From the Department of Clinical Neuroscience
Karolinska Institutet, Stockholm, Sweden

# ON MAPPING EPILEPSY: MAGNETO- AND ELECTROENCEPHALOGRAPHIC CHARACTERIZATIONS OF EPILEPTIC ACTIVITIES

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Cover illustration:
Magnetoencephalographic raw sensor data containing epileptic activity

# On mapping epilepsy: Magneto- and electroencephalographic characterizations of epileptic activities

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

**Karin Westin**

The thesis will be defended in public at Karolinska Institutet, Stockholm, 8th of April 2022

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*For my beloved daughter Ada Cornelia,*

*May you always choose your own path.*

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Karin Westin

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# POPULAR SCIENCE SUMMARY

Epilepsy is one of the most common brain disorders. The patients suffer from recurring epileptic seizures associated with a wide range of symptoms depending on which brain region that is affected. These symptoms can include involuntary movements, inability to speak or understand others, or loss of consciousness. There is also an increased risk of developing cognitive problems such as memory impairment. Although many epilepsy patients can be successfully treated using anti-epileptic drugs, approximately a third of all patients continue to develop seizures despite of treatment. These patients can sometimes undergo epilepsy surgery where the seizure generating region can be removed. Unfortunately, it can be very difficult to delineate this region, resulting in poor surgical results. This is in part due to insufficient understanding of how epileptic activity, such as seizure activity, develop and propagate within the brain. This study thus aims to both increase the understanding of how epileptic activities develop (*part 1*), and to improve techniques to localize seizure generating brain areas (*part 2*). *Part 1* consists of two studies. In **Study I** we analyzed data recorded from the surface of a seizure generating brain area. This area was stimulated with an electric stimulation with slow 2 Hz-pulse that reduces the amount of seizure experienced by the patient. The study analyzes how this stimulation modifies this area, and its likelihood to generate epileptic activities. **Study II** analyzes magnetoencephalography (MEG) recordings of epilepsy patients. MEG detects very weak magnetic fields produced by the brain cells. This technique can be used to visualize epileptic activity. Here, we analyzed occurrence of slow brain activity that emerges before onset of epileptic activity. **Study I** and **Study II** both demonstrate that slow activity exerts a possible inhibitory effect in the epileptic brain. Thus, part 1 gives an improved insight into the dynamical processes that underlie development of epilepsy. *Part 2* analyzed a novel, cutting-edge MEG sensor that can be placed much closer to the brain than conventional MEG sensors. Moving the MEG sensor closer to the brain might improve both strength and resolution of the detected brain activity. Part 2 thus aimed to analyze the potential clinical value of these sensors. In **Study III**, we performed the first-ever on scalp MEG of an epilepsy patient. We demonstrated that this technique might detect more epileptic activity than conventional MEG. In **Study IV,** we compared how well on scalp MEG sensors detect and localize epileptic activity compared to the techniques that are routinely used in clinical epilepsy evaluations. Together, the studies of part 2 demonstrate that on scalp MEG might improve future epilepsy evaluations.

# ABSTRACT

Epilepsy is one of the most common neurological disorder, affecting up to 10 individuals per 1000 persons. The disorder have been known for several thousand years, with the first clinical descriptions dating back to ancient times. Nonetheless, characterization of the dynamics underlying epilepsy remains largely unknown. Understanding these patophysiological processes requires unifying both a neurobiological perspective, as well as a technically advanced neuroimaging perspective. The incomplete insight into epilepsy dynamics is reflected by the insufficient treatment options. Approximately 30% of all patients do not respond to anti-epileptic drugs (AEDs) and thus suffers from recurrent seizures despite adequate pharmacological treatments. These pharmacoresistant patients often undergo epilepsy surgery evaluations. Epilepsy surgery aims to resect the part of the brain that generates the epileptic seizure activity (seizure onset zone, SOZ). Nonetheless, up to 50% of all patients relapse after surgery. This can be due to incomplete mapping of both the SOZ and of other structures that might be involved in seizure initiation and propagation. Such cortical and subcortical structures are collectively referred to as the epileptic network. Historically, epilepsy was considered to be either a generalized disorder involving the entire brain, or a highly localized, focal, disorder. The modern technological development of both structural and functional neuroimaging has drastically altered this view. This development has made significant contributions to the now prevailing view that both generalized and focal epilepsies arise from more or less widespread pathological network pathways. Visualization of these pathways play an important role in the presurgical planning. Thus, both improved characterization and understanding of such pathways are pivotal in improvement of epilepsy diagnostics and treatments. It is evident that epilepsy research needs to stand on two legs: Both improved understanding of pathological, neurobiological and neurophysiological process, and improved neuroimaging instrumentation.

Epilepsy research do not only span from visualization to understanding of neurophysiological processes, but also from cellular, neuronal, microscopic processes, to dynamical, large-scale network processes. It is well known that neurons involved in epileptic activities exhibit specific, pathological firing patterns. Genetic mutations resulting in neuronal ion channel defects can cause severe, and even lethal, epileptic syndromes in children, clearly illustrating a role for neuron membrane properties in epilepsy. However, cellular processes themselves cannot explain how epileptic seizures can involve, and propagate across, large cortical areas and generate seizure-specific symptomatologies. A strict cellular perspective can neither

explain epilepsy-associated pathological interactions between larger distant regions in between seizures. Instead, the dynamical effects of cellular synchronization across both mesoscopic and macroscopic scales also need to be considered. Today, the only means to study such effects in human subjects are by combinations of neuroimaging modalities. However, as all measurement techniques, these exhibit individual limitations that affect the kind of information that can be inferred from these. Thus, once more we reach the conclusion that epilepsy research needs to rest upon both a neurophysiological/neurobiological leg, and a technical/instrumentational leg. In accordance with this necessity of a dual approach to epilepsy, this thesis covers both neurophysiological aspects of epileptic activity development, as well as functional neuroimaging instrumentation development with focus on epileptic activity detection and localization. **Part 1 *(neurophysiological part)*** is concerned with the neurophysiological dynamical changes that underlie development of so called interictal epileptiform discharges (IEDs) with special focus on the role of low-frequency oscillations. To this aim, both conventional magnetoencephalography (MEG) and intracranial electroencephalography (iEEG) with neurostimulation is analyzed. **Part 2 *(instrumentation part)*** is concerned with development of cutting-edge, novel on-scalp magnetoencephalography (osMEG) within clinical epilepsy evaluations and research with special focus on IEDs. The theses cover both modeling of osMEG characteristics, as well as the first-ever osMEG recording of a temporal lobe epilepsy patient.

# LIST OF SCIENTIFIC PAPERS

I. **Westin K,** Lundstrom B, Van Gompel J, Cooray G. Neurophysiological effects of continuous cortical stimulation in epilepsy – Spike and spontaneous ECoG activity. Clin Neurophysiol 2019;130:38–45. https://doi.org/10.1016/j.clinph.2018.10.009.

II. **Westin K**, Cooray G, Lundqvist D. Interictal epileptiform discharges in focal epilepsy are preceded by a gradual increase in low-frequency oscillations. Clin Neurophysiol 2022. https://doi.org/10.1101/2020.05.27.118802.

III. **Westin K,** Pfeiffer C, Andersen LM, Ruffieux S, Cooray G, Kalaboukhov A, et al. Clinical Neurophysiology Detection of interictal epileptiform discharges : A comparison of on-scalp MEG and conventional MEG measurements Clin Neurophysiol 2020;131:1711–20. https://doi.org/10.1016/j.clinph.2020.03.041.

