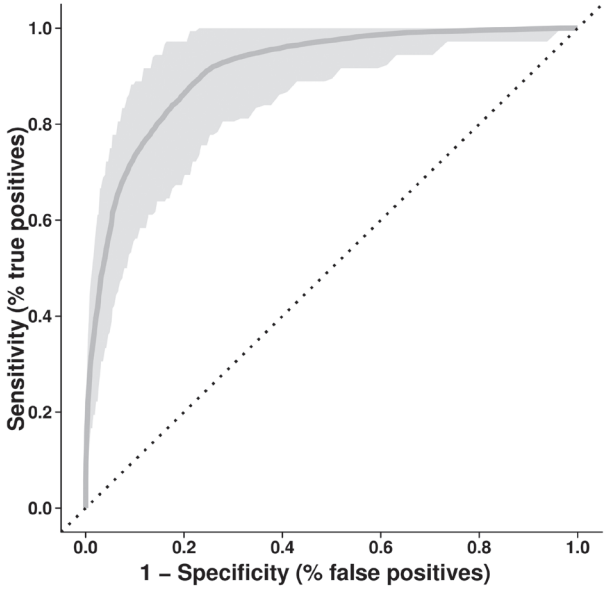
F3



Correlation matrix included network characteristics

Pearson correlation matrix of all network characteristics for the broadband frequency. Red color indicates positive correlation, blue color indicates negative correlation. Tensor anisotropy indicates strength of correlation (0 correlation, circular; full correlation, single line).

F4



ROC curve

ROC curve and 95% confidence interval for the network characteristics based on the broadband frequency.

performance if the analysis was repeated for specific frequency bands including delta band (0.5 – 4Hz), theta band (4 – 8Hz), alpha band (8 – 12Hz), beta band (12 – 30Hz) and gamma band (30 – 45Hz) (data not shown).

In an additional sub analysis, we bootstrap validated the model specifically using only the subset of 8 epilepsy patients of whom routinely performed EEG recordings were judged normal, resulting in a sensitivity of 0.86 [CI: 0.64 – 1.00]. Similarly, we tested the subset of 3 controls in whom routinely performed EEG recordings contained aspecific abnormalities and in these patients the model was found to have a good performance as well with a specificity of 0.81 [CI: 0.33 – 1.00].

CONCLUSION

In this study we were able to build a highly accurate diagnostic prediction model to distinguish children with partial epilepsy from children who were judged to have had non-epileptic events, with network analysis on resting-state epochs of routinely performed interictal EEG recordings. The diagnostic power was high: sensitivity and specificity of 0.96 and 0.95 respectively. The values obtained by the diagnostic model clearly exceed the sensitivity and specificity based on epileptiform EEG activity only, namely 0.77 and 0.91 respectively.

There is a rising interest in understanding brain functioning from a network perspective. However, only few studies have aimed to explore the clinical value of these functional networks in epilepsy. For example, two studies have used a functional network approach to predict post-surgical outcome in epilepsy surgery. Wilke and colleagues studied invasive corticography recordings during epilepsy surgery to correlate betweenness centrality, to the resected cortical regions. They found that the betweenness centrality was correlated with the resected areas of patients who became seizure-free after surgery (Wilke et al., 2011). Yet another study, found that functional connectivity in patients with seizure-recurrence after epilepsy surgery was less lateralized compared to those who were seizure free (Negishi et al., 2011; Ortega et al., 2008a). Although these results are promising, the study domain has been limited to epilepsy surgery and data acquisition has been applied to specific patients only.

This study was undertaken to bridge the gap between the field of more fundamental computational neuroscience and daily clinical practice. The use of network characteristics in diagnosing epilepsy extends the method used in a recent study (Douw et al., 2010a). Douw and colleagues used EEG functional connectivity to diagnose epilepsy after a first suspected seizure and found a specificity of 0.76 and sensitivity of 0.62. The higher sensitivity and specificity values obtained in our study argue for a multivariable prediction design and machine learning models, such as the random forest classifier, although one may argue that the high sensitivity and specificity may be partly due to the homogeneity of our patients. Douw and colleagues found the theta frequency band to be most sensitive in terms of prediction. In our study we found excellent results using the broadband frequency, suggesting that the discriminatory power is not restricted to one specific frequency band.

A potential limitation of our study is that the inclusion was restricted to children diagnosed with partial epilepsy. Hence, this prediction model can be applied in children with partial epilepsy only. In addition, selecting resting-state EEG epochs in very young children can be challenging since EEG recordings may have motion-induced artifacts. Clear instructions and longer registrations could potentially overcome this limitation. Clearly, its clinical value will increase with extended inclusion of adults and other epilepsy syndromes.

Early accurate diagnosis is particularly valuable in young children to inform and guide parents, to prompt treatment decisions and to limit the period of uncertainty and unnecessary risks (Hamiwka et al., 2007). To explore the true diagnostic value of our proposed predictive model, larger studies are required, especially using external validation, although we corrected for too optimistic model results using stringent internal validation. The use of freely available software packages for model development validation should facilitate the process of external validation (Breiman, 2001; Kung, 2007; Liaw and Wiener, 2002; Ripley, 2001; Robin et al., 2011). Our prediction model, based on routinely performed EEG, is appealing due to the immediate clinical availability. Further, standard EEG epoch selection is straightforward, network calculations are relatively fast and software is freely available. Although current selection of epochs was performed manually, software is available to reduce the time spent to select 'resting state' epochs semi-automatically (Dalponte et al., 2007).

Indications for future research are multiple. Use of other modalities such as resting-state functional magnetic resonance imaging, high density EEG and magnetoencephalogram may lead to development of diagnostic models with even higher accuracy due to their superior spatial resolution of functional networks.

In conclusion, although more and larger studies are needed, this study clearly shows that functional network characteristics are promising and clinically useful in the early diagnosis of partial epilepsy in children after their first seizure(s).

Acknowledgments

The authors would like to thank Anja Ezendam, EEG technician, UMC Utrecht, for her help in retrieving and selecting interictal EEG recordings for our study.

ST1

	Gender (Female/Male)	Age (years) (at presentation)	Number of seizures (prior to referral)	diagnosis	EEG report	Neuroimaging report
1	Female	4.6	2	Idiopathic epilepsy	IEDs in left central region	No abnormalities
2	Male	5.1	1	Crypotogenic epilepsy	Bilateral IEDs in occipital region	No abnormalities
3	Female	5.1	36	Crypotogenic epilepsy	IEDs in right central region	No abnormalities
4	Male	5.3	1	Idiopathic epilepsy	Abnormal slowing in right central region	No abnormalities
5	Female	6.2	1	Idiopathic epilepsy	Spikes in left frontopolar region	No abnormalities
6	Female	6.8	unknown	Symptomatic epilepsy	IEDs and spikes in left occipital region	Small hyperintense lesions in occiptal region
7	Male	7.0	3	Idiopathic epilepsy	IEDs in left central region	No abnormalities
8	Female	7.1	5	Idiopathic epilepsy	IEDs and spikes in right centrotemporal region	No abnormalities
9	Male	7.2	1	Symptomatic epilepsy	No abnormalities	Several hyperintense lesions in basal ganglia
10	Male	7.4	1	Idiopathic epilepsy	IEDs in left occipital region	No abnormalities
11	Male	7.9	1	Idiopathic epilepsy	IEDs in left centrotemporal region	No abnormalities
12	Female	8.4	1	Idiopathic epilepsy	IEDs and spikes in right centrotemporal region	No abnormalities
13	Male	9.0	4	Idiopathic epilepsy	Spikes in left central region	No abnormalities
14	Male	9.0	1	Idiopathic epilepsy	IEDs in left parietal region	No abnormalities
15	Male	9.2	1	Crypotogenic epilepsy	IEDs in left parietal region	No abnormalities
16	Male	9.2	5	Crypotogenic epilepsy	No abnormalities	No abnormalities
17	Female	9.4	unknown	Crypotogenic epilepsy	Spikes in left temporal region	Small subcortical lesion in cerebellum
18	Male	9.6	30	Crypotogenic epilepsy	No abnormalities	Several perivascular abnormalities
19	Male	9.6	1	Idiopathic epilepsy	No abnormalities	No neuroimaging available
20	Male	9.9	2	Idiopathic epilepsy	Bilateral spikes in centroparietal region	No abnormalities
21	Male	10.4	1	Symptomatic epilepsy	Abnormal slowing and spikes in right centroparietal region	Several (sub)cortical lesions
22	Female	10.8	unknown	Crypotogenic epilepsy	No abnormalities	No abnormalities
23	Female	12.1	3	Symptomatic epilepsy	Abnormal slowing in left frontal region	Arachnoidal cyst in left temporal lobe
24	Male	12.3	15	Symptomatic epilepsy	No abnormalities	Gliosis and atrophy in left parietal lobe

Table S1. Continued

	Gender (Female/Male)	Age (years) (at presentation)	Number of seizures (prior to referral)	diagnosis	EEG report	Neuroimaging report
25	Male	12.5	2	Crypotogenic epilepsy	No abnormalities	No abnormalities
26	Male	12.9	7	Idiopathic epilepsy	Bilateral IEDs in temporal regions	Small cortical dysplasia in left frontal/ insular region
27	Male	13.1	3	Crypotogenic epilepsy	Abnormal slowing and IED in right frontopolar region	No abnormalities
28	Male	13.3	1	Idiopathic epilepsy	Spikes and IED in temporal region	No neuroimaging available
29	Male	13.3	unknown	Symptomatic epilepsy	IEDs in left temporal region	Left hippocampus atrophy
30	Female	14.0	10	Symptomatic epilepsy	IEDs in right temporal region	Right temporal arteriovenous malformation
31	Male	14.2	2	Crypotogenic epilepsy	IEDs in left frontopolar region	Several hyperintense lesions
32	Male	15.3	2	Crypotogenic epilepsy	Abnormal slowing and spikes in left temporal region	No abnormalities
33	Male	15.3	unknown	Crypotogenic epilepsy	Spikes in left frontotemporal region	No abnormalities
34	Female	15.4	2	Symptomatic epilepsy	Spikes in midtemporal region	No neuroimaging available
35	Male	16.8	2	Crypotogenic epilepsy	No abnormalities	No abnormalities

Clinical characteristics of 35 children with new onset partial epilepsy

ST2

	Gender (Female/Male)	Age (years) (at presentation)	diagnosis	EEG report
1	Female	5.5	Syncope	No abnormalities
2	Male	5.4	Staring/non-attendance	No abnormalities
3	Female	5.5	Breath holding spells	No abnormalities
4	Male	6.1	Behavioural/psychological	No abnormalities
5	Female	6.1	Behavioural/psychological	No abnormalities
6	Female	6.1	Night terrors	No abnormalities
7	Male	6.7	Staring/non-attendance	Abnormal slowing in temporal regions
8	Female	7.3	Night terrors	No abnormalities
9	Male	7.1	Behavioural/psychological	No abnormalities
10	Male	7.3	Behavioural/psychological	No abnormalities
11	Male	8.2	Behavioural/psychological	No abnormalities
12	Female	8.5	Night terrors	No abnormalities
13	Male	8.3	Syncope	No abnormalities
14	Male	8.6	Behavioural/psychological	No abnormalities
15	Male	8.8	Behavioural/psychological	No abnormalities
16	Male	9.2	Syncope	No abnormalities
17	Female	8.9	Behavioural/psychological	No abnormalities
18	Male	9.4	Arrhythmia	No abnormalities
19	Male	9.7	Staring/non-attendance	No abnormalities
20	Male	9.7	Behavioural/psychological	No abnormalities
21	Male	10.1	Syncope	No abnormalities

Table S2. Continued

	Gender (Female/Male)	Age (years) (at presentation)	diagnosis	EEG report
22	Female	11.5	Behavioural/psychological	No abnormalities
23	Female	11.8	Staring/non-attendance	Abnormal slowing and aspecific spikes in central regions
24	Male	10.2	Staring/non-attendance	No abnormalities
25	Male	11.5	Staring/non-attendance	No abnormalities
26	Male	11.6	Syncope	No abnormalities
27	Male	11.9	Syncope	No abnormalities
28	Male	12.2	Staring/non-attendance	No abnormalities
29	Male	12.6	Syncope	Aspecific spikes in frontal regions
30	Female	14.5	Syncope	No abnormalities
31	Male	12.9	Behavioural/psychological	No abnormalities
32	Male	14.6	Syncope	No abnormalities
33	Male	15.6	Syncope	No abnormalities
34	Female	15.5	Segmental myclonus	No abnormalities
35	Male	15.8	Syncope	No abnormalities