IV. **Westin K,** Beniczky S, Hämäläinen M, Lundqvist D. On the clinical benefit of on-scalp MEG: A modeling study of on-scalp MEG epileptic activity source estimation ability. *Manuscript in preparation*

# TABLE OF CONTENT

# ABBREVIATIONS

| | |
|---|---|
| AED | Anti-Epileptic Drugs |
| Am | Ampèremeter |
| cm | Centimeter |
| convMEG | conventional magnetoencephalography |
| CSCS | Chronic Subtreshold Cortical Stimulation |
| DICS | Dynamical Imaging of Coherent Sources |
| dSPM | dynamic Statistical Parametric Mapping |
| ECD | Equivalent Current Dipoles |
| EEG | ElectroEncephaloGraphy |
| EOG | ElectroOculoGraphy |
| ERD-ERS | Event-Related Desynchronization/Synchronization |
| FCD | Focal Cortical Dysplasia |
| FDG-PET | $^{18}$F-fluorodeoxyglucose Positron Emisson Tomography |
| fMRI | functional Magnetic Resonance Imaging |
| fT | fentoTesla |
| GABA | Gamma-AminoButyric Acid |
| GNT | developmental GlioNeuronal Tumors |
| hdEEG | high-density EEG |
| htc-SQUIDS | high critical temperature Superconducting Quantum Interference Devices |
| Hz | Hertz |
| IED | Interictal Epileptiform Discharges |
| iEEG | intracranial ElectroEncephaloGraphy |
| ILAE | International League Against Epilepsy |
| ltc-SQUIDS | low critical temperature Superconducting Quantum Interference Devices |
| K | Kelvin |

| | |
|---|---|
| MEG | MagnetoEncephaloGraphy |
| MNE | Minimum-Norm Estimate |
| mm | Millimeter |
| MRI | Magnetic Resonance Imaging |
| mTOR | mammalian Target Of Rapamycin |
| OPM | Optically Pumped Magnetometers |
| osMEG | on scalp MagnetoEncephaloGraphy |
| PDS | Paroxysmal Depolarizing Shift |
| PSP | Post-Synaptic Potential |
| RSA | Representational Similarity Analysis |
| sEEG | stereo ElectroEncephaloGraphy |
| sbdEEG | subdural ElectroEncephaloGraphy |
| SOZ | Seizure Onset Zone |
| SUDEP | SUdden onset Death in Epilepsy |
| SVM | Support Vector Machine |
| VNS | Vagus Nerve Stimulation |
| TMS | Transcranial Magnetic Stimulation |

# 1. INTRODUCTION

## 1.1 EPILEPSY

### 1.1.1 Diagnostic criteriae

The International League Against Epilepsy (ILAE) defines epilepsy as a brain disorder that fulfills at least one of following criteriae:

1) "At least two unprovoked (or reflex) seizures occurring more than 24 hours apart
2) One unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years
3) Diagnosis of an epilepsy syndrome".

However, epilepsy is not a unanimous disorder but should be considered a syndrome covering a large range of etiologies and seizure types. Epileptic seizures types are defined as *generalized* seizures, *focal* seizures or *unknown* seizure types. While so-called generalized seizures rapidly engage bilaterally distributed both cortical and subcortical networks, focal seizures originate from a more limited network within one hemisphere. These different seizure types can be further subdivided into at least ten subcategories. Further describing these are outside the scope of this thesis. Epilepsy can arise from several different etiologies, including *structural, genetic, infectious, metabolic, immune* or *unknown* etiologies. The final epilepsy diagnosis given to the patient reflects both seizure type, as well as underlying etiology (Fisher et al., 2017, 2014; Scheffer et al., 2017).

By definition, the epileptic brain produces seizure activity that originates from a region called the seizure onset zone (SOZ). The seizure activity can hereafter propagate along patient-specific pathways within the epileptic network (Jehi, 2018; Stefan and da Silva, 2013). The clinical symptoms experienced by the patient, the epileptic semiology, is closely related to the anatomical localization of both the SOZ, and of the epileptic network seizure propagation pathways. Although the semiology can vary from patient to patient, the semiology of focal epilepsy stemming from the different lobes exhibit common traits. For instance, temporal lobe epilepsy is characterized slow onset, aura symptoms, amnesia, speech arrest and automatisms while frontal lobe seizures are characterized by very rapid onset, often from sleep, brief duration and hypermotor behavior (Blair, 2012; McGonigal et al., 2021; Noachtar and Peters, 2009). From

a neurophysiological perspective, epileptic seizure are accompanied by specific seizure patterns that can be visualized using neurophysiological recording modalities such as electroencephalography (EEG), magnetoencephalography (MEG) or intracranial EEG (iEEG) (Jayakar et al., 2016, 2014, 2008). By definition, epileptic seizure activity constitutes neural time series exhibiting a quasi-rhythmic appearance with gradual changes in amplitude and frequency content (spatiotemporal evolution). Apart from seizure activity, the epileptic brain often produces so-called interictal epileptiform discharges (IEDs) in between seizures. These are transient, short-lasting events not associated with any clinical symptoms. On the EEG, IEDs are typically sharp, high-amplitude waveforms clearly distinguishable from the background activity (Kane et al., 2017). These IEDs originate from the so called irritative zone. The irritative zone often, but not always, include the SOZ. The irritative zone can also extended beyond the borders of the SOZ, and might even involve the contralateral hemisphere (Jehi, 2018). The SOZ and the irritative zone might also be completely separate regions. The exact relationship between IEDs and seizures still remains elusive (Avoli, 2019). In the remaining thesis, the term "epileptic activities" will be used to refer collectively to both epileptic seizures, and IEDs.

While many generalized epilepsies can be treated successfully using anti-epileptic pharmacological drugs, approximately 30% of all focal epilepsy patient are so-called pharmacoresistant. These patients do not respond to pharmacological treatment (REF). Many of these patients suffer from severe epilepsy, sometimes with daily seizures with a great risk of developing co-morbidities and reduced quality of life. The main remaining treatment options consist in epilepsy surgery, that aims to remove the SOZ. However, a minority of patients are not eligible for surgery due to SOZ situated within eloquent cortex with severe adverse effects associated with surgery, or due to widespread SOZs that cannot be resected (Kwan et al., 2011; Sander, 2003). For these patients, only different neurostimulory paradigms remains (Starnes et al., 2019).

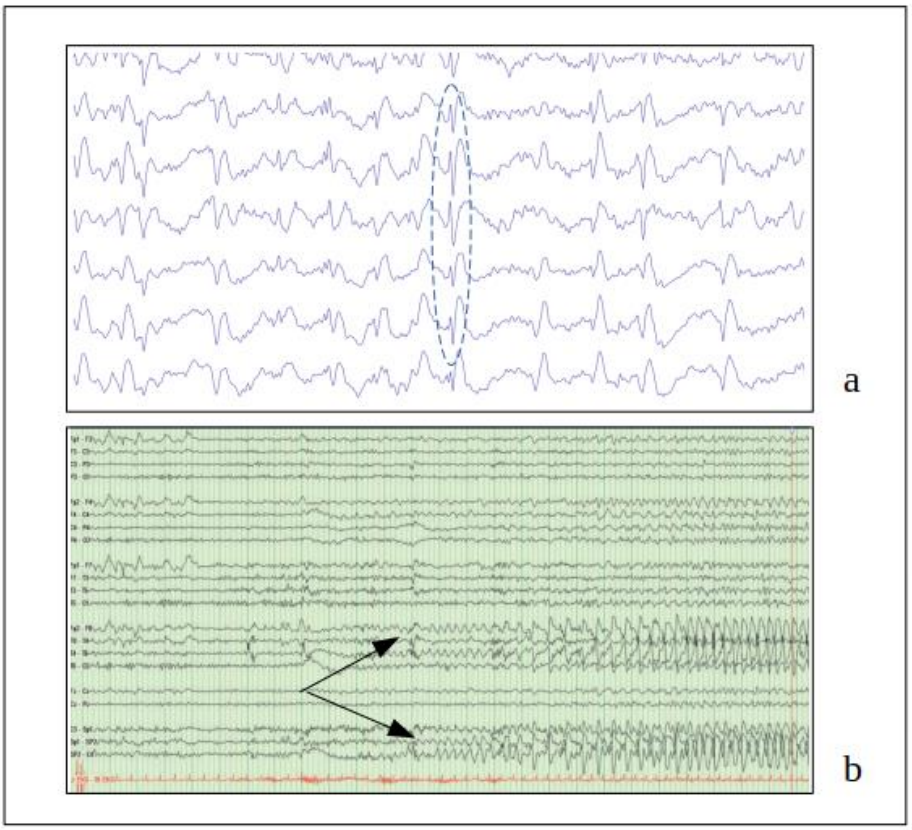



***Figure 1***
***Epileptic activities: Interictal epileptiform discharges (IEDs) and epileptic seizure activity***
*a) Raw MEG sensor traces with multiple IEDs*
*b) Example of a focal temporal lobe seizure with spatiotemporal resolution in clinical EEG recording with 21 electrodes. From (Javidan, 2012).*

### 1.1.2 Epidemiology

Epilepsy is one of the most common neurological disorder, and the most common if all ages are taken in considerations. The incidence of epilepsy is approximately 61 per 10000 person-years, with higher incidence in low- and middle-income countries than in high-income countries. The prevalence, on the other hand, is 7.60 per 1000 individuals. Epilepsy is slightly more common in men than in women, and the incidence of the disorder is highest in young children and in the oldest individuals (Beghi, 2020; Sander, 2003). Furthermore, epilepsy is associated with an increased mortality compared to the general population. The death causes include injuries, status epilepticus, and so-called sudden onset death in epilepsy (SUDEP). Epilepsy is associated with several co-morbidities, such as cognitive impairment, depression, anxiety, dementia as well as

heart disease and arthritis. The general co-morbidity risk is eight times higher than in the general population (Keezer et al., 2016; Yuen et al., 2018). 60 % of all epilepsy patients suffer from generalized epilepsy, and these patients often respond well to pharmacological treatments. In fact, the majority of epilepsy patients can be rendered seizure-free with anti-epileptic drugs (AEDs). On the contrary, focal epilepsy patients are, on average, more prone to be pharmacoresistant, with recurring seizures despite three different anti-epileptic drugs. An analysis of a large number of tertiary care centers referrals shows that approximately 60% of these patients suffered focal epilepsy. Of these, 60% suffered from temporal lobe epilepsy, 24% frontal lobe epilepsy, 5% had parietal or occipital epilepsy and 3% had multilobar epilepsy. (Beghi, 2020; Keezer et al., 2016; Téllez-Zenteno and Hernández-Ronquillo, 2012; Sander 2013). Among the focal epilepsies, approximately 60% are pharmacoresistant, and might become eligible for epilepsy surgery. The outcome of epilepsy surgery varies with the underlying etiology and localization. Anterior temporal lobe resections exhibit approximately 50% seizure freedom in a ten-year follow-up study. In comparison, seizure freedom after five years was approximately 15% in all extratemporal epilepsy cases. However, this group is far from homogeneous. For instance, frontal lobe resections result in approximately 40% seizure freedom. Furthermore, seizure outcome depends on the underlying etiology as well as disease duration (Ryvlin et al., 2014).

### 1.1.3 Brief Overview of Neurobiological Mechanisms in Epilepsy

Under normal, physiological conditions, neurons connect through synapses. A synapse can elicit a postsynaptic potential (PSP) in the post-synaptic neuron, hereby changing the neuron cell membrane voltage. If the PSP is excitatory, the membrane voltage moves towards the neuron's threshold for action potential firing. The action potential gives rise to a time-scale specific dynamic change in membrane voltage potential. However, the epileptogenic neurons eliciting an IED produce a different, pathological, potential called *paroxysmal depolarizing shift (PDS)*. The PDS is initiated by a plateau potential created by both excitatory post-synaptic potentials and multiple ionic conductance changes. The PDS is characterized both by a higher amplitude and a longer duration than the action potential. Several experimental animal studies with cortical application of epileptogenic substances have recorded such events accompanied by IEDs using both intracellular and extracellular recordings and clamp voltage studies. (Ayala et al., 1970; Dichter and Spencer, 1969; Dichter and Spencer, 1968; Matsumoto and Marsan, 1964) Although PDS might also play a role in development of sustained seizure activity, (Meijer et al., 2015;

Tryba et al., 2019), the cellular underpinnings of epileptic seizures are less well understood than for IEDs. Nonetheless, a large number of both local field potentials and microelectrode recordings from both humans and animals have been performed to understand the neuronal activities that underlie seizure activity development. Also, dynamical aspects of both synaptic potentials and action potentials that rule these processes have also been investigated using advanced mathematical models. Historically, seizure development has been considered to arise from an excitation/inhibition imbalance. This notion stems from both *in vivo* and *in vitro* animal studies, and studies on human epileptogenic tissue resected in epilepsy surgery. These experiments often involved application of either epileptogenic substances (such as for example penicillium), or excitating GABA-antagonistic substances, with subsequent recording of seizure activities. It is thus well known that epileptic seizures can be elicited experimentally through application of exciting substances, or through blockage of inhibition (Matsumoto and Marsan, 1964; Schwartzkroin and Prince, 1979). Here, inhibition corresponds to up regulation of inhibitory post-synaptic potentials, while neuronal excitation results from excitatory post-synaptic potentials mediated by excitatory neurotransmitters. These processes can also be mathematically modeled using computational simulations of strong post-synaptic excitatory potentials (Wilson and Cowan, 1972). Nonetheless, describing human epileptic activities as instantaneous changes in excitation/inhibition balances is an unrealistic simplification. There exist rare patient cases with rare neurotransmitter mutations, resulting in excitation up regulation. However, these patients develop very severe, continuous epileptic seizures that cannot be treated, often with high childhood mortality. Thus, such simplified models cannot alone explain human epilepsies. (Shields, 2000; Staley, 2015) Furthermore, neither seizure activity or IEDs are isolated cellular events, but reflect sustained, hypersynchronized activity from a large neuronal population (Destexhe, 1998; Kramer and Cash, 2012; Tao et al., 2007; Wendling et al., 2009). A microscopic perspective cannot alone explain how an epileptic network can come to involve a sufficiently large cortical area to give rise to clinical symptoms. Rather, combined analyses of microscopic, mesoscopic and macroscopic scales are required. While a mesoscopic scale refers to a smaller brain region ($mm^2$-$cm^2$) (Kleinfeld et al., 2017; Zhang et al., 2011) the macroscopic scale refers to large-scale, whole-head dynamics (Bressler and Menon, 2010; Horn et al., 2014; Wang and Kennedy, 2016). These levels are also deeply connected, and changes on one level can translate to activity changes on other levels. For instance, pharmacologically induced epilepsy in cat do not only produce cellular changes as those described above, but result in spontaneous development of ripples in neocortical slices (Grenier et al., 2001) that propagate to thalamic neurons that hereafter give rise to 3 Hz spike and wave activity as seen in absence seizures

(Grenier et al., 2001; Grenier et al., 2003; Steriade and Contreras, 1995). Similarly, Eissa et al demonstrated that synchronized, pathological cellular activity is required for seizure initiation within small mesoscopic networks. Voltage clamp studies on human focal epilepsy cortical slices alongside mesoscopic microelectrode recordings elegantly demonstrated that simultaneous PDS and high-frequency oscillations associated with seizure initiation (Eissa et al., 2016). This process has also been simulated mathematically, where high-frequency activity behaving as seizure activity produced from a neuronal population can be produced simply by tuning the post-synaptic potentials of individual neurons. (Wendling et al., 2016).