Clinical characteristics of 35 controls

CHAPTER 9

A PREDICTION MODEL TO DIAGNOSE CHILDHOOD EPILEPSY AFTER A POSSIBLE SEIZURE

Submitted

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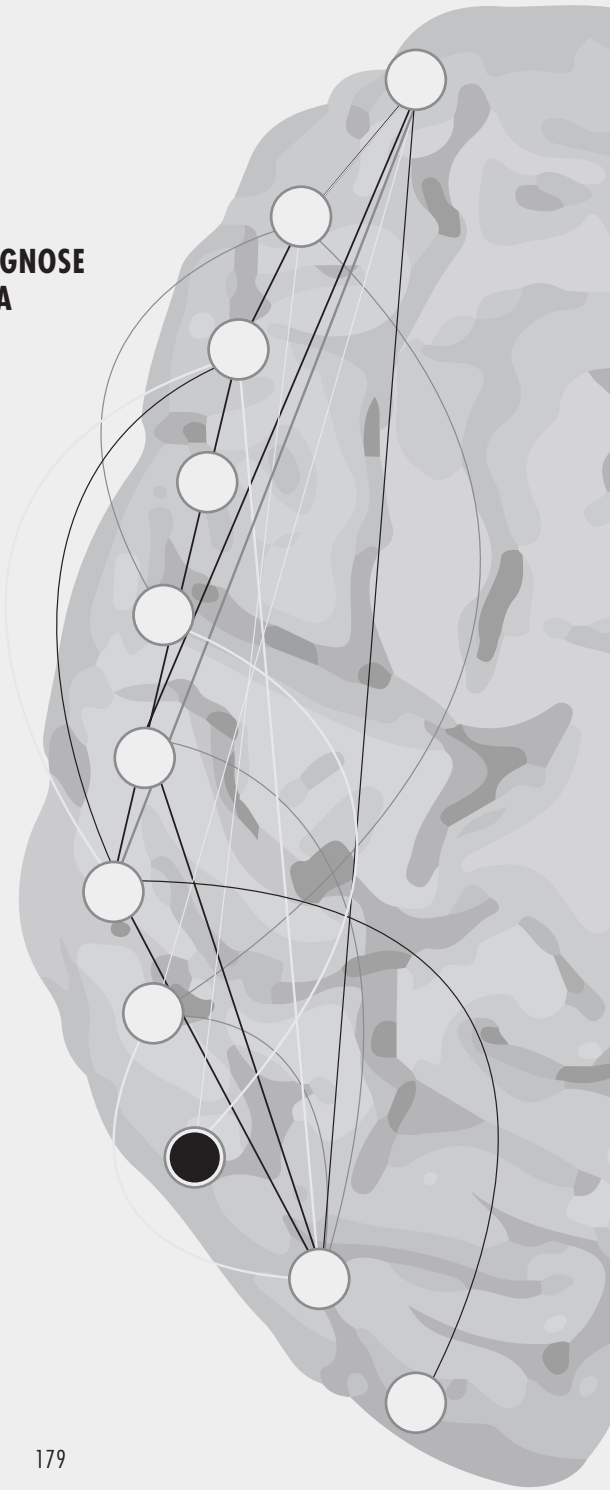
*Equal contribution



ABSTRACT

Objective: the clinical profile of children who suffered from a possible seizure is heterogeneous and the sensitivity of diagnostic testing is limited. This hampers early and accurate diagnosis. We developed a model to reliably predict diagnosis of epilepsy in children after a possible seizure by combining routinely available information at first consultation.

Methods: we collected clinical data of 451 children who visited our first seizure clinic between January 2008 and May 2013. The gold standard outcome, classified as epilepsy or no epilepsy, was based on the eventual diagnosis after clinical follow-up. A multivariate logistic regression model was fitted on data from 70% of randomly selected children. Patient characteristics (age, gender), clinical characteristics (age of first seizure, event frequency, developmental status, event description, medical and family history) and standard electroencephalography report were used as predictor variables. Model performance was validated with data from the remaining 30% of children using a receiver operating characteristic (ROC) curve.

Results: overall model performance was excellent, with an area under the ROC curve (AUC) of 0.90 [95% confidence interval (CI) 0.83 to 0.97]. Model performance in a selective sub-population of 177 children with unclear diagnosis at initial consultation was good with an AUC of 0.76 [CI 0.68 to 0.83].

Significance: this model may prove to be a valuable tool in clinical practice as all predictor variables are routinely available at first consultation. A web-application is provided to facilitate the diagnostic process for clinicians who are confronted with children with paroxysmal events, suspected of suffering from epilepsy.

INTRODUCTION

A first possible epileptic seizure is a frightful event for children and their caregivers (Besag et al., 2005). When the diagnosis of epilepsy is considered, the associated psychosocial aspects may negatively influence the child's health-related quality of life (Hamiwka et al., 2008; Modi et al., 2009). Possibly, recurrent epileptic seizures are harmful to the developing brain, leading to cognitive and behavioral deficits (Berg et al., 2014; Berg et al., 2012; O'Callaghan et al., 2011). This emphasizes the need for an early and accurate diagnosis. However, in clinical practice epileptic origin of seizures in children is often underrecognized due to low specificity and sensitivity of the heterogeneous clinical symptoms and due to the limited sensitivity of interictal electroencephalography (EEG) recordings (Dayan et al., 2011; Hamiwka et al., 2007; Stroink et al., 1998). In children with unclear epilepsy diagnosis additional investigations, such as prolonged or sleep deprived EEG recordings and magnetic resonance imaging, are required, with subsequent delay in assessment of the final diagnosis (Hamiwka et al., 2007; King et al., 1998; Shinnar et al., 1994). Otherwise, diagnostic inaccuracy could also lead to a false diagnosis of epilepsy resulting in unnecessary antiepileptic drug treatment or outpatient admissions. To this end, a clinical tool that facilitates early and accurate diagnosis of epilepsy in children would be highly valuable.

Previous efforts were made to predict the development of epilepsy after first presentation (Camfield et al., 1985; Hirtz et al., 1984; Shinnar et al., 1990; Shinnar et al., 1994; Stroink et al., 1998), identifying prognostic clinical variables – including seizure types, neurological status and EEG abnormalities – that were associated with an increased risk of seizure recurrence. These studies, however, focused on first indisputable epileptic seizures and patients with ambiguous events were excluded from further analysis. These restrictions may hamper its value in daily practice, as diagnostic inaccuracy of first seizures is common (Leung et al., 2008). Also, children do not always present directly after a first possible seizure due to delayed recognition (Berg et al., 2014; Hamiwka et al., 2007).

In the present study we determined the value of routinely available predictor variables combined in a single prediction model to estimate the probability of epilepsy at first consultation after a possible seizure. To that aim we constructed and validated a multivariate prediction model built with data extracted from a

high-quality cohort database of clinical information available at first consultation. To improve the implementation in clinical practice, we constructed a web-application that may assist caregivers to estimate the individualized probability of a future diagnosis of epilepsy in children who present after a first possible seizure.

METHODS

Subject selection

We collected the clinical data of 451 children who visited the first seizure clinic (FSC) of the department of pediatric neurology, University Medical Center Utrecht, the Netherlands, between January 2008 and May 2013. Children were referred to the FSC by a general practitioner, pediatrician, neurologist, or via the emergency department after one or more possible seizures. In addition to clinical history and physical examination, a routine EEG recording performed at time of first consultation was available from each child. At least one year of clinical follow-up was available for all children who were eventually diagnosed with epilepsy, or when their diagnosis remained inconclusive. Children in whom diagnosis of epilepsy was discarded, directly after first consultation, were referred back to their general practitioner with explicit instructions to report new possible seizures. Excluded from our study were children in whom the diagnosis of epilepsy had already been confirmed by the referring health care professional prior to presentation at the FSC. The institutional ethical committee approved the study and concluded that the Dutch Medical Research Involving Human Subjects Act did not apply, and written informed consent was not required.

Outcome definition

The outcome measure was binary, namely the presence or absence of epilepsy. Eventual diagnosis of epilepsy was defined in two ways: *i*) at least two unprovoked seizures within one year judged by an experienced child neurologist to be epileptic of origin, or *ii*) one unprovoked seizure supported by clear EEG abnormalities at first presentation or at later sleep-deprived or prolonged EEG recordings, or the demonstration of a typical epileptogenic brain lesion on MRI. Children in whom the diagnosis remained unclear were explicitly instructed to return for a clinical revision when suspected new events would occur. If diagnosis of epilepsy was discarded, based on clinical history, EEG results, other ancillary

investigations or at least one year of uneventful follow up, we attempted to reappoint the diagnosis. Children in whom a definitive diagnosis (epilepsy or not) was inconclusive, or who were lost to follow-up were excluded from the analysis.

Predictor variables

Potential predictor variables were selected from previous cohort studies that found individual characteristics in children with newly diagnosed epilepsies (Arts et al., 2004; Berg et al., 2011; Camfield et al., 1993; Geerts et al., 2010; Sillanpää et al., 1998). Predictor variables were required to be routinely available at time of first consultation. From all children we selected 25 predictor variables. Information on age at presentation, gender, age of first event, medical history, relevant family history, psychomotor development, number of events and detailed event description, and the EEG report, was available and extracted into separate variables in a database. Age and age at first event were entered as continues variables truncated at month level. The medical history of a child was categorized into three binary predictor variables, namely 'neurological history' in the presence of a history of birth asphyxia, congenital or acquired brain lesions, head trauma, central nervous system infections, or migraine; 'psychiatric history' in the presence of a diagnoses of autism, attention deficit (hyperactivity) disorder or other psychiatric disorders, and the category 'known metabolic or genetic syndrome'. Relevant family history was categorized into the presence or absence of epilepsy, febrile seizures, migraine and seizures in first- or second-degree relatives or as 'other' when consanguinity of parents, cerebrovascular accidents or retardation was present in first- or second-degree relatives. Psychomotor development was categorized as normal or delayed, based on the information on the intelligence quotient (IQ of ≤ 70 was considered as intellectual disability) and type of schooling available at first consultation. Categorization was not mutually exclusive in these clinical categorical variables. EEG reports were categorized as presence or absence of 'focal epileptiform abnormalities' if (multi) focal spike or focal spike-wave-complexes were reported, 'generalized epileptic abnormalities' when generalized spikes or spike-wave complexes were reported, and 'aspecific non-epileptiform abnormalities' if no clear epileptiform activity was stated but focal or generalized slowing or other aspecific abnormalities were reported.

Clinical symptoms extracted from the event descriptions provided by caregivers and children were categorized into multiple predictor variables, namely: *i*) presence or absence of staring or non-attendance, *ii*) presence or absence of bilateral jerking or shaking, *iii*) presence or absence of stiffening or tonic posturing, *iv*) presence or absence of lateralizing symptoms, *v*) presence or absence of weakness or loss of muscle tone, *vi*) presence or absence of sensory signs (such as a sensation of tingling and visual or auditory hallucinations), and *vii*) presence or absence of automatism or viii) autonomic symptoms. Standard seizure classification, as proposed by the international league against epilepsy (ILAE) (Berg et al., 2010), could not be applied to categorize event description as a considerable part of the events were eventually non-epileptic in nature.

Quality assessment of event description

One author [EvD], blinded for the diagnosis at follow-up, classified the event description into the aforementioned predictor variables based on the clinical report. The reproducibility of this classification was tested in a random sample of twenty percent of patients by an experienced pediatric epileptologist [FEJ]. Inter-observer agreement was calculated with Cohen's kappa coefficient (Viera and Garrett, 2005). A kappa of one indicates perfect agreement; a kappa of zero indicates agreement equivalent to chance.

Multivariate prediction model

Data was missing for 8.0% of the database records. We imputed the missing values with single imputation to prevent biased results towards the complete case subjects (Donders et al., 2006; Greenland and Finkle, 1995).

Based on the imputed dataset a logistic regression model was used to estimate the log odds of the epilepsy probability as a linear function of the 25 predictor variables: log [probability (epilepsy) / probability (no epilepsy)] = $\alpha + \beta 1 \times x1 + \ldots + \beta n \times xn = \eta$ in which α is the intercept and $\beta 1$ to βn are the regression coefficients of predictor variables x1 to xn, and η the sum of the intercept and regression coefficient multiplied by the predictor variables. The exponent of the regression coefficients for the binary predictor variables provides the odds ratios between the two categories. A high odds ratio indicates a strong association between the predictor variable and the presence of epilepsy. The epilepsy probability in individual children is subsequently determined as:

$$P = \frac{1}{1 + e^{-\eta}}$$

The regression model was fitted based on data from seventy percent of randomly selected children. The remaining thirty percent was kept separate for internal model validation. We tested whether fitting the continuous predictor variables as restricted splines improved the logistic model, which was not the case, so these were kept included with their original values (Durrleman and Simon, 1989).

To assess the contribution of the EEG recording to the predictive value of multivariate models we developed, in addition to the full linear logistic regression model, two sub-models: one based only on clinical predictor variables, and one with only EEG predictor variables.

Internal model validation

The performance of the full model and the two sub-models was validated with data from the remaining thirty percent of children, using a receiver operating characteristic (ROC) curve. Sensitivity, specificity, balanced accuracy, positive and negative predictive values were computed for different epilepsy probability thresholds, covering the relevant range for clinical decision-making (20% to 80%). Sensitivity and specificity were corrected for prevalence of epilepsy in our cohort (Mercaldo et al., 2007). In addition, to test the potential value of the model in less straightforward cases, we separately tested the model in a subgroup of children in whom diagnosis remained uncertain after first consultation at the FSC. The modeling was done in R (http://www.R-project.org).

RESULTS

Patient characteristics

Data of 451 children who were referred to the FSC (mean age 6.6 ± 4.5 years; 195 girls) were eligible for this study. Demographics and clinical characteristics are provided in Table 1. Accurate information on exact age at first event was available for 302 children (67.0%). The number of events could be reliably determined for 275 children (61.0%). In 203 children (45.0%) the diagnosis was inconclusive after first consultation at the FSC and ancillary investigations or follow-up was required. After at least one year of follow-up, a definite classifying diagnosis – epilepsy or not – was available for 425 children (94.2%). In thirteen (2.9%) the diagnosis remained inconclusive and an additional thirteen were

lost to follow-up (Figure 1). Eventually 32.2% of the children referred to the FSC received the diagnosis of epilepsy. We provided the etiology and differential diagnosis (Table 2) according to the newly proposed ILAE classification when possible (Berg et al., 2010).