Thus, it is evident that understanding of the development of both IEDs and seizures require neuroimaging modalities such as intracranial EEG, scalp EEG and MEG especially designed to capture mesoscopic and macroscopic scales. As mentioned above, IEDs and seizure activity can be identified on non-invasive neurophysiological recordings only after hypersynchronization of at least 3 $cm^2$ cortex (Oishi et al., 2002; Tao et al., 2007). This further underline that epilepsy arise from the mass effect of pathological interactions between a large number of neuronal populations. Neuroimaging studies have also demonstrated that neither seizures nor IEDs arise from instantaneous shifts excitation/inhibition (Hawco et al., 2007; Jacobs et al., 2009). Indeed, both intracranial and scalp EEG recordings exhibit pre-ictal frequency changes several hours before seizure onset. Even IEDs, which are transient events often lasting less than 200 milliseconds, are preceded both neurophysiological and hemodynamical tens of seconds prior to IED onset (Bourel-Ponchel et al., 2017; Jabran et al., 2020; Jacobs et al., 2009). Interestingly, mesoscopic local field potential recordings have also demonstrated a role for inhibitory interneuronal communication patterns in epileptogenic foci. ((Foci et al., 1967; Schevon et al., 2012a). Human and animal epilepsy multiarray recordings recorded hypersynchronized firing from an ictal core, while the surrounding regions exhibited low-level, inhibitory firing that might prevent seizure activity propagation (Schevon et al., 2012a). Similarly, Keller et al reported a decrement in single unit firing up to 500 ms prior to IED onset (Keller et al., 2010).

Thus, it is evident that epileptic activities arise from joint, synchronized neuronal activity across an entire epileptic network. Both the pathological activity of the individual neuron, and the dynamical effects of massive, synchronized interneuronal communication is required for IEDs and symptomatic seizures to be visible on EEG, or MEG.

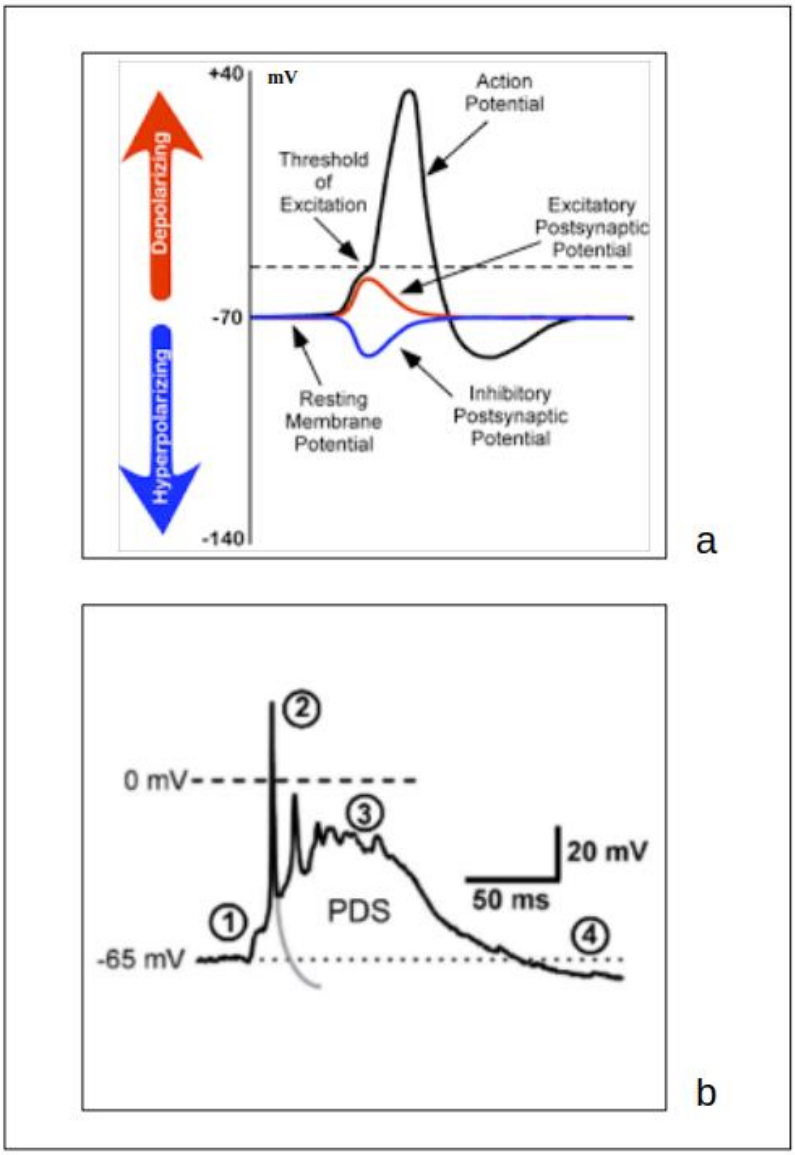



***Figure 2***

***Postsynaptic potentials and Paroxysmal depolarizing shifts (PDS)***

*a) Illustration of physiological neuronal membrane voltage potential changes. While an excitatory postsynaptic potential moves the membrane voltage potential closer to the threshold where the neuron fires an action potential, an inhibitory post-synaptic potential decreases the likelihood of action potential firing. Adapted from (Furtak S, 2022)*

*b) Illustration of the paroxysmal depolarizing shift. Postsynaptic firing result in repetitive action potentials with diminishing amplitudes (1, 2) until a membrane voltage plateau (3) is reached. The PDS can also be followed by a after-hyperpolarization with membrane voltage potential values below membrane resting state values (4). From (Kubista et al., 2019)*

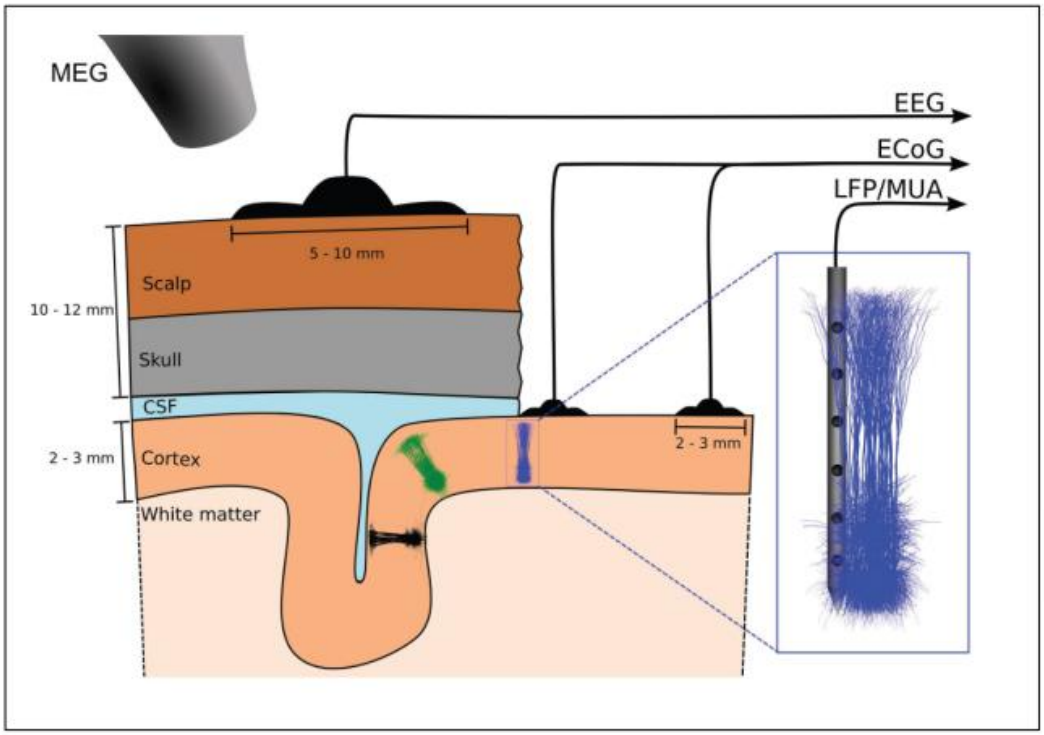



***Figure 3***
***Micro-, meso- and macroscopic scales of neuroimaging***
*Illustration of detection of neural activity at different scales. On the most detailed, microscopic, level, multi-unit arrays (MUA) and local field potential (LFP) measurements both record from within the cortex. Hereby, the extracellular potentials resulting from neuronal firing can be detected. Electrocorticography (ECoG) records summated mesoscopic neuronal activity from a small section of cortical surface, while both MEG and EEG records macroscopic neuronal signals summated from larger cortical sections. Three different neuronal populations (blue, green and black) and their geometrical orientations are illustrated here. From (Hagen et al., 2018)*

### 1.1.4 Introduction to Epilepsy Etiologies

As mentioned in section *1.1 Diagnosis criteriae*, epilepsy etiologies include *structural, genetic, infectious, metabolic, immune* and *unknown causes*. The following section will give a brief introduction to some of these, mainly to shed a light on the widespread dynamical processes that lead to epilepsy development. The section is not an exhaustive description of epilepsy etiologies.

*Some structural etiologies in epilepsy*

By definition, an epilepsy-associated structural abnormality is any abnormality that substantially increases the risk of being associated with epilepsy. In addition, the abnormality should also be

visible on structural magnetic resonance imaging MRI, and the finding should be in accordance with other epilepsy evaluation findings. These findings can be explained by both genetic mutations and acquired insults such has stroke or traumatic brain injuries (Berg et al., 2010; Gaillard et al., 2009; Scheffer et al., 2017). Reviewing genetic pathways and resulting neuronal properties involved in some common developmental structural abnormalities provide an insight into possible mechanistic processes involved in epilepsy development. One highly epileptogenic lesion is so-called developmental glioneuronal tumors (GNT). These occur in young patients with pharmacoresistant epilepsy with several years of duration. The patients typically do not exhibit other symptoms and can be treated only be total tumor resection (Aronica and Crino, 2014; Thom et al., 2012). Often, these tumors contain dysplastic neurons with random orientation, contrary to the parallel organization of healthy pyramidal cells. (Aronica and Crino, 2014; Becker et al., 2006). Interestingly, another common etiology to pharmacoresistant focal epilepsy is so-called focal cortical dysplasias (FCD). These also exhibit an abnormal organization of the neocortical layers with changed neuron orientation. Even with preserved neuronal histology, this reorganization alone can render the cortical tissue highly epileptogenic (Blümcke et al., 2009; Tassi et al., 2002). Several structural abnormalities, including both GNTs, cortical dysplasias and tuberous sclerosis, tumor-like changes strongly correlated with epilepsy, result from genetic mutations in the mammalian target of rapamycin (mTOR)-pathways. mTOR is critically involved in cortical development and neuronal migration. Mutations here result in neuronal hyperexcitability, mediated by up-regulation of excitating neurotransmitters. Interestingly, ion channel gene expressions of these developmental malformation resemble those of the immature neuron. (Baybis et al., 2004; Boer et al., 2010). The immature brain with its different neurotransmitter profile is especially prone to develop seizures compared to the adult brain. (Katsarou et al., 2018).

In summary, evidence from developmental abnormalities, a common etiology to pharmacoresistant epilepsy, indicates that neuronal organization, as well as neurotransmitter profiles across a cortical region plays important roles.

*Some genetic etiologies in epilepsy*

Epilepsy genetics is a growing field that has revolutionized epilepsy diagnostics with targeted sequencing and whole-exome/genome sequencing. These genetic etiologies cover both multigenetic etiologies that increase the risk of pharmacorespondent generalized epilepsy in otherwise healthy patients, to monogenic epilepsy often resulting in a severe phenotype with both

epilepsy and cognitive deficits. Approximately 40% of all patients with severe epilepsy have monogenic epilepsy (Perucca et al., 2020; Rasia-Filho et al., 2021). The development of genetic evaluations has become especially important in diagnostics of the developmental and epileptic encephalopathies A large number of epilepsy-associated mutations with varying phenotypes have been identified. Some of these involve ion channel mutations with a direct effect on membrane potentials and the individual neuron's ability to elicit action potentials. For instance, mutations in the SCN1A gene controlling sodium channels can result in Dravet syndrome characterized by severe epilepsy with cognitive deficits. Similarly, mutations in STXBP1 coding for proteins in neurotransmitter release; KCNQ2 coding for potassium ion channels and CACNA1A genes coding for calcium channels have severe epileptic syndrome phenotypes (Perucca et al., 2020). However, as discussed above, it is important to have in mind that these are rare diagnoses. The most common epilepsies occur in otherwise healthy individuals that might exhibit multigenetic inheritance with an increased familiar risk for epilepsy. Thus, ion channel mutations directly influencing neuron membrane voltages cannot alone explain epilepsy development (Staley, 2015).

### 1.1.5 Clinical Epilepsy Evaluations

Epilepsy evaluations include several both non-invasive and invasive neuroimaging investigations. These aim to both diagnose the disorder, and to estimate the localization of the SOZ. Almost all epilepsy patients undergo clinical scalp EEG recordings with 21 electrodes to detect IEDs, and possibly seizures, in order to support the epilepsy diagnosis. The majority of patients never undergo further epilepsy evaluations, and can hereafter be successfully treated with AEDs. Approximately 30% of all patients do not achieve adequate seizure frequency reduction despite pharmacological treatment. (Jayakar et al., 2016, 2014; Sander, 2003). Most commonly, these patients suffer from focal epilepsy and might be eligible for epilepsy surgery with resection of the seizure onset zone. The anatomical localization of the SOZ can vary significantly between patients. It can be a demanding clinical challenge to properly locate a SOZ. Consequently, these patients often undergo several neuroimaging investigations to capture both functional, structural and metabolic changes associated with an epileptic focus. These often include non-invasive investigations such as long-term scalp EEG to capture seizures, structural MRI to reveal any abnormality, MEG for localization of IEDs and nuclear medicine FDG-PET scans to reveal metabolic changes. These results are used to plan implantation of stereo-EEG (sEEG) where a

limited number of needle electrodes are implanted into the brain. SEEG aim to find the exact SOZ. Several factors influence how easily the SOZ can be identified in an individual patient. A widespread epileptic network might render SOZ delineation difficult. Recording epileptic activity from deep anatomical sites such as basal frontal lobe or insula can be complicated using conventional non-invasive neurophysiological modalities, making adequate sEEG implantation difficult. Inconsistent or inconclusive non-invasive findings might also impair stereo-EEG planning resulting in no electrodes positioned within the SOZ. SOZ localization might fall in between whole-head investigations with limited spatial resolution, and invasive recordings with superior spatial resolution but very limited sampling.