Quality assessment and model description

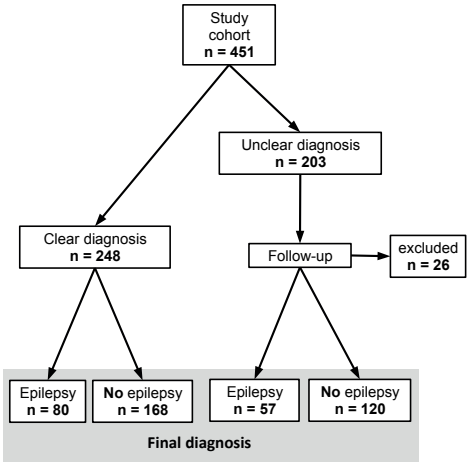
The interobserver agreement of event description was excellent, with a Cohen's kappa coefficient of 0.91. The multivariable odds ratio of each predictor variable for the full model is provided in Table 3. The predictor variables with the highest odds ratios were those from the EEG report: the presence of absence of generalized epileptiform activity, focal epileptiform activity or aspecific non-epileptic abnormalities. High odds ratios were also found for the event description predictor variables on automatisms and lateralizing symptoms and for the predictor variable on the presence of a neurological history.

Performance of prediction models

Figure 2 shows the ROC curves for the full multivariate model and the two sub-models, as tested on the validation cohort of 30% of the total group of children. Excellent performance was found for the full model, combining clinical and EEG predictor variables: AUC of 0.90 [CI 0.83 to 0.97]. The corresponding sensitivity, specificity, positive predictive value, negative predictive values are provided in Table 4. A low threshold (20%) results in a high negative predictive value of 0.95 [CI 0.87 to 0.98] but a lower positive predictive value (0.71 [CI 0.61 to 0.80]). Conversely, a high threshold (80%) corresponded with a high positive predictive value of 0.85 [CI 0.56 to 0.96] but a lower negative predictive value (0.74 [CI 0.70 to 0.77]) (Table 4). The sub-model based on only EEG predictor variables out-performed the model based on clinical predictor variables only: AUC of 0.85 [CI 0.77 to 0.92] versus AUC of 0.67 [CI 0.57 to 0.78], respectively.

The model performance for the prediction of epilepsy diagnosis based on the subgroup of children in whom diagnosis was inconclusive after first consultation was good, with an AUC of 0.76 [CI 0.68 to 0.83] when combining EEG and clinical factors (ROC curve in Figure S1). Corresponding sensitivity, specificity, positive predictive value, negative predictive value and balance accuracy values are provided in the supplementary materials (Table S1).

F1



Cohort flow-chart

451 children were referred to the FSC after (a) suspected event(s). In 248 children (54.9%), a definitive clinical diagnosis was given directly after clinical intake and routine EEG recording. In the remaining children, clinical follow-up and ancillary investigations were needed to give a definitive diagnosis. Eventually, 26 children were excluded due to lost to follow-up or diagnosis was inconclusive at the moment of the study. At least one year of clinical follow-up was available for all those children and the children who were eventually diagnosed with epilepsy.

T1

	All	Epilepsy	No Epilepsy
Number of children	451	137	288
Gender male (%)	256 (56.8)	85 (62.0)	160 (55.5)
Age (years)	6.6±4.5	7.6±4.0	6.2±4.6
Age at first event (years)	5.8±4.6	7.0±4.1	5.1±4.6
Event frequency (mean/median)	20/2	24/3	20/2
Seizure history (no/yes/febrile seizures)	365/38/48	99/19/19	247/14/27
Development (normal/delayed)	343/108	97/40	228/60
Medical history (%)			
- Normal	343 (76.1)	90 (65.7)	227 (78.9)
- Neurological	54 (12.0)	28 (20.4)	24 (8.3)
- Psychiatric	46 (10.2)	9 (6.7)	30 (10.4)
- Metabolic or genetic syndrome	21 (4.7)	10 (7.3)	7 (2.4)
Family history (%)			
- Normal	279 (61.9)	80 (58.4)	182(63.2)
- Epilepsy	91 (20.2)	32 (23.4)	55 (19.1)
- Migraine	34 (7.5)	7 (5.1)	22 (7.6)
- Febrile seizures	29 (6.4)	8 (5.8)	17 (5.9)
- Other	30 (6.7)	10 (7.3)	12 (4.1)

General characteristics of included children

Values are expressed as mean ± standard deviation unless otherwise indicated. 26 children did not receive a definitive diagnose due to lost to follow-up (n = 13) or diagnosis was unclear at the moment of the study (n = 13)

T2

Definitive Diagnosis	Number of children
Epilepsy	*137*
Etiology	
- Presumed genetic*	45
- Structural-metabolic	41
- Unknown	51
No epilepsy	*288*
Cause of paroxysmal event	
- Behavioural	133
- Febrile seizures	38
- Single seizure	33
- Syncope	30
- Migraine	13
- Breath holding spells	4
- Segmental myoclonus	3
- Alice in Wonderland syndrome	2
- Narcolepsia	2
- Tinnitus	1
- Not further specified	29

Definitive diagnosis after clinical examination and follow-up

ILAE classification of etiology is according to the newly proposed classification in 2010. *The presumed genetic group included children with myoclonic epilepsy of infancy or juvenile myoclonic epilepsy (n = 7), childhood or juvenile absence epilepsy (n = 15), benign epilepsy with centrotemporal spikes (n = 17) or benign epilepsy of childhood with occipital paroxysms (n = 6).

T3

Predictor variables	Odds ratio (95% confidence interval)
(Intercept)	0.01 (0.00 – 0.04)
Male	1.81 (0.84 – 4.09)
Age	1.04 (0.91 – 1.19)
Age at first event	1.00 (0.87 – 1.15)
Number of events	1.00 (1.00 – 1.01)
Delayed development	0.73 (0.28 – 1.78)
Event description	
Automatism	4.56 (1.80 – 12.00)
Lateralizing symptoms	3.39 (1.25 – 9.49)
Weakness or loss of muscle tone	2.91 (0.94 – 8.98)
Stiffening or tonic posturing	2.35 (0.74 – 7.19)
Staring or non-attendance	2.20 (0.63 – 8.28)
Bilateral jerking	1.74 (0.73 – 4.13)
Autonomic signs	1.10 (0.46 – 2.60)
Sensoric signs	0.51 (0.04 – 4.37)
Seizure history	
Non febrile seizures	3.07 (0.86 – 11.01)
Febrile seizures	2.10 (0.62 – 6.74)
Medical history	
Neurological	4.84 (1.63 – 15.69)
Metabolic or genetic syndrome	1.41 (0.24 – 8.85)
Psychiatric	1.19 (0.31 – 4.32)
Family history	
Epilepsy	2.35 (0.92 – 6.07)
Migraine	1.33 (0.26 – 5.56)
Other	1.21 (0.23 – 5.71)
Febrile seizures	0.66 (0.11 – 3.45)
EEG findings	
Focal epileptiform abnormalities	37.20 (13.67 – 115.37)
Generalized epileptiform abnormalities	26.01 (6.57 – 123.21)
Aspecific non-epileptiform abnormalities	4.90 (1.74 – 14.16)

Relative odds ratio of each predictor variable for epilepsy

Determined in a multivariate model including both clinical and EEG predictor variables, based on a randomly selected group of 70% of all patients.

DISCUSSION

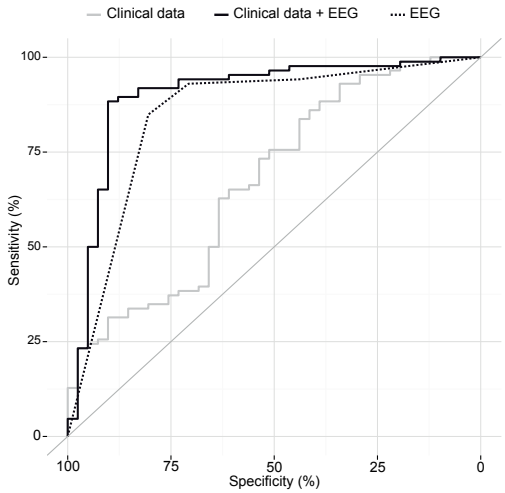
This study shows that the diagnosis of epilepsy can be predicted with a high likelihood in children at first presentation after one or more suspected events, with a limited set of predictor variables. The multivariate prediction model is a potentially valuable tool in clinical practice, as all predictor variables are routinely available at first clinical consultation.

Implications of the model

Clinicians increasingly rely on multivariate models to predict the presence of disease or disease prognosis based on combined clinical characteristics. Previous epilepsy studies have already shown that, by combining clinical data, it is possible to identify the most relevant clinical variables, which reliably predict disease outcome in children with newly diagnosed epilepsy. These studies report a variety of outcome measures, including the success rate of antiepileptic drug treatment (Arts et al., 2004; Geerts et al., 2010), the likelihood of complete remission of epileptic seizures(Berg et al., 2011) and the risk for patients to develop cognitive or behavioral deficits (Berg et al., 2012; Sillanpää et al., 1998). The results of these studies may improve counseling of patients once the diagnosis of epilepsy is established, and can guide adequate treatment during follow-up.

Similarly, early and accurate diagnosis – or exclusion – of epilepsy after a suspected event will diminish patients' ignorance but also wrongly applied stigmata, improve self-management, limit potential seizure-induced damage to the developing brain, prevent unnecessary antiepileptic drug treatment and unnecessary burden of ancillary investigations in children who are wrongly diagnosed. Our prediction model provides a rational approach to assist the clinician during the diagnostic process by combining routinely available clinical information in a multivariate way. More specifically, we expect our model to be useful as a screening tool to assess the likelihood of a possible seizure to be epileptic in origin and to help the clinician decide on the need for ancillary investigations. This model might be particularly of use for clinically more complicated cases in which each of the individual routinely available clinical variables proves insufficient to assess a diagnosis at first presentation. Our model performed well in children in whom diagnosis remained unclear after first presentation (AUC of 0.76).

F2



ROC curves

The ROC areas for the multivariate model with clinical predictor variables only (grey), was 0.67 [CI 0.57 to 0.78]. The ROC area for the EEG predictor variables only (dashed line), was 0.85 [CI 0.77 to 0.92]. The black line represents the ROC of the full model with both the clinical and EEG predictor variables, with an area of 0.90 [CI 0.83 to 0.97].

T4

Risk threshold	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Balanced Accuracy (95% CI)
0.2	0.90 (0.77 – 0.97)	0.83 (0.73 – 0.90)	0.71 (0.61 – 0.80)	0.95 (0.87 – 0.98)	0.85 (0.78 – 0.91)
0.35	0.88 (0.74 – 0.96)	0.88 (0.80 – 0.94)	0.78 (0.66 – 0.87)	0.94 (0.87 – 0.97)	0.88 (0.81 – 0.93)
0.5	0.73 (0.57 – 0.86)	0.94 (0.87 – 0.98)	0.86 (0.71 – 0.93)	0.88 (0.82 – 0.92)	0.87 (0.80 – 0.93)
0.65	0.59 (0.42 – 0.74)	0.95 (0.89 – 0.99)	0.86 (0.69 – 0.94)	0.83 (0.77 – 0.87)	0.83 (0.76 – 0.89)
0.8	0.27 (0.14 – 0.43)	0.98 (0.92 – 1.00)	0.85 (0.56 – 0.96)	0.74 (0.70 – 0.77)	0.75 (0.66 – 0.82)

Diagnostic performance measures at different probability thresholds.

Obtained from the multivariate model including both clinical and EEG predictor variables. CI = confidence interval, PPV = positive predictive value, NPV = negative predictive value.

As expected, predictor variables obtained from the EEG report strongly contributed to the discriminative power of the full multivariate model. This reflects the actual situation in daily practice. History taking is without doubt an important diagnostic instrument as it provides a coherent sequence of all the information provided (Guerrini, 2006). However, subjective reportage might obscure its value for clinical decision-making. The sub-model based on EEG predictors alone showed a superior performance as compared to the sub-model with only clinical predictor variables. Nevertheless, after assessing the most important aspect – whether the event is epileptic in origin – a treating physician needs to classify the specific epilepsy syndrome to initiate adequate treatment and predict prognosis. Here, clinical information is indispensible and a routine EEG recording alone will be insufficient. Conversely, EEG predictors showed an inferior performance compared to clinical predictors when tested on children in whom diagnosis remained unclear after first presentation (Figure S1). Again, this reflects the actual clinical situation in which incongruent EEG findings might obfuscate clinical diagnosis.

It would be valuable to explore possibilities to further improve performance of the model, and thus increasing positive predictive values in differentiating between the diagnosis of epilepsy and non-epileptic events. A possibility might be to include higher order information on brain connectivity derived from standard interictal EEG recordings (Douw et al., 2010b; Kerr et al., 2012; van Diessen et al., 2013c).