In the following, we will revise some of the most common neuroimaging modalities used in epilepsy evaluations. We wish to give the reader a brief impression of benefits and limitations of some neuroimaging modalities in epilepsy evaluations. MEG is discussed separately in section 1.2.

*Scalp EEG modalities*

Scalp EEG (or simply EEG) constitutes a routine clinical evaluation functional neuroimaging modality. EEG measures the potential differences between two electrodes. These potentials result from the summated PSP of tens of thousands of neurons. The most commonly utilized electrode montage consists in 21 electrodes placed across the scalp. This might reveal the existence of IEDs or seizure activity but cannot estimate the epileptic activity source localization with any higher precision (Benbadis et al., 2020; Seeck et al., 2017). Epilepsy patients often undergo long-term EEG monitoring that might last several days. These aim to capture epileptic seizures so that the origin of the seizure activity can be roughly estimated (Tatum et al., 2022). The number of electrodes can be increased up to 256 electrodes to significantly improve the spatial resolution of the recording. Such high-density EEG (hdEEG) recordings are, for practical reasons, mainly limited to detection of IEDs which are typically much more abundant than seizures (Stoyell et al., 2021). The IED origin do not necessarily coincide with the SOZ, why surgical resection cannot be performed solely on IED source estimations.

*Structural structural MRI*

Structural MRIs are routinely performed to characterize the underlying etiology. A focal, epilepsy generating lesion can also be delineated. The structural MRI can also be used together with MEG

recordings, where IED source estimations can be mapped onto the patient's MRI. (Sidhu et al., 2018)

*IEEG modalities*

Although some epilepsies such as neurodevelopmental tumors stem from focal lesions seen on MRI, many presurgical cases require intracranial recordings to pinpoint the SOZ, and map the epileptic network. There are a large variety of iEEG recording techniques, including subdural EEG (sbdEEG) with rectangular grids or arrays implanted on the cortical surface. However, the most commonly used intracranial modality employed today is sEEG. Here, needle electrodes are implanted deeply into the brain. Apart from all other neurophysiological modalities, sEEG can measure initial seizure activity from deep sites such as the hippocampus or insula. However, individual sEEG electrodes detect activity from only a very small cortical region why SOZ activity can be missed if the electrodes are slightly misplaced. The modality relies heavily on a well performed non-invasive evaluation (Arévalo-Astrada et al., 2021; Kappen et al., 2020; Lesser et al., 2010; Rossi Sebastiano et al., 2020).

### 1.1.6 Epileptic networks

Historically, epilepsy was thought to involve either the entire brain (generalized epilepsy), or only a small, restricted brain region (focal epilepsy). Modern neuroscience has radically changed this view. Nowadays, both so-called focal and generalized epilepsies are considered networks disorders with varying distribution of the epileptic network (Lee et al., 2020; Stefan and da Silva, 2013). The epileptic network can be understood as a more or less widespread network regions and pathways exhibiting pathological function and structure. This can be easily visualized by analyzing propagation of seizure activity. Here, patients initially exhibit one set of symptoms, that gradually changes as the seizure activity moves to other regions, eliciting different symptoms. Correspondingly, inspection of scalp EEG or iEEG demonstrates a spatiotemporal evolution of epileptic seizure activity (Janca et al., 2018; Wendling et al., 2009; Zhang et al., 2017). Several studies have also shown that epileptic networks are active in between seizures. Regions distant from the epileptic focus exhibits pathological changes on both structural and functional MRI (Fahoum et al., 2012). Temporal epilepsy patients often exhibit changes to the contralateral, healthy temporal lobe (Coito et al., 2015; Seidenberg et al., 2005). Even FCDs can be associated with widespread epileptic networks. Pathological high-frequency activity propagates from the

dysplasia to distant regions, during both ictal and interictal periods. (Jeong et al., 2014; Varotto et al., 2012)

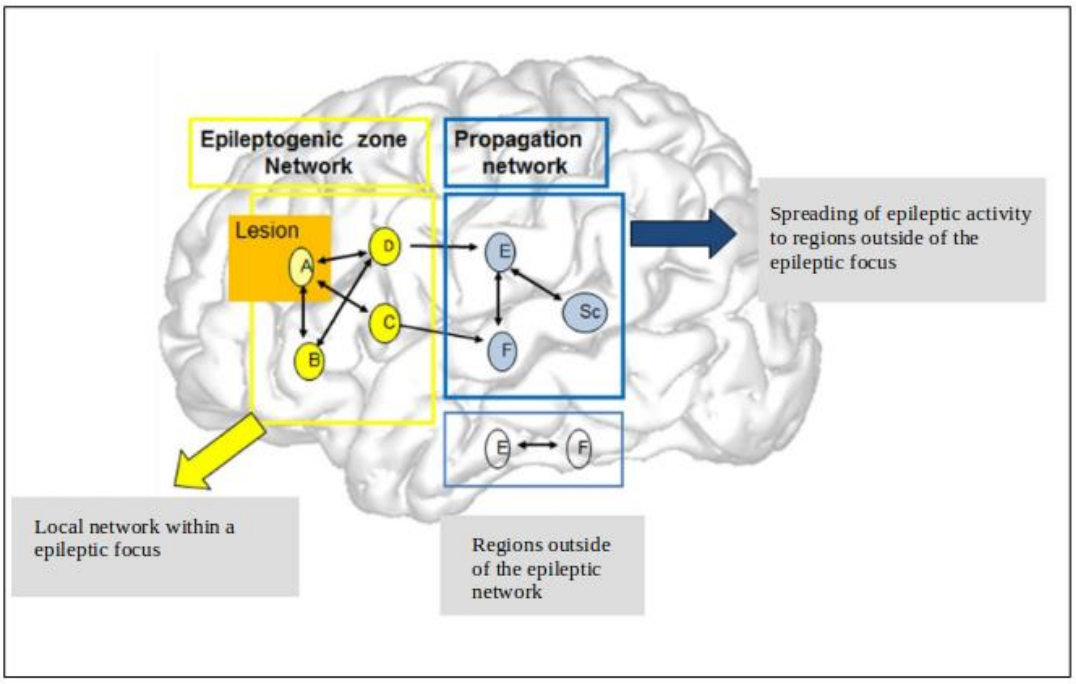



***Figure 4***
***Epileptic networks***
*Schematic overview over an epileptic network with a lesion as the seizure-generating region (yellow). After seizure initiation, the seizure activity propagates to other remote regions (blue). Adapted from (An et al., 2019)*

## 1.2 NEUROSTIMULATION WITH SPECIAL FOCUS ON NEOCORTICAL STIMULATION

A minority of focal epilepsy patients are not eligible for surgery. This might be due to a too widespread epileptic networks that would not benefit from surgery. The SOZ might also be placed within eligible cortex. Only neurostimulation remains as a treatment option for these patients. There are several types of neurostimulations. Some of these have been approved by both American and European drug administration authorities. Relatively little is known about the underlying processes. Given the variation in target sites and stimulation paradigms, it is likely that the underlying dynamics depend on the individual neurostimulation paradigm. Both continuous and responsive stimulation with onset during the preictal phase can reduce seizure frequency.

Vagus nerve stimulation (VNS) has been approved for usage in therapy resistant epilepsy for over 20 years. Both high-frequency (30 Hz) and low frequency (1 Hz) paradigms exist. VNS targets central anatomical sites such as the thalamus. Thalamic nuclei can also be targeted directly using deep brain stimulation. It is assumed, but not proven, that the stimulation interrupts seizure propagation pathways (Starnes et al., 2019; Torres Diaz et al., 2021).

Quite differently, chronic subtreshold cortical stimulation (CSCS) directly simulates a neocortical SOZ and significantly reduces the seizure burden (Starnes 2019, Lundstrom 2016). CSCS delivers low-frequency (2 Hz) pulses to SOZ contacts of a sbdEEG grid. SbdEEG electrodes consists of grids or arrays with fixed electrode spacing, often 1 cm. One individual electrode touches a cortical region with diameter 2-5 millimeter (mm) (Lundstrom et al., 2016). The number of electrodes have to be restricted due to potential severe adverse effect including midline shifts. The electrodes pick up very little from the cortex surrounding the individual electrode – there is little field spread. Unfortunately, this is also a limitation, as epileptic activity originating from cortex between two electrodes can be missed (Lesser et al., 2010). Although the mechanisms of CSCS remains unknown, CSCS opens up interesting discussions on an inhibitory role for low-frequency activity. In support of such a role, some evidence has indicated that low-frequency non-invasive transcranial stimulation (TMS) directed at the SOZ also reduce the seizure burden (Kile et al., 2010). It is well known that low-frequency activity gates information-routing through cortical inhibition (Jensen and Mazaheri, 2010). This can be seen in studies of experimental sensory gating. Here, low-frequency TMS stimulation can be used to inhibit visual gamma oscillations, resulting in a drop in visual accuracy (Herring et al.,

2019; Hwang et al., 2019). Low-frequency oscillations might be coupled to inhibition in epilepsy as well. For instance, Smith et al found that seizure activity is surrounded by a brim of cortical inhibition mediated by low-frequency oscillations (Smith et al., 2016).

## 1.3 MAGNETOENCEPHALOGRAPHY: INSTRUMENTATION AND CLINICAL APPLICATIONS OF CONVENTIONAL AND ON SCALP MEG

### 1.3.1 Neural Basis for MEG

MEG, and EEG, detects the summed PSP of pyramidal cells aligned tangentially to the cortical surface. The resting pyramidal cell maintains a membrane potential close to -70 mV. This is mainly due to intracellular and extracellular concentrations of Na and K ions. If a synapse mediates the release of neurotransmitters, the membrane permeability changes and allows for ion flow across the membrane, hereby creating the PSP. Depending on whether the post-synaptic potential is excitatory or inhibitory, the neuron becomes more or less likely to fire an action potential, which occurs when the membrane potential reaches a specific threshold value. Since the action potential current flows in opposite directions, cancellation occurs and summed action potentials cannot be recorded by distantly placed electrodes or sensors. However, the post-synaptic potential results in a ion flow that create a current. Seen from a distance, this current behaves as a current dipole accompanied by a magnetic field that can be measured by MEG sensors. The magnetic field of a single PSP has a strength of approximately 20 fAm (fentoAmpere meter). For the magnetic field to be detectable by conventional MEG sensors outside of the skull, summation of approximately $10^5$ neurons are required, resulting in a dipole current of 10 nAm. Based upon neuron density, activation of approximately 0.5 $mm^2$ should be detectable by conventional MEG. However, due to cancellation of opposite sources, approximately 40 $mm^2$ is required for a signal to be detectable (Hämäläinen et al., 1993).

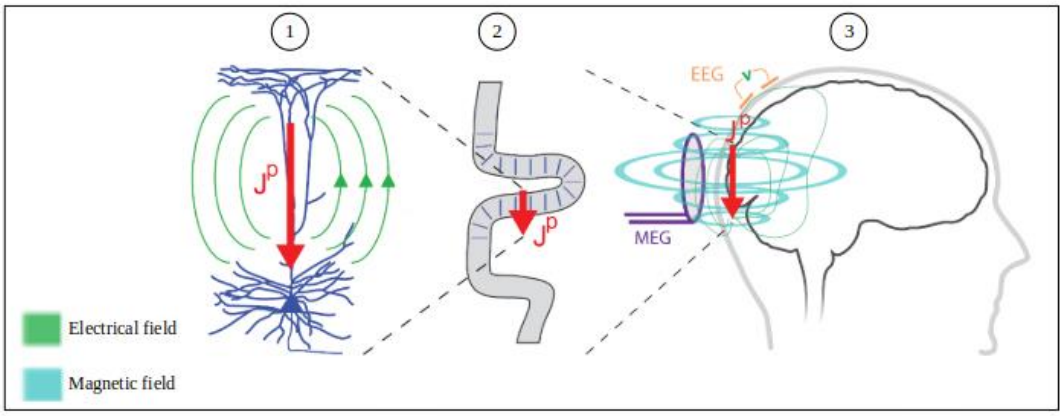



***Figure 5***
***Overview over MEG, and EEG, signal generation.***
*Parallel organization of the pyramidal cells (1) aligned perpendicularly to the cortical surface (2) generate postsynaptic potentials giving rise to an electrical current ($J^P$). The MEG sensors (3) pick up the magnetic field from a large number of activated pyramidal cells.*
*EEG measures the voltage difference (V) between two sensors (3) resulting from the electrical field generated by the electrical current ($J^P$). Adapted from (Neymotin et al., 2020)*

### 1.3.2 Conventional MEG Instrumentation and Limitations

The sensors of the conventional MEG (convMEG) systems are superconducting quantum interference devices (ltc-SQUIDS). These can detect the very weak magnetic fields of the brain, ranging approximately from 10-100 fT. Important for signal-to-noise ratio, ltc-SQUIDS exhibit a very favorable noise profile, with an internal noise level of only 3 $fT/Hz^{1/2.}$. The modern day convMEG system consist of 306 ltc-SQUID sensors, with 102 magnetometers and 204 planar gradiometers (Garcés et al., 2017; Vrba and Robinson, 2002). These sensor types exhibit different sensitivity profiles (so-called lead fields). While magnetometers (unit: Tesla) have a circular sensitivity detecting magnetic fields from afar, planar gradiometers (unit: $Tesla/m^2$) detect the magnetic field underneath the sensor. The sensitivity of the gradiometer decreases rapidly with distance from the sensor. (Malmivuo, 1976) As ltc-SQUIDs require cooling to approximately 4 K (Kelvin) to operate, the sensors are cooled with liquid helium housed within a thermally insulated dewar (Cohen, 1968; Heiden, 1991). As the environmental magnetic field strength is much higher than that of the brain, the entire MEG system needs to be housed within a custom Faraday's cage, a magnetically shielded room. The need for cooling and consequent placement

of the sensors within a fixed, one-sized helmet constitutes the main limitation to the conventional MEG system. The fixed sensor array makes the system sensitive to any head movement, requiring patients to sit still. Thus, neither seizure recordings nor long lasting recordings are feasible. The arrangement of the conventional MEG sensors results in a scalp-sensor distance of approximately 2-4 centimeters (cm) . As the magnetic field strength diminishes rapidly with the distance to the source, this distance have a negative effect on MEG spatial resolution and information content. This especially effects pediatric patients with smaller heads, and thus possibly even longer sensor-scalp distances. (Riaz et al., 2017; Wehner et al., 2008). In order to handle these limitations, osMEG sensors systems have been developed (see below).