Strengths and limitations

A main strength of this study is the clinical applicability of the model. We collected data from a cohort of children after first presentation at our FSC with at least one year of follow up, rather than restricting ourselves to a predefined patient cohort. That is, we did not select children with first epileptic seizures but we included all newly presented patients irrespective of the number and nature of possible events prior to presentation. We believe that this approach will allow a better translation to the daily practice, as first seizures are often underrecognized (Berg et al., 2014; Hamiwka et al., 2007; Leung et al., 2008). Secondly, the model is based on clinical data that is routinely collected in standard care. Direct use of our model would therefore not financially burden the diagnostic work-up. We built a web application (www.invivonmr.uu.nl/epilepsy [online should the

paper be accepted for publication]) that enables clinicians to explore the added clinical value of the model in their daily practice and compute the probability for the diagnosis of epilepsy for an individual child at first presentation. Obviously, it is up to the clinician to finally conclude whether a child may truly have epilepsy or not, and whether ancillary diagnostics or treatment is indicated.

This study has limitations. Our model was developed on a randomly selected subgroup of patients and validated on the remaining cohort of children, both being recruited at the same first seizure clinic in a single institution. Furthermore, the incidence of epilepsy in our first seizure cohort was lower than in other comparable patient cohorts (Hamiwka et al., 2007; Stroink et al., 2003; Uldall et al., 2006), which might reflect a more liberal referral policy to our clinic. External validation is thus needed to investigate the value of the model in other cohorts and requires a separate dataset from one or more different institutions. Second, although positive and negative predicted values remain excellent at higher risk thresholds (Table 4), sensitivity is lower at high thresholds. This is a limitation of the model, but not necessarily a problem in daily practice. If, for a specific patient, the clinician wants to minimize the risk of falsely rejecting the diagnosis of epilepsy (comparable to setting a high risk threshold), clinical follow-up and ancillary investigations will likely be performed. Increasing the number of children in future validation studies allows the inclusion of more predictors that could be useful to boost the sensitivity of the model at higher risk thresholds. Third, we used generic categories based on unambiguous symptoms provided by the children or their caregivers to classify the event description. Although the interobserver variability was low in our study – suggesting a reliable classification – an accurate event description depends on a good observation and some symptoms might be difficult to recognize or interpret. This may have reduced the discriminative power of our model. Finally, no explicit follow-up data was collected for the children in whom the diagnosis of epilepsy was discarded directly after first consultation, which could have introduced false-negatives in our outcome variable. Nonetheless, we think the percentage of false-negatives is very low as these children were referred back to their general practitioner or referral specialist with explicit instructions to report new suspected events – if any – and there is a close collaboration between health care providers in the region and our institution. In addition, previous research has shown that the risk of false-negatives in a similar cohort is very small (Stroink et al., 2003). Similarly,

a subset of children in whom a single seizure was evaluated to be epileptic in origin was not diagnosed with epilepsy if ancillary investigations did not reveal epileptiform abnormalities or underlying epileptogenic brain lesions. Some of those children might develop epilepsy in the time beyond our follow-up period (Camfield et al., 1985; Shinnar et al., 1990).

Conclusion

Accurate and robust clinical models to predict a diagnosis of epilepsy based on clinical information available at first presentation in children with first suspected seizures are currently lacking. Our results indicate that multivariable prediction models may be valuable for decision-making at first presentation in children presenting with possible seizures and underscore the importance of an EEG recording in combination with a structured and extensive history taking at first consultation. Future research should evaluate the added value of a prediction model in clinical practice to manage expectations of patients, parents or caregivers and to prompt treatment in some cases.

SUPPLEMENTARY MATERIALS

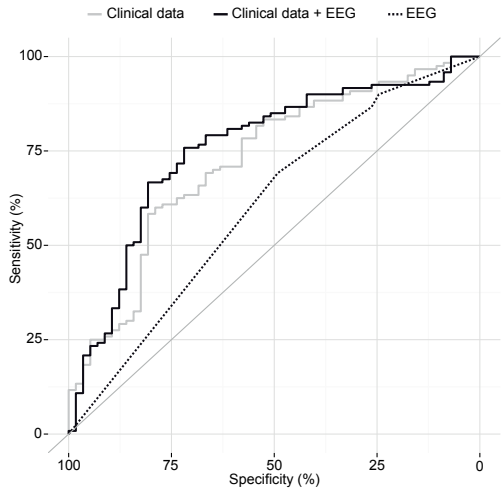
ST1

Risk threshold	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Balanced Accuracy (95% CI)
0.2	0.75 (0.62 – 0.86)	0.69 (0.60 – 0.77)	0.54 (0.46 – 0.61)	0.86 (0.79 – 0.90)	0.71 (0.64 – 0.78)
0.35	0.67 (0.53 – 0.79)	0.77 (0.68 – 0.84)	0.58 (0.48 – 0.66)	0.83 (0.77 – 0.88)	0.73 (0.66 – 0.80)
0.5	0.47 (0.34 – 0.61)	0.87 (0.79 – 0.92)	0.63 (0.50 – 0.74)	0.78 (0.73 – 0.82)	0.74 (0.67 – 0.80)
0.65	0.39 (0.26 – 0.52)	0.90 (0.83 – 0.95)	0.65 (0.49 – 0.77)	0.76 (0.71 – 0.79)	0.73 (0.66 – 0.80)
0.8	0.21 (0.11 – 0.34)	0.93 (0.86 – 0.97)	0.57 (0.37 – 0.75)	0.71 (0.68 – 0.74)	0.69 (0.62 – 0.76)

Diagnostic performance measures at different probability thresholds tested on children in whom diagnosis remained unclear after first presentation

CI = confidence interval, PPV = positive predictive value, NPV = negative predictive value.

SF1

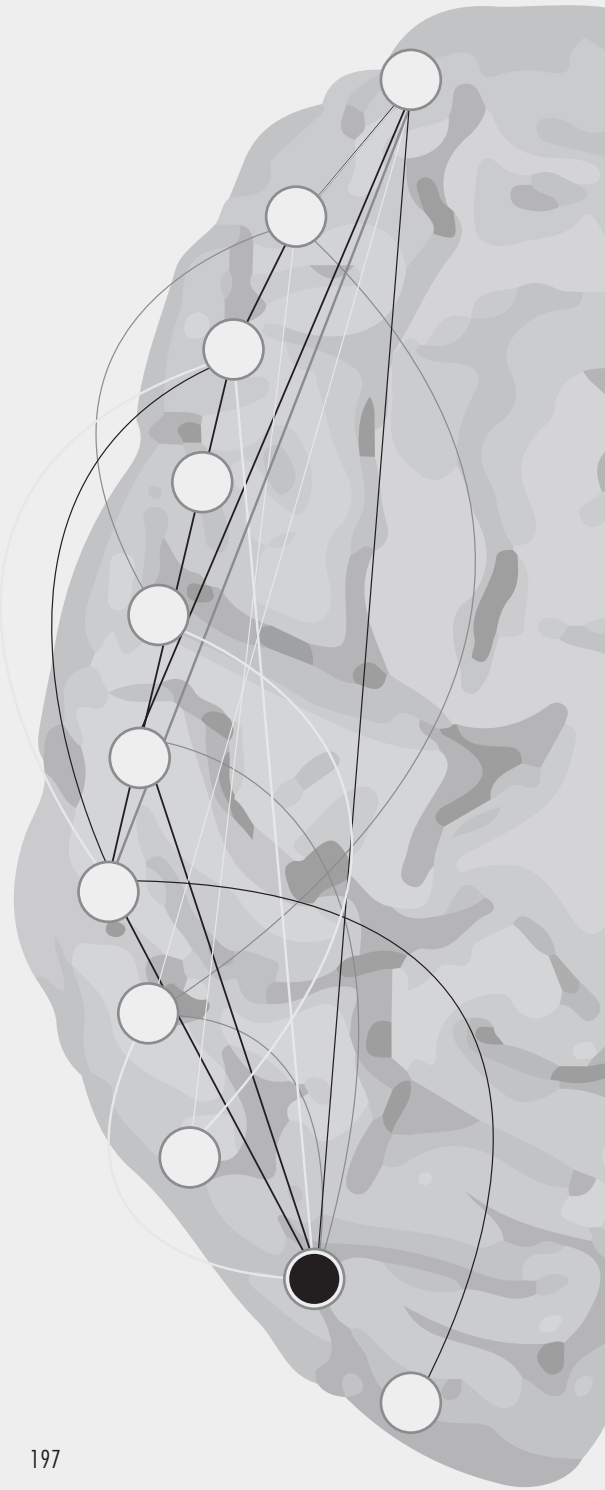


Receiver operating characteristic (ROC) curves of model performance when tested on all children in whom diagnosis remained unclear at first consultation

The grey line represents ROC of only clinical predictors with an AUC of 0.72 [CI 0.64 to 0.80], the dashed line represents ROC of EEG predictors with an AUC of 0.60 [CI 0.52 to 0.68] and the black line represents ROC of clinical and EEG predictor variables combined with AUC of 0.76 [CI 0.68 to 0.83].

CHAPTER 10

SUMMARY AND GENERAL DISCUSSION



SUMMARY

Although history taking is considered as the main diagnostic instrument when epilepsy is suspected, the electroencephalogram (EEG) is an essential part in the diagnostic work-up. It is a straightforward, low-cost investigation that can be adapted according to the clinical needs. Its wide range of clinical applications includes standard interictal EEG recordings after a suspected paroxysmal event, nocturnal and prolonged video-EEG registrations to relate clinical symptoms to epileptiform activity, and intracranial recordings to localize the epileptogenic zone for the purpose of surgical interventions. Despite the diagnostic usefulness of EEG recordings, it remains unclear how the recorded activity reflects underlying mechanisms of brain dysfunctioning in epilepsy. The aim of the present thesis was to investigate if a network analytical approach could: *i*) provide a theoretical framework to better understand how epileptic activity reflects the underlying pathological mechanisms, and *ii*) contribute to the diagnostic process in new-onset childhood epilepsy.

PART I: Systematic reviews of networks in epilepsy. Throughout this thesis, different EEG recording techniques were used to obtain information on the functional brain organization in childhood epilepsy. Chapter 2 provided a methodological review on the current best practice to reliably construct functional brain networks from neurophysiological registrations such as EEG. The extent to which different brain areas are functionally connected depends on any statistical interdependency between neurophysiological time series. This so-called functional connectivity is needed to attribute an appropriate weight to the level of connectedness between brain areas. Together with brain areas – or recording sites in the case of EEG and magnetoencephalography (MEG) research – the connections between brain areas are used to build a functional brain network. A network analytical approach enables to deduct information in a quantitative manner on the efficient organization of these networks. We discussed various pitfalls during the acquisition process and data analysis in EEG and MEG registrations that possibly confound the reliable construction of functional networks and we provided recommendations that could be of use for planning and the comparison of functional EEG or MEG network studies. In chapter 3, we reviewed the literature on studies that have used a network

analytical approach to gain insights into seizure generation, deviant network organization in epilepsy, and the associated behavioral and cognitive deficits often seen in chronic epilepsies. Relevant confounders in epilepsy research that might influence the construction of functional networks were discussed including use of antiepileptic medication, seizure load and presence of structural abnormalities. During a seizure, the results from previous studies consistently revealed a more regular functional network organization, which could reflect the more hypersynchronous state of the brain. Results from interictal studies showed a less consistent shift in functional network organization in epilepsy. Possibly, contradictive and inconclusive results were due to a small sample size and a considerable heterogeneity of patients included. To characterize changes in network organization with sufficient power, a systematic review and meta-analysis was conducted in **chapter 4**. Here, we were specifically interested in focal epilepsy patients and restricted the analysis to conventional network metrics of whole-brain networks, namely average path length, a measure of network integration, and average clustering coefficient, a measure of network segregation. In total, twelve studies met the inclusion criteria for our systematic review and meta-analysis. The pooled analysis showed a more segregated and less integrated network in focal epilepsy, suggesting a more regular network organization interictally. Subanalysis of structural networks, based on various MRI techniques, revealed a similar change in network organization as for functional networks derived from EEG, MEG or functional MRI measurements. This supports the hypothesis that focal epilepsy has a widespread detrimental effect on brain organization, which may relate to co-morbid cognitive and behavioral deficits. We further suggested the application of more advanced network metrics, such as so-called 'hub measures' and new methodological approaches to better illustrate network changes in epilepsy.

PART II: Brain network studies in epilepsy. Instead of addressing whole-brain network alterations, we focused in **chapter 5** on local network properties to explore how focal epileptic activity coincides with local network alterations. Brain activity was measured with intracranial EEG depth electrodes in patients with pharmacoresistant focal epilepsies who were elected for epilepsy surgery. High frequency oscillations (HFOs), a highly sensitive electrophysiological biomarker, were used to delineate the epileptogenic zone. Local network alterations were

quantified with the eigenvector centrality; a network metric increasingly used in network analytical studies to express the level of centrality of a node in a network. The results showed that brain tissue associated with the presence of HFOs appeared to be less central in the functional network, suggesting a counter-intuitive functional isolation of the epileptogenic zone when the epileptic brain is in an interictal state.

In **chapter 6** we took a complementary methodological approach to explain the increased diagnostic yield of an EEG recording after sleep deprivation (SD-EEG). Often, an SD-EEG recording is performed when the routinely obtained interictal EEG recording is not able to detect epileptiform abnormalities or is insufficient to determine the classification of the epilepsy syndrome. Instead of using the more conventional network method, we applied a minimum spanning tree (MST) approach to characterize the network organization. The MST approach reconstructs a unique network based on the weighted connections between nodes without forming cycles. In this way, networks are obtained with an identical number of connections, thereby facilitating comparison between groups by taking only the most important connections into account. MST networks were constructed from a standard interictal EEG and an SD-EEG recording, both in children with new onset focal epilepsy as in a control group. Compared to the control group, we found a shift towards a less integrated and more segregated MST network organization in patients after sleep deprivation. This shift in network organization is in accordance with previous studies showing a more regular network organization in the ictal state and might relate to the increased epileptiform EEG abnormalities found in patients after sleep deprivation.

Whereas previous research has mainly focused on describing network alterations in chronic epilepsies, we investigated in **chapter 7** if – and to what extent – these changes are already present at an early stage of the disease. Functional networks were constructed from standard interictal EEG recordings obtained in drug-naïve children. Functional networks of focal and generalized epilepsies were compared to healthy controls. We only found a less integrated MST network organization in children with focal epilepsies, which suggests that the network shift seen at later stages of the disease is already present at an early stage. Since no network changes were found with conventional network metrics, we hypothesize that these network changes may be subtler than at later stage and could be related to seizure load. As no network alterations were

found in children with generalized epilepsies compared with healthy controls, we conclude that focal and generalized epilepsies might differ in terms of network organization.

PART III: Prediction models in children with suspected epilepsy. Besides the insights into the pathological mechanisms underlying epileptic activity in focal epilepsies, we investigated the clinical value of a network analytical approach in diagnosing epilepsy. More specifically, in **chapter 8** we compared the value of EEG-derived functional networks to a conventional EEG evaluation for diagnosing focal epilepsy in children. Again, standard interictal EEG recordings were obtained at first consultation to construct functional brain networks. Although network metrics separately did not reveal differences between patients and controls, we were able to build a robust diagnostic model to accurately predict the diagnosis of focal epilepsy based on EEG recordings by combining network metrics in a multivariate way. The model outperformed conventional EEG evaluation. This suggests that standard interictal EEG recordings contain a significant amount of information, which is largely neglected in conventional evaluation of EEG recordings, and can be used to improve early diagnosis of focal epilepsy.

In **chapter 9** we took a more practical approach by combining clinical characteristics and the information from the EEG report available at first presentation into a prediction model. Considering that the clinical profile of children who had a possible seizure is heterogeneous and the sensitivity of diagnostic testing is limited, such a model would be highly valuable. Patient data from 451 children who were referred to our first seizure clinic over a period of five years was used to build a multivariable logistic regression model. The model performance was excellent, also when tested in a clinically more complicated group of children. EEG report, clinical description of paroxysmal events and neurological history were the most important predictors in this model. This model shows that it is possible to reliably predict the diagnosis epilepsy after a suspected event and may be directly used in daily practice for clinical decision-making.

Finally, the main conclusions from this thesis are:

i) Focal epilepsies are characterized by a more regular network organization as compared with healthy controls, which may relate to the wide-range of cognitive deficits (chapter 4)

ii) The epileptogenic zone in refractory focal epilepsy is associated with a local functional network isolation (chapter 5)

iii) Sleep deprivation in children with focal epilepsy causes a deviation towards a less integrated functional network, which may explain the increased diagnostic yield of sleep deprived EEG recordings (chapter 6)

iv) A less integrated network organization was found in newly onset focal epilepsies and not in generalized epilepsies, suggesting that network alterations are already present at an early stage of focal epilepsy only, which may relate to the distinct processes in seizure dynamics of focal and generalized epilepsies (chapter 7)

v) Combining network metrics in a multivariate model improves early diagnosis of focal epilepsy, as compared to conventional EEG evaluation (chapter 8)

vi) Accurate prediction of the diagnosis epilepsy in children after a suspected event is achieved by combining clinical and EEG characteristics into a multivariate model (chapter 9)

GENERAL DISCUSSION

The objective of this thesis was two-fold. First, we investigated if a network analytical approach could increase our understanding of the pathological mechanisms underlying epilepsy, particularly in newly diagnosed (focal) childhood epilepsy. To that aim, we used interictal and intracranial EEG recordings that were part of clinical investigations in children suspected of having focal epilepsy or in patients with refractory focal epilepsy. More specifically, we characterized functional network alterations to investigate how these global and local network changes coincide with clinical signs and symptoms. Secondly, we aimed to develop prediction models for diagnosing epilepsy in children to improve clinical decision-making in two different ways: *i*) based on network analytical data and *ii*) by means of routinely available clinical information and EEG report at first presentation. The results and implications from our studies are discussed here in a broader perspective. In addition, we will elaborate on methodological limitations and provide a future perspective for EEG network studies in epilepsy.

Do we actually need networks in epilepsy?

Focal epilepsy, global pathology

Historically, the epileptogenic zone in patients with focal epilepsy has been attributed to a single source or a combination of isolated sources (ILAE, 1989). The cognitive and behavioral deficits in these patients suggest, however, that more regions are involved (Bell et al., 2011; Risse, 2006; Vlooswijk et al., 2010). Over the past two decades, efforts were made to clarify this. For example, structural MRI studies revealed that the integrity of the epileptic brain is disrupted beyond the epileptogenic zone (Gross, 2011; Otte et al., 2012b), which may be responsible for a wide range of cognitive deficits (Baxendale et al., 1998; Braakman et al., 2012; Stewart et al., 2009). Studies focusing on functional dynamics of the brain found similar widespread disease specific alterations of increased and decreased connectivity in focal epilepsy (Bettus et al., 2009; Laufs et al., 2007; Luo et al., 2011; Mankinen et al., 2011). Even for seizure generation it has been proposed that activity does not arise from a focal 'pacemaker' but that a system of highly interconnected brain regions is involved in seizure initiation and secondary generalization (Franaszczuk et al., 1994; Lemieux et al., 2011).

Together, these findings suggest the importance of considering regions outside the epileptogenic zone when investigating underlying mechanisms in focal epilepsy.

Network analysis: a new window of opportunities for research

Despite these insights into the functional and structural integrity of the epileptic brain, the aforementioned studies only provide information if – and to what extent – brain regions are altered in terms of structural or functional properties. Consequently, any possible significant relations with cognitive or behavioral deficits tell us little about the underlying mechanisms that drive these changes in the epileptic brain. To better appreciate the way the brain is interconnected, it helps to think of the brain in terms of a complex network. This more holistic approach enables scientists to go beyond the superficial activation and deactivation patterns of independent brain regions and, instead, focus on the topological organization of the brain. There is now a growing consensus to perceive the healthy brain as a cost-efficient network with an optimal balance of global integration, local segregation and highly interconnected regions (Bullmore and Sporns, 2012; Stam, 2014). Brain disorders, including epilepsy, are associated with local and global disturbances of this cost-efficient network configuration. In particular, there is a growing consensus that many different types of brain disease, including epilepsy, primarily affect hub areas as a kind of "weak spots" (Crossley et al., 2014; Stam, 2014). Although a network analytical approach may not reveal the underlying pathological substrate of epilepsy, it does provide a theoretical framework to better understand the relation between focal pathology and global structural or functional disturbances that may lead to clinical deficits in patients. The next sections will discuss how the results obtained in this thesis contributed to an improved understanding of functional brain networks and focal epilepsy.

How does this thesis contribute?

The research questions we highlighted in the aim and outline of this thesis (chapter 1) are discussed in three separate parts:

PART I: Systematic reviews of networks in epilepsy

Interictal networks in focal epilepsy are characterized by a regular topology

Chapter 3 provides an overview of studies that applied a network analytical approach to quantify epileptic brain networks. Whereas ictal network configuration revealed a consistent deviation towards a more regular EEG network topology, interictal studies showed a less conclusive change in brain network topology. A possible explanation for the inconclusive characterization of interictal networks is a modest sample size of these studies. Typically, the number of included subjects was in the range of 20-40 per study cohort. The chance of detecting a true effect might therefore be reduced and, consequently, results are less reliable (Ioannidis, 2005). The meta-analysis in chapter 4 circumvents this problem by pooling studies that have focused on whole-brain network alterations in patients with focal epilepsy. The results showed that the interictal network configuration is pointing towards a less integrated and more segregated network organization, which suggests a more regular network topology. A loss of network integration indicates that long distance connections of the brain, allowing an efficient global communication, are affected (weakened and/or reduced in number). An increase of network segregation suggests that relatively more short distance connections between brain areas are present. This network topology could support the tendency of the brain to generate seizures. Support for this hypothesis comes from previous work on ictal network dynamics showing that network organization has a regular topology pre-ictally, and gradually progresses into an even more regular network when the brain is seizing (Kramer et al., 2008; Ponten et al., 2007; Schindler et al., 2007).

Despite the valuable insights into global topological network alterations, it remains unclear how focal neuroanatomical disturbances are related to local network properties. To this end, new methodological approaches have been developed that allow a further clarification of local network topology, including the detection of highly connected hub-nodes and the presence of a stable network core (Bullmore and Sporns, 2012; Stam and van Straaten, 2012b; van den Heuvel and Sporns, 2013b). Both approaches share the fundamental idea that

not all network nodes are equally connected and that information is processed in a hierarchical manner. Another concern is to what extent network changes are driven by seizures or the duration of epilepsy. Previous experimental work by our group reported incremental network changes in the direction of a regular network topology as a result of persisting seizures (Otte et al., 2012a). Our meta-regression on duration of epilepsy was not able to reproduce this gradual increase of network changes, possibly due to the limited number of studies that focused on new onset epilepsy.

PART II: Brain network studies in epilepsy

Focal epileptic activity coincides with local network alterations

The intuitively appealing idea that not all nodes are connected equally in a network was used in chapter 5 to quantify centrality values of brain areas based on EEG depth-electrode recordings in patients with refractory focal epilepsy. In contrast to previous work, which revealed an increased centrality of nodes in the ictal state (van Mierlo et al., 2013; Varotto et al., 2012; Wilke et al., 2011), network nodes associated with the epileptogenic zone were less centrally connected in the interictal state. What does this mean? Apparently, the role of the epileptogenic zone in a functional network changes as the seizure progresses, which is supported by previous work on seizure dynamics in relation to (local) network alterations (Kramer et al., 2010). It could be hypothesized that the functional isolation of the epileptogenic zone interictally is the result of a compensatory strategy of the brain to prevent spreading of epileptic activity. This may change in the ictal state once the epileptogenic zone makes contact with spatially separated and highly connected pathological hubs that are present in epileptic networks (Amini et al., 2010; Palmigiano et al., 2012). A recent study supported this hypothesis, showing indeed that the epileptogenic zone is functionally isolated interictally, but becomes more connected during seizure progression due to specific network transitions (Burns et al., 2014).

Is it possible to translate these findings into clinical practice? The results possibly provide a new perspective for localization of the epileptogenic zone that is currently done by visual inspection of the EEG recording and may be particularly helpful in cases where conventional identification of the epileptogenic zone has failed (Thom et al., 2010). A possible next step

could be to retrospectively analyze if seizure freedom after epilepsy surgery is associated with the removal of these critical (isolated) network nodes (van Dellen et al., 2014a).

MST network alterations are present at an early stage of epilepsy

The studies in both chapter 4 and chapter 5 primarily focused on patients with chronic epilepsies and revealed both global and local pathological network alterations. Little is known, however, to what extent these networks are affected at disease onset. By focusing on EEG recordings obtained at first presentation of children suspected of having epilepsy, we were able to construct functional networks at an early stage of the disease in chapter 6 and chapter 7.

When studying brain networks in children, it is important to consider age-related changes in network density (Dennis et al., 2013; Hagmann et al., 2010; Smit et al., 2012), which are directly related to maturation and myelination of the developing brain (Gogtay et al., 2004; Sporns et al., 2004). Recent studies have acknowledged that these changes in network density influence the construction of brain networks and, consequently, may hamper the comparison of networks (Fornito et al., 2013; van Wijk et al., 2010). In chapter 2 it is argued that the minimum spanning tree (MST) adequately deals with the possible bias due to differences in network density. The MST is a unique acyclic subnetwork that contains the most important connections of the original weighted network, resulting in networks with an equal number of connections (Stam et al., 2014). Recent experimental work revealed that MST is a robust approach to construct unbiased networks (Tewarie et al., 2014b). A possible disadvantage of the MST approach is that it may omit information on network topology that is contained in the weaker connections of the EEG network. We therefore compared conventional network metrics with MST networks in chapter 6 and chapter 7.

Chapter 6 revealed that functional networks in children with newly diagnosed focal epilepsy were differently affected by sleep deprivation. A less integrated functional network configuration was found after sleep deprivation, which could be accountable for the increased presence of focal epileptic activity. This study not only provides a mechanistic explanation from a network analytical perspective for the increased diagnostic yield of sleep deprived EEG recordings in patients suspected of epilepsy (Ellingson et al., 1984; Fountain et al., 1998), it also shows that dynamical changes following a seizure precipitant, such as sleep

deprivation, differently affect epilepsy (section 10.5). In **chapter 7** functional networks of focal and generalized epilepsies were compared and we found a significantly less integrated network only in children diagnosed with focal epilepsy. This finding is in accordance with **chapter 4** and shows that network alterations are already present at an early stage of the disease. Furthermore, the results suggest a mechanistic dichotomy from a network analytical perspective between focal and generalized epilepsies and may contribute to a more scientific rationale for the new classification of seizures and epilepsies (Berg et al., 2010).