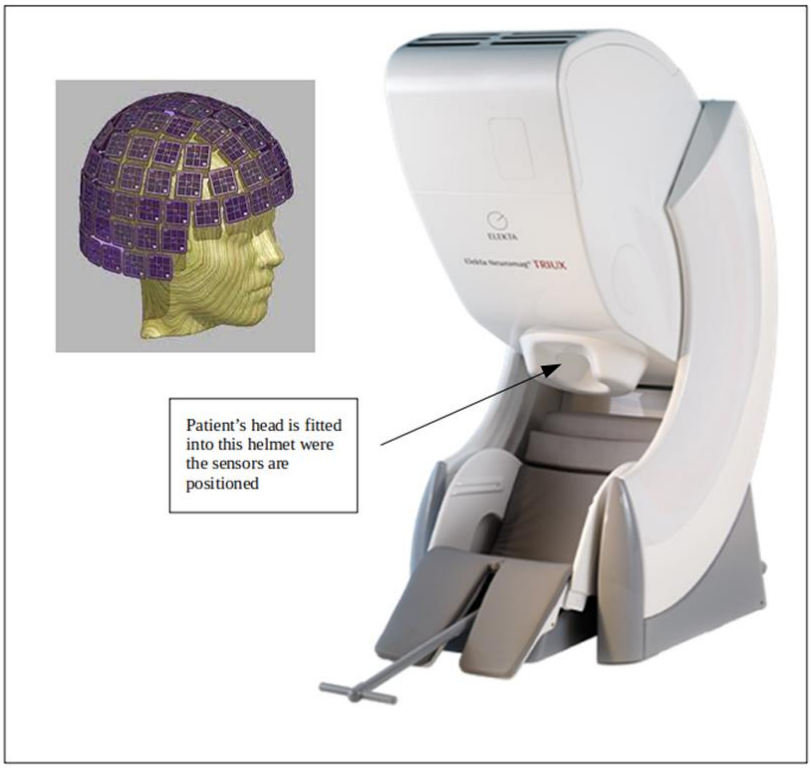



***Figure 6***
***Conventional MEG sensor system***
*Left: Conventional MEG sensor array*
*Right: A conventional MEG sensor system*

### 1.3.3 MEG Signal Analysis with Special Application to Epilepsy Evaluations

MEG records the magnetic fields that originate from the interneuronal communication of several billion of pyramidal cells with "only" 306 sensors. Directly after the recording, pre-processing of the data filter out extracranial magnetic field as well as effects of head movement (MaxFilter) (Taulu and Simola, 2006). Hereafter, the first post-processing step is often a mathematical estimation of the origin of the recorded signals. This is an ill-posed problem with infinitely many solutions. The general approach is to determine the forward and inverse model of the data. First, the forward model theoretically determines how the scalp magnetic field from a well-known dipole would look on the scalp. The magnetic field outside of the head can be determined as a function of the current and of the distance between a source and the scalp by application of a quasistatic approximation of Maxwell's equations and with neural generators assumed to be point-sized dipoles. This model also depends on conductive properties of the tissues (meninges, bones, skin et cetera) surrounding the brain. Hereafter, the inverse solution computes from where the recorded magnetic field stems. A first step in calculation of the inverse solution is determination of the lead field that describes the sensitivity of individual sensors. Especially, the lead fields of magnetometers (circular sensitivity) and gradiometers (steeply decreasing sensitivity of distant fields) are markedly different (Hämäläinen et al., 1993; Malmivuo, 1976). As mentioned, there is no definitive solution to this problem why prior assumptions about the solution is required. One such assumption could be that the solution has to be placed on the cortex . Hereby, mathematically possible solutions that place neural generators outside of the brain are removed a priori (Hämäläinen and Ilmoniemi, 1994). There are several consequences of both the underdetermined nature of the problem, and the requirement of assumptions that are relevant to epilepsy evaluations. Small experimental errors, such as movement or muscle artifacts, translates to large solution errors with inexact localization of the epileptic activity. This would be particularly relevant in seizure activity recordings, or in pediatric epilepsy recordings (Wehner 2008). Furthermore, the inverse solution always contains a degree of blurring. Simplified, the inverse solution of a point-sized neural generator would be smeared out across a larger cortical area (Dale et al., 2000; Wehner et al., 2008).

There exist multiple techniques for computation of the inverse solution. As this is the single most important step in MEG epileptic activity source estimations, we will shortly discuss two different approaches to inverse modeling. Lastly, we will touch upon cancellation within extended sources.

*Equivalent current dipole*s

Computation of the equivalent current dipole (ECD) of the epileptic activity is one of the most commonly used inverse solutions within epilepsy source imaging. ECDs are based upon the simplifying assumption that the sum of currents generated from a large number of neurons looked upon from afar behaves as one single dipole. The model fits the data to the dipole that explain the data distribution best. The dipole is thus assumed to be positioned at the origin of the maximum activity (Sarvas, 1987). In epilepsy evaluations, this means that the placement of the ECD on the patient's MRI indicate the activity center of the irritative zone. However, evaluation of the goodness-of-fit of ECDs reveals several important aspects that could potentially influence the interpretation of MEG source estimations. The orientation of the sources within the active patch influences the final dipole. Thus, it could be speculated that the pyramidal cell disorganization seen in FCDs and GNTs could influence the resulting source estimation. Another critical aspect is whether a single dipole is a suitable model for the data distribution. For instance, bilaterally occurring IEDs would be unsuitable for ECD modeling. Epileptic foci can involve large, extended cortical areas. A larger region is less well described by a single dipole. An extended area could theoretically be better described by a multipole expansion, but no such technique has been validated for clinical usage (Jerbi et al., 2002).

*Distributed source solutions*

Contrary to ECDs that explain the entire cortex' activity as one dipole, the majority of inverse solutions handle distributed sources. The models thus compute individual inverse solutions for all points on the cortex. Accordingly, these can be considered as more realistic than an ECD. Several distributed source models are based upon minimum-norm estimations (MNE). Here, the cortical current distribution is expressed as a linear combination of the lead fields. The linear combination exhibiting the shortest L2-norm (Euclidean distance) is considered to be the solution of the inverse problem. These inverse solutions are however associated with blurring of the inverted data (field spread), as well as varying sensitivity profile across the cortex with better source estimations of superficial sources. It should be noted, however, that alternative distributed source solutions such as dynamical statistical parametric mapping (dSPM) and sLORETA have been developed to better handle both deeper sources and activity field spread (Hämäläinen and Ilmoniemi 1994, Hauk 2011, Dale 2000).

*Cancellation index*

MEG can detect only the net current of the activated patch. This means that activity with opposite polarity cancel out, and cannot at all be detected from a distance. This means that only some parts of an epileptic focus can be characterized using MEG. This effect is quantified using cancellation index. Without going into mathematical details, cancellation index compares the magnetic field generated by simultaneously activated source, to the magnetic fields generated by individually activated sources. Analysis of cancellation index of the cortical