Interestingly, network changes in both studies were only identified using MST networks. Possibly, epileptic network alterations are subtle at an early stage of the disease and aggravate at later stages as a result of persisting seizures (Liao et al., 2010; van Dellen et al., 2009). Considering the observation that network changes were only detected when studying MST network metrics, one could conclude that subtle network changes selectively affect the strongest connections of the original network, which are more likely to contain important information. In contrast, weaker (and more noisy) connections are taken into account when computing conventional network metrics, which may obfuscate network differences between groups. To find further support for this hypothesis, future studies should compare more established measurements of the network core – such as the default mode network and the rich-club connections – with the MST network properties in newly diagnosed epilepsy patients.

PART III: Prediction models in children with suspected epilepsy

Multivariate models to improve diagnosis of epilepsy

Another aim of this thesis was to investigate the added value of EEG network metrics for early and accurate diagnosis of childhood epilepsy. Diagnosing focal epilepsy in children can be particularly challenging, as focal epileptic activity may be absent in the initial EEG recording (King et al., 1998; Shinnar et al., 1994; Stroink et al., 1998). In **chapter 8** we constructed a robust network-based model that was able to distinguish patients from controls with a high sensitivity and specificity. Additionally, the performance was superior to the conventional EEG evaluation, which leads to the conclusion that standard EEG recordings contain valuable information – beyond the presence of epileptiform changes – for diagnosing epilepsy that is currently neglected in clinical practice.

What is the clinical value of this model? It is a relatively easy and low-cost approach to boost the diagnostic yield of standard interictal EEG recordings and could make ancillary investigation, such as prolonged or sleep deprived EEG recordings, unnecessary. Previous studies have suggested similar approaches for the early identification of epilepsy patients based on the added value of higher order information derived from EEG recordings, such as spectral power and the level of connectivity (Douw et al., 2010a; Kerr et al., 2012). This promising study has its limitations as well. First, the model is based on a highly selected subgroup of patients, namely children with new onset focal epilepsy. This may hamper clinical implementation, as this would require selecting a pre-defined group of children at risk for developing focal epilepsy. Considering the challenges to identify these patients, it would be better for future models not to make an a priori selection of patients. However, models based on unselected cohorts will probably have a lower performance due to differences in network configuration between different types of epilepsies (chapter 7). Secondly, a random forest classifier was used to build an aggregated set of hierarchical decision trees (Breiman, 2001). In these trees, decision rules are formed (so-called split values), which are based on the information provided by subsets of individual network metrics. Each tree generates a binary outcome and the average of all tree outcomes provides the probability of epilepsy that might be useful to the clinician. However, it does not give a mechanistic explanation how networks differ between patients and controls. Furthermore, considering the results from chapter 7, model performance might further increase when using MST metrics instead of conventional network metrics. Finally, a regular bootstrapping procedure for internal validation was used. This procedure is generally suggested for smaller studies (Steyerberg et al., 2003), but may have led to an overestimation of our model performance. It can therefore be concluded that the added clinical value of this model will depend on the external validation in a larger, and more heterogeneous patient population.

A different approach was taken in chapter 9. The central question was whether it is possible to predict, with a high accuracy, the probability of epilepsy after a possible seizure. Instead of including patients with focal epilepsy only and disregarding other clinical information available at first presentation, both clinical data and the EEG report were combined into a multivariate model. This resulted in a straightforward prediction tool with an excellent performance. In contrast to chapter 8, no information from functional networks was used and no restrictions

were made regarding patient inclusion, which facilitates its application in the daily practice. In essence, this clinical prediction model can be seen as a rational approach for clinicians to accurately manage patients who present after a possible seizure. Similar to an experienced (pediatric) epileptologist, who uses implicit knowledge obtained during previous cases to evaluate another, this model combines the information obtained from multiple individuals to compute the probability of having epilepsy in the next patient.

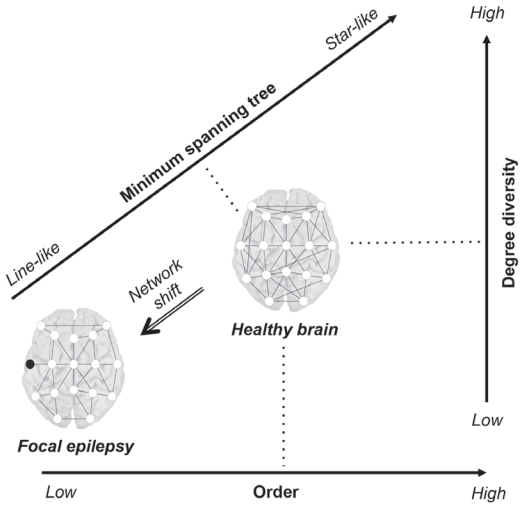
Is this model directly applicable in clinical practice? This could be the case. The clinical data used to build this model is standard information obtained during the diagnostic work-up (Guerrini, 2006). To underline this, a freely accessible web-based application tool was created that enables clinicians to use it in daily clinical practice (chapter 9). Since all consecutive patients referred to the outpatient clinic after a possible seizure were included, the model is not biased towards a specific age group, medical history or clinical presentation. One might argue that this liberal inclusion of patients inflated the model performance. For example, patients who have experienced tonic-clonic seizures (and therefore very likely to have epilepsy) were also included in the model. The model was therefore separately tested with the clinically more complicated cases. Although the model performance dropped, it was still able to predict the probability of epilepsy with a high likelihood (full model correct overall discrimination of 90% versus 76%). A way to further increase model performance is by including the information from EEG network metrics. Regretfully, it was not possible to select EEG epochs of sufficient quality to infer reliable network metrics from all patients. Particularly in children of younger age (under five years) and patients who were difficult to instruct, resting state epochs of sufficient quality were absent. This problem can be overcome by prospectively collecting EEG recordings that are dedicated for network construction. This would include a longer and artifact free resting state condition in the EEG recording (chapter 2). Finally, all patients were included in a single tertiary referral medical center. Despite the fact that the model was trained and tested on different patients, future validation studies should include patients from other institutions.

Integrated view on network changes in focal epilepsy

The results of the presented studies in this thesis suggest a less integrated and more segregated network topology in focal epilepsies. These functional network

disturbances are subtle at an early stage of the disease (chapter 7) and become more prevalent following a seizure precipitant, such as sleep deprivation (chapter 6). Based on the meta-analysis in chapter 4, it is plausible that these network alterations will aggravate at later stages as a result of recurrent seizures. Furthermore, chapter 7 reveals that crucial network nodes, with a high node centrality, become less important in newly diagnosed focal epilepsy. A possible explanation for this reduced node centrality is proposed in chapter 5, where the epileptogenic zone was found to be associated with a reduced node centrality in the interictal state. Together, these results consistently indicate a failure of the normal hierarchical network in focal epilepsy, illustrated by reduced network integrity and failure of highly interconnected nodes (Figure 1).

F1



Integrated view on network changes in focal epilepsy

This figures summarizes the network shift from a healthy brain to focal epilepsy in the interictal state, further clarified by this thesis. The healthy brain (central figure) is characterized by i) an optimal balance of global integration and local segregation (and thus a normal network ordering, horizontal axis) and ii) some highly interconnected regions, which implies that some nodes have a higher degree with more connections than other nodes (and thus a normal degree diversity, vertical axis). The presented studies consistently revealed a more regular network topology (high ordering) and a reduced node centrality (low degree diversity) in focal epilepsy (bottom left figure). Furthermore, MST subnetworks revealed a more line-like network in focal epilepsies (reduced network integrity and a reduced node centrality, diagonal axis).

Methodological consideration

The most important methodological challenges when constructing functional networks from EEG are summarized in chapter 2. Epilepsy related confounders that may directly influence the construction of functional brain networks, such as structural abnormalities, antiepileptic drug usage and cognitive impairments, are discussed in chapter 3. This section will specifically focus on methodological considerations that were encountered in the studies.

Selecting EEG epochs from clinical recordings

The EEG epochs that are needed for the construction of functional networks were selected from clinical EEG recordings. Since these recordings are obtained according standardized procedures and generally include a resting state assessment for the evaluation of background EEG activity (Pillai and Sperling, 2006; Smith, 2005), they could be used for the presented studies. However, the recordings were not acquired and evaluated on the quality needed for functional network construction, which meant that a considerable number of children did not have an EEG of sufficient quality (mainly due to myogenic or movement artifacts). A practical consequence of this limitation is that insufficient data was available to include EEG network metrics as a predictor variable in the diagnostic model presented in chapter 9. This problem might be overcome when EEG recordings are conducted in a prospective manner. Doing so, EEG recordings can be directly evaluated and if the resting state assessment appears to be of insufficient quality, necessary adjustments can be made, such as prolonging the recording or giving new instructions to the patient.

Another challenge concerns the presence of epileptic activity in the EEG recordings. Since no current literature is available if – and to what extent – this activity influences the computation of resting state functional networks, EEG epochs with epileptic activity were not included. With this conservative criterion many EEG recordings were not suitable for further network analyses. Future studies should systematically investigate the possible influence of epileptic activity on the construction of functional networks. If this influence is negligible, or highly dependent on the connectivity measure of choice (chapter 2), more data can be eventually used for patient studies.

Interpreting frequency-dependent network changes in epilepsy

In chapter 2 it was suggested to study frequency specific networks, as information processed by the brain is encoded at different time scales. Previous studies based on non-invasive recordings revealed that lower frequencies – in particular the theta-band – are most commonly associated with *global* network alterations in patients with focal epilepsy (Bartolomei et al., 2006; Douw et al., 2010a; Horstmann et al., 2010; van Dellen et al., 2012). Conversely, intracranial EEG recordings revealed that *local* network alterations, associated with the epileptogenic zone, are primarily associated with the gamma frequency band (Ibrahim et al., 2013; Varotto et al., 2012; Wilke et al., 2011). How to relate these seemingly opposing results between surface and invasive recordings?

Previous research suggested that low and high frequency bands reflect different aspects of information processing. Large-scale integration is primarily processed in the lower frequencies while local activity evolves in higher frequency bands (Koenig et al., 2005; von Stein and Sarnthein, 2000). It could be that the results obtained in focal epilepsy reflect two different ways of information processing. A way to investigate this hypothesis is by relating functional changes in the different frequency bands with the structural integrity of the epileptic brain. Possibly, widespread structural abnormalities in focal epilepsy are primarily related to network changes in lower frequency bands, whereas structural changes directly related to the epileptogenic zone are reflected by changes in higher frequency bands. An alternative suggestion is provided by a recent insight that high frequency oscillations – including gamma activity – are nested in the theta-frequency band (Tort et al., 2013). Consequently, alterations described in the gamma and theta band in epilepsy research (chapter 5) could be reflections of the same underlying mechanism. More research is needed, not only to better understand the results obtained in different frequency bands, but also to grasp what these changes in different frequency bands mean. Do they reflect a corrupted functional network or merely an (inadequate) compensatory mechanism of the brain?

Significance of EEG-derived networks

In all presented studies, EEG channels were used as nodes of the network, and the functional connectivity between nodes as edges. As discussed previously, this approach has its limitations (chapter 2). For example, one might argue that the placement of EEG electrodes does not coincide with anatomically relevant

regions. Strictly speaking, this would thus mean that constructing EEG-derived networks in 'signal space' results more in a *virtual* than a *functional* brain network with spatially defined brain areas. To obtain more reliable and accurate information on the communication between brain areas, studies have therefore proposed to project the activity measured at the electrode or sensor (signal space) back to the underlying sources, the so-called 'source space' (Schoffelen and Gross, 2009), which is currently considered the standard approach in MEG network studies. Although anatomically more correct, this approach comes with several methodological problems (chapter 2). In addition, when aiming to relate epileptic activity measured at an electrode level with functional network changes (chapter 5), constructing functional networks in signal space enables a direct comparison.

Related to this subject is the question what we actually measure when we construct EEG-derived functional networks. Functional connectivity is essential for the construction of functional networks as it attributes an *appropriate* weight to the level of connectedness between brain areas based upon the statistical interdependency between neurophysiological time series measured in two (or more) distinct brain areas. Although this thesis provides converging evidence that a specific blue print exists for the network failure in focal epilepsy (section 10.5), it does not provide a physiological substrate for the level of connectedness and what happens when it fails. In vivo and in vitro studies aiming to relate functional connectivity with, for example, neurotransmitter activity are crucial in understanding how functional network alterations reflect neurobiological activity (Huntsman et al., 1999; Pita-Almenar et al., 2014).

Where do we go from here?

Several opportunities for future research were mentioned. First of all, the field is in need of methodological studies that address the influence of various choices throughout the analysis pipeline systematically (chapter 2). This information will improve the comparison between studies that have used a different methodology and the results obtained in different frequency bands. Secondly, future studies should combine different modalities into one integrated model. Although we provided evidence for functional network alterations in focal epilepsy, it is unclear how these results relate to the structural integrity of the brain. Studies should focus on combining structural and functional networks, which could provide new

insights into how functional networks are constrained by the structural integrity and how this relates to cognitive and structural impairments in epilepsy (Vaessen et al., 2014; Zhang et al., 2011).

Another challenge for studies is to explore the clinical applicability of a network analytical approach in epilepsy. Here, it should be noted that the current literature is biased towards more advanced neuroimaging techniques, including MEG and fMRI (chapter 3). Despite their advantages, it is important not to neglect the practical appeal and the unique properties of EEG recordings. Throughout the diagnostic work-up and to evaluate treatment of epilepsy, EEG recordings are a standard measurement. This will eventually facilitate a possible implementation of functional brain networks in clinical practice. The next sections will therefore specifically focus on future EEG-based research projects that could extend the findings of the studies discussed in this thesis.

Visualizing network dynamics in epilepsy using EEG recordings

Current EEG network studies are neglecting an important advantage of EEG recordings: its temporal resolution. As a result, EEG studies often envisage functional networks as *static* entities whereas a *dynamical* representation could provide additional information on functional epileptic networks. This advantage has previously been used to study network alterations in relation to seizure dynamics (Burns et al., 2014; Kramer et al., 2010; Ponten et al., 2007; Ponten et al., 2009; Schindler et al., 2007). This section briefly discusses three new perspectives how to make optimal use of the high temporal resolution of EEG recordings in the construction of functional brain dynamics:

- How dynamical models used in social epidemiology, such as the suspected-infected-suspected (SIS) model, could help to understand secondary generalization of a seizure
- How temporal properties of EEG networks can lead to a better characterization of interictal network changes in epilepsy
- How longitudinal measurements of brain networks can be used to monitor the success of treatment strategies in epilepsy

SIS modeling and ictal networks

An important clinical question is why some focal seizures develop into a generalized tonic-clonic seizure. This type of seizure is the most consistent risk

factor for sudden unexpected death in epilepsy (Hesdorffer et al., 2011). A better understanding of the mechanisms underlying this secondary generalization might lead to an early recognition of the patients who are at risk, and possibly, adequate preventive strategies (Shorvon and Tomson, 2011). How can a network analytical approach be of value to predict secondary generalization? Previous work from the field of social epidemics may provide a helpful clue in this context.

Mathematical models have provided us with new insights to understand and predict the spread of an infection in social networks (Anderson and May, 1991). A relatively simple model is the so-called susceptible-infected-susceptible (SIS) model, in which individuals are classified as 'susceptible' or 'infected' depending on the disease status (susceptible means no presence of the disease; the second S in the model indicates that immunization or death does not occur). Although this reductionistic approach has been very informative describing dynamics of epidemics, it assumes that all individuals are connected equally. Incorporating information on the number of social contacts of each individual will enable a more natural modeling of disease spread (Keeling and Eames, 2005). Previous studies have therefore combined SIS modeling and characterization of social networks – by means of network analytical approach – to describe disease spread in communities (Christakis and Fowler, 2007; Eames and Keeling, 2002) and copying health behavior between individuals (Christakis and Fowler, 2008; Smith and Christakis, 2008). A common finding of these studies suggests that highly interconnected individuals are crucial for the fast spread of information in a social community.

This might be an interesting method to study secondary generalization in epilepsy, using ictal EEG registrations. Initially, all EEG channels are 'susceptible'. With the start of focal epileptic activity – and the possible spread into a generalized seizure – an increasing number of channels will become 'affected'. By the simultaneous quantification of EEG networks, it should be possible to identify the EEG channels (or network nodes) that are crucial for secondary generalization. These crucial nodes might become candidate regions for surgical removal to prevent seizure progression, particularly when the epileptogenic zone is situated in an eloquent area and can therefore not be resected. From a mechanistic point of view, it would be interesting to see how these crucial nodes coincide with the interictally isolated network regions that are associated with the epileptogenic zone (**chapter 5**).

Dynamical changes of epileptic networks to seizure precipitants

Paradoxically, EEG functional network studies in epilepsy have mainly focused on the static properties of interictal epileptic networks. This is related to the way EEG networks are currently constructed (chapter 2). Typically, studies compute network properties per selected epoch and consequently an average value (over epochs) per subject to have a more robust EEG network (Chu et al., 2012). It is likely that the differences between networks computed from individual epochs reflect ongoing dynamics of brain functioning (Kuhnert et al., 2010), which may lead to an unclear image of the interictal epileptic network. As consequence, it may be challenging to differentiate interictal epileptic networks from healthy networks. This is comparable to the situation where a photograph of two moving objects is taken with a prolonged shutter time, resulting in a blurred picture. To get an accurate image on how the objects differ from each other, one should adjust the shutter time or shoot a movie instead. Could a similar solution be used to get a clearer image of the interictal epileptic network? This might be possible (Breakspear et al., 2004).

In clinical practice patients often report that seizures are provoked by various internal and external precipitants (Kasteleijn-Nolst Trenite, 2012). This information can be used to explore network properties of patients by focusing on how they respond to these provoking factors such as hyperventilation or stress. Similar to investigating the effect of sleep deprivation (chapter 6), these provoking factors may cause the interictal network in patients to shift towards a more epileptic network. This could be tested in a straightforward research design were patients are exposed to a specific seizure precipitant to see how the functional network will change before and after exposure. Integrating the computation of networks properties in EEG recording software could allow a direct visualization – and evaluation – of network changes. Doing so, relevant information can be obtained to what extent provoking factors influence the development of seizures and may improve clinical counseling and guidance of patients and caregivers on lifestyle decisions.

Longitudinal measurements of EEG networks for treatment evaluation

We have discussed the limited sensitivity of conventional EEG evaluation in epilepsy and proposed the implementation of EEG networks to increase the diagnostic yield. Besides its use in the diagnostic work-up, EEG recordings are commonly

acquired during clinical follow-up to evaluate treatment, such as monitoring the effect of antiepileptic drugs (Stefan and Hopfengartner, 2009). A practical problem frequently encountered in clinical practice is that electrographical improvement of the patient – as tested with conventional evaluation of epileptic activity – does not always coincide with patients' clinical status, which urges the implementation of new EEG strategies to improve the evaluation of clinical treatments. Recent studies have suggested that EEG networks can be used to monitor success of antiepileptic drug treatment (Clemens et al., 2014) and the effects of vagal nerve stimulation in refractory patients, implanted to reduce seizure load (Fraschini et al., 2014). An interesting question is whether it is possible to use EEG networks for the intermediate evaluation of a treatment; this would allow clinicians a timely reconsideration of treatment when needed in the case of non-responders. In addition, distinguishing responders from non-responders could provide us with new information to answer the question whether epileptic networks reflect functional failure or a compensatory mechanism of the brain.

To investigate if an intermediate clinical evaluation by network quantification is possible and useful, one should start to conduct a sufficiently powered study to characterize a baseline EEG network, for example at the start of antiepileptic drug treatment. Then, follow-up recordings are needed during and at the end of the treatment; this could be both at the moment of a desirable effect, for example seizure freedom, or when treatment has failed. By characterizing consecutive EEG networks during the treatment, it is possible to construct a therapeutic bandwidth for desirable network alterations: from an epileptic network at baseline towards a normalized network at the end of (successful) treatment.

Increasing the gain of prediction models in diagnosing epilepsy

The clinical presentation after a possible seizure is diverse and may vary according to the age, underlying pathology and medical situation. Together with the great variety of seizure semiology in children, diagnosing epilepsy may be challenging, even to the experienced epileptologist. An elegant way to guide the clinician through this process is by providing a prediction tool that helps to estimate the probability of epilepsy. In **chapter 8** and **chapter 9** two of such models were provided that could accurately predict the diagnosis of epilepsy. Despite the practical appeal, improvements can be made to increase the diagnostic yield of both models.

To start, both models generate a binary outcome. This is a practical limitation of both models as this will automatically lead to a pre-defined cohort (chapter 8) or a less specific outcome (chapter 9). Considering the heterogeneity of the clinical presentation and the variety of diagnoses, increasing the number of model outcomes would be needed. For example, it would be interesting to assess the prognosis of patients and thereby identifying children at risk of developing refractory epilepsy. Similarly, constructing a model that is able to distinguish various types of epilepsies could prompt start of the most appropriate treatment strategy. Increasing the number of model outcomes will, however, automatically mean that more input is needed for the model. To maintain its practical appeal, we propose to raise the number of clinical predictors and EEG network features combined in a single integrated model.

A logical consequence when including more prediction variables in a model is that it requires a larger number of cases for validation and test procedures. Besides collaborations with other institutions the web-based calculator provided in chapter 9 can be of use here. Once clinicians start to use this online calculator for assessing the likelihood of epilepsy in daily practice, this anonymized information could be used for validation procedures. This might be a less time-consuming, and thus more efficient way to obtain a sufficiently powered patient cohort. Obviously, this requires the active participation of the users of the web-based calculator since it is essential to obtain the eventual – and reliable – diagnosis of the patient once revealed.

Concluding thoughts

Modern network science revolutionized the field of neuroscience and revealed significant insights into the organization of the brain. Throughout this thesis we applied a network analytical approach to improve our understanding of the pathological mechanisms underlying focal epilepsy. The presented studies suggest a network shift towards a less integrated network configuration and a failure of the normal hierarchical network in focal epilepsy, which is already present at an early stage of the disease and may aggravate at later stages. Furthermore, the network shift in focal epilepsy may be crucial to understand how the brain generates epileptic activity and the consequential development of behavioral and cognitive deficits in patients, which could provide new clinical opportunities for disease monitoring. Network differences between healthy controls and patients

have already been proven to be useful in the process of diagnosing epilepsy. Together the studies illustrate the great potential of a network analytical approach to be ultimately used in clinical practice.

SAMENVATTING

In de huidige klinische praktijk vormt het electroencephalogram (EEG) een belangrijk onderdeel bij het diagnosticeren van epilepsie. Het betreft een relatief eenvoudig aanvullend onderzoek waarmee epileptische hersenactiviteit gemeten kan worden. Deze informatie is van belang bij het stellen van de diagnose epilepsie en het bepalen van de behandeling.

Hoewel zeer waardevol in de klinische praktijk, biedt het EEG ons geen inzicht in het onderliggende mechanisme van epilepsie. Hoe komt het dat er epileptische activiteit ontstaat? Waar komen de klinische- en gedragsproblemen vandaan die vaak gezien worden bij patiënten met (chronische) epilepsie? Het centrale thema van dit proefschrift is het toepassen van de moderne netwerktheorie om meer inzicht te krijgen in deze onderliggende pathologie, in het bijzonder bij kinderen met (focale) epilepsie. Netwerktheorie is gebaseerd op een wiskundige methode waarbij complexe systemen, zoals de hersenen, vereenvoudigd worden tot knopen – die afzonderlijke hersengebieden representeren – en de verbindingen daartussen. Op deze manier kan er onderzocht worden hoe efficiënt hersennetwerken geordend zijn en wat er met deze ordening gebeurt als er sprake is van een hersenziekte, zoals epilepsie. Voor het construeren van deze hersennetwerken kunnen verschillende metingen gebruikt worden, onder andere een EEG-registratie. De voornaamste doelen van dit proefschrift zijn: *i*) onderzoeken hoe epileptische activiteit in het EEG samenhangt met het onderliggende hersennetwerk en *ii*) of netwerktheorie van toegevoegde waarde is voor het verbeteren van de diagnostiek. De uitkomsten van de studies worden in verschillende paragrafen besproken.

Deel I: Overzicht van netwerkstudies in epilepsie. In dit proefschrift gebruiken we EEG-registraties om hersennetwerken te construeren. In hoofdstuk 2 wordt uiteengezet hoe op basis van de huidige beschikbare kennis een betrouwbaar en accuraat hersennetwerk gemaakt kan worden. Een essentieel onderdeel hiervan is het berekenen van de verbindingssterkte tussen verschillende hersenengebieden, ook wel 'functionele connectiviteit' genoemd. De mate van overeenkomst tussen hersenactiviteit van de hersengebieden bepaalt de sterkte van de netwerkverbinding en vormt daarmee de basis voor functionele netwerken. De voor- en nadelen van de verschillende methoden om de

connectiviteit te bepalen komen aan bod, net als de methodologische valkuilen tijdens het selecteren van de benodigde EEG-data voor het construeren van hersennetwerken. Uit deze hersennetwerken kunnen verschillende netwerkmaten bepaald worden. De netwerkmaten die gebruikt worden in de studies uit dit proefschrift worden in dit overzicht verder beschreven. Na deze methodologische introductie, richt **hoofdstuk 3** zich op de netwerkstudies die tot op heden zijn verricht binnen het epilepsieonderzoek. Er wordt een overzicht gegeven van zowel netwerkstudies die zich richten op netwerkveranderingen tijdens een epileptische aanval (*ictaal*) als tussen de aanvallen door (*interictaal*) en gewezen op de mogelijke epilepsie-afhankelijke invloeden op netwerken zoals het gebruik van anti-epileptische medicatie, het effect van herhaaldelijke epileptische aanvallen op hersennetwerken en de invloed van structurele hersenafwijkingen (dat is, afwijkingen in de 'hardware' van het brein) die vaak de oorzaak vormen van het ontstaan van epilepsie. Hoewel in het algemeen geconcludeerd wordt dat er sprake is van een minder efficiënte ordening van het netwerk in epilepsie, zijn de uitkomsten van afzonderlijke netwerkstudies vaak tegenstrijdig. Mogelijk is dit het gevolg van te kleine patiëntgroepen binnen studies of een te grote verscheidenheid aan patiënten tussen de studies wat een goede vergelijking bemoeilijkt. In **hoofdstuk 4** is er daarom gekozen voor het combineren van de data van voorgaande netwerkstudies die zich richten op patiënten met focale epilepsie in een meta-analyse. Voor het karakteriseren van de mogelijke veranderingen in hersenennetwerken werd er gebruik gemaakt van de gemiddelde *padlengte*, een netwerkmaat voor de mate van integratie, en de gemiddelde *clustering coëfficiënt*, een netwerkmaat voor de mate van segregatie. De combinatie van de resultaten van twaalf afzonderlijke studies lieten een meer gesegregeerd en minder geïntegreerd netwerk zien, ongeacht de gebruikte meetmethode. Dit lijkt erop te wijzen dat lange verbindingen die hersengebieden op afstand verbinden verdwijnen of afnemen in sterkte, terwijl ze op korte afstand toenemen. Deze resultaten ondersteunen de hypothese dat focale epilepsie leidt tot wijdverspreide veranderingen in het hersennetwerk, wat mogelijk de oorzaak is de cognitieve en gedragsmatige problemen die vaak worden gezien bij patiënten met epilepsie.

Deel II: Netwerkstudies in epilepsie. In plaats van globale netwerkmaten te gebruiken voor het typeren van veranderingen in de gehele hersenen, richtte

hoofdstuk 5 zich op de mate waarin focale epileptische activiteit samenvalt met lokale netwerkveranderingen. Voor deze studie werd gebruik gemaakt van intracraniële diepte-electroden registraties. Deze electrodes worden gebruikt voor het meten van hersenactiviteit bij medicatieresistente epilepsie patiënten die in aanmerking komen voor epilepsiechirurgie. Hoog frequente oscillaties, een sensitieve elektrofysiologische biomarker, werd gebruikt om de epileptogene zone vast te stellen. Lokale netwerkveranderingen werden berekend door middel van de *eigenvector centrality*, een netwerkmaat die de mate van centraliteit van een punt in het netwerk karakteriseert. De resultaten toonden dat hersengebieden met meer epileptische activiteit een minder centrale plek in het hersennetwerk innemen. Hieruit werd geconcludeerd dat er in de interictale fase er sprake is van een 'functionele isolatie' van de epileptogene zone. Deze resultaten geven een beter inzicht in hoe de epileptogene zone zich verhoudt tot overige hersengebieden en kan mogelijk in de toekomst gebruikt worden voor het vaststellen van de epileptogene zone ten behoeve van epilepsiechirurgie.

In **hoofdstuk 6** werd onderzocht of de toegenomen diagnostische waarde van een EEG na slaaponthouding bij kinderen met de verdenking epilepsie, verklaard kon worden door een veranderingen in de organisatie van functionele hersennetwerken. Een EEG na slaaponthouding wordt verricht wanneer de standaard EEG-registratie niet conclusief is. In tegenstelling tot de analysemethode van de vorige twee hoofdstukken, werd hierbij gebruik gemaakt van de *minimum spanning tree* (MST) methode. Ten opzichte van de conventionele netwerkanalyse richt de MST zich op de belangrijkste netwerkverbindingen en zorgt dat zowel het aantal punten als het aantal verbindingen in het netwerk gelijk zijn, wat uiteindelijk leidt tot een accuratere vergelijking tussen hersennetwerken. Er werd gekeken naar de veranderingen in het functionele hersennetwerk voor- en na slaaponthouding. In tegenstelling tot gezonde kinderen was er bij kinderen met epilepsie na slaaponthouding sprake van een minder geïntegreerd netwerk. Deze netwerkverandering is overeenkomstig met de resultaten beschreven in de meta-analyse en vormt een mogelijke verklaring voor de toegenomen epileptische activiteit in een EEG-registratie na slaaponthouding.

Hoofdstuk 7 richtte zich op de vraag of – en in hoeverre – netwerkveranderingen al aanwezig zijn in een vroeg stadium van epilepsie. Functionele hersennetwerken werden geconstrueerd uit de eerste EEG-registratie in kinderen die recent de diagnose epilepsie hadden gekregen en nog niet gestart zijn met een

medicamenteuze behandeling. In een later stadium is het lastig om de effecten van epilepsie en antiepileptica op hersennetwerken te onderscheiden. Voor deze medicatievrije studie werden zowel kinderen met focale- als generaliseerde epilepsieën vergeleken met gezonde kinderen. Alleen bij focale epilepsie bleek er sprake te zijn van een afwijkende netwerkordening en werd er een minder geïntegreerd netwerk vastgesteld met behulp van de MST-methode. De conventionele methode liet echter geen veranderingen zien. Hieruit werd geconcludeerd dat subtiele veranderingen in hersennetwerken al in een vroeg stadium van de aandoening aanwezig zijn en met de tijd toenemen in ernst, mogelijk als gevolg van herhaaldelijke epileptische aanvallen. Gezien dat hersennetwerken van kinderen met generaliseerde epilepsieën niet verschilden van gezonde controles kan dit wijzen op een verschil tussen focale en gegeneraliseerde epilepsie in termen van netwerkordening. Deze informatie kan bruikbaar zijn om de huidige classificatie van verschillende epilepsietypen te verbeteren.

Deel III: Predictiemodellen bij kinderen verdacht van epilepsie. Naast de inzichten in de onderliggende pathologische mechanismen bij focale epilepsie werd onderzocht of moderne netwerktheorie een toegevoegde klinische waarde heeft bij het diagnosticeren van epilepsie. In de praktijk blijkt dat epileptische activiteit vaak ontbreekt bij een eerste EEG-registratie in kinderen die uiteindelijk gediagnosticeerd worden met focale epilepsie. De beperkte gevoeligheid van de conventionele EEG-beoordeling kan zodoende leiden tot een verlate diagnose en start van een eventuele behandeling. In **hoofdstuk 8** werd onderzocht of een voorspellingsmodel gebaseerd op functionele hersennetwerken gebruikt kon worden om het diagnostisch proces te verbeteren. Er werd hierbij teruggegaan naar de eerste EEG-registratie bij kinderen die uiteindelijk de diagnose focale epilepsie kregen en kinderen waarbij de diagnose epilepsie uitgesloten werd. Uit deze initiële EEG-registratie werden verschillende netwerkmaten bepaald die gecombineerd werden in het voorspellingsmodel. Het model bleek heel accuraat, en beter dan de conventionele EEG-beoordeling, te kunnen bepalen welk kind al dan niet focale epilepsie had. Concluderend laat dit model zien dat er veel bruikbare informatie zit in EEG-registraties waarvan momenteel in de kliniek geen gebruik van wordt gemaakt, terwijl deze informatie kan bijdragen aan het sneller vaststellen van de diagnose en een eerdere start van de behandeling.

Een ander probleem bij de diagnostiek van epilepsie op de kinderleeftijd betreft de grote verscheidenheid aan klinische symptomen. Ook dit kan de diagnose van epilepsie bemoeilijken. In **hoofdstuk 9** werd onderzocht of op basis van de klinische informatie die beschikbaar is tijdens het eerste gesprek met de dokter in combinatie met de uitslag van de eerste conventionele EEG-registratie een betere voorspelling kon worden gedaan over de aanwezigheid van epilepsie. In totaal werden de gegevens van 451 kinderen die de afgelopen jaren gezien waren op de polikliniek met de vraagstelling epilepsie gecombineerd in een multivariaat voorspellingsmodel. Het model bleek uitstekend te functioneren, ook wanneer het getest werd op de klinisch meer ingewikkelde patiënten. De EEG-uitslag, klinische beschrijving van de aanval en een neurologische voorgeschiedenis bleken de meest bijdragende voorspellers binnen het model. Dit model laat zien dat het mogelijk is om op basis van klinische gegevens na een mogelijke epileptische aanval een accurate voorspelling te doen over de aanwezigheid van epilepsie. Gezien de eenvoud van het model en de benodigde data zou het direct kunnen worden toegepast in de klinische praktijk. Om dit te bevorderen werd er op basis van het model een web-applicatie ontwikkeld.

Deel IV: Samenvatting en algemene discussie. In **hoofdstuk 10** werden de belangrijkste resultaten van dit proefschrift samengevat en geplaatst in een breder wetenschappelijk kader. Daarbij kwam eveneens een toekomstvisie aan bod over hoe de uitkomsten van de besproken studies gebruikt kunnen worden in de klinische praktijk.

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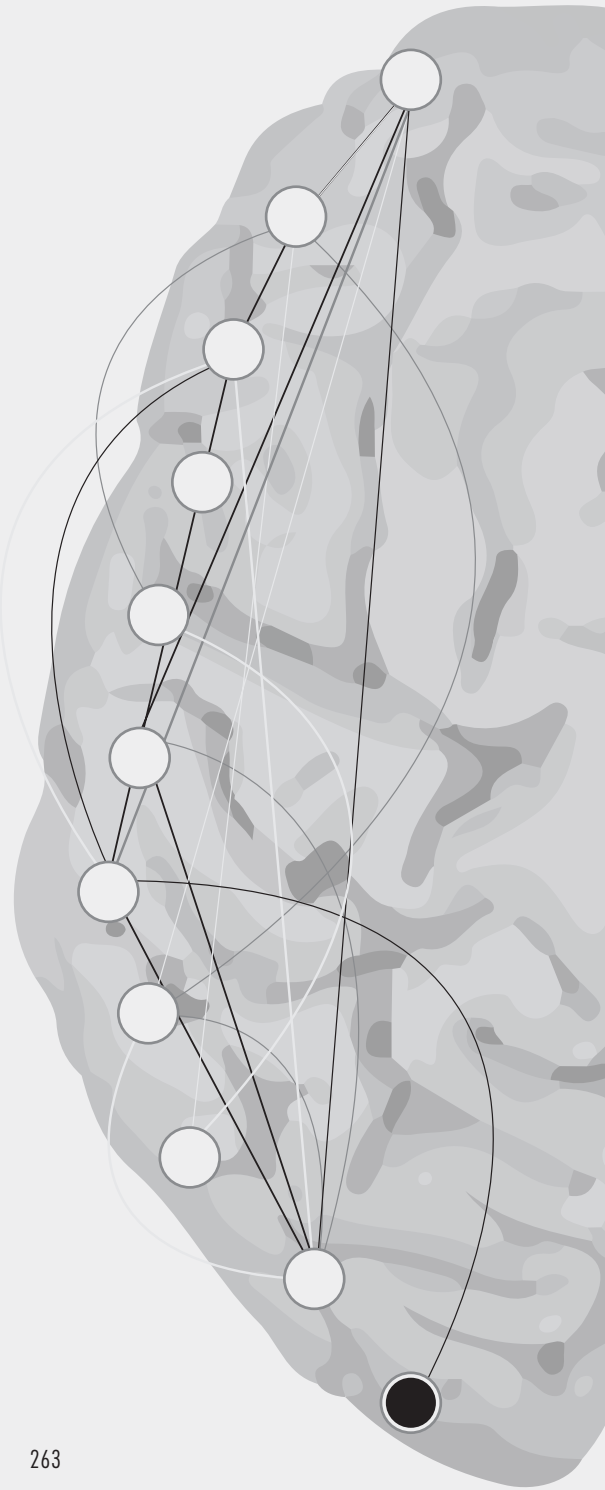
CHAPTER 11

DANKWOORD & PERSONALIA



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PUBLICATION LIST

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van Diessen E*, Numan T*, van Dellen E, van der Kooi AW, Boersma M, Hofman D, van Lutterveld R, van Dijk BW, van Straaten EC, Hillebrand A, Stam CJ. Problems and challenges in resting state EEG and MEG research. Clinical Neurophysiology. 2014 Nov 28.

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CURRICULUM VITAE

Eric van Diessen werd geboren op 25 mei 1982. Na een onbezorgde jeugd in Tilburg en de lange zomers bij zijn Italiaanse familie in Abano Terme begon hij in 2000 met de studie Psychologie in Utrecht. Als uitwisselingsstudent studeerde hij een semester Filosofie en Neurowetenschappen aan Brock University (Canada) wat zijn interesse in de hersenen heeft aangewakkerd. Hij rondde zijn studie Psychologie af in 2005 na zijn wetenschappelijke stage aan het *Centro di Neuroscienze Cognitive* van de Università di Bologna (Italië). In 2006 begon Eric aan een vierjarige master opleiding Geneeskunde in Utrecht (SUMMA) om opgeleid te worden tot arts en klinisch onderzoeker. Zijn laatste jaar bestond uit een tropenstage in het Bamu Hospital (Oeganda) en een afstudeerproject in de kinderneurologie, (UMC Utrecht) wat resulteerde in een promotieplek en uiteindelijk dit proefschrift. Recent is Eric begonnen aan zijn opleiding tot kinderarts in het UMC Utrecht. Wanneer hij niet aan het werk is achter de computer of in het ziekenhuis, kun je Eric overal op de wereld tegenkomen op de fiets of aan tafel etend met zijn vrienden en familie.

CURRICULUM VITAE

Eric van Diessen was born on the 25th of May, 1982. After an untroubled childhood in Tilburg and spending long summers with his Italian family in Abano Terme, he started his study Psychology at Utrecht University. As an exchange student, he spent a semester at Brock University (Canada) studying Philosophy and Neurosciences, which inspired him to study the brain. He graduated in Psychology (MSc) in 2005 after completing a scientific internship at the *Centro di Neuroscienze Cognitive* of the University of Bologna (Italy). In 2006, Eric started his 4-year medical training program (SUMMA). His last year included a tropical internship at Bamu Hospital (Uganda) and a scientific internship in Child Neurology at the UMC Utrecht, which resulted in a PhD position and – ultimately – this thesis. Recently, he started his residency in Pediatrics at the UMC Utrecht. When he is not working in the hospital or behind his computer, you could come across Eric anywhere in the world on a bicycle, or eating at a table with his friends and family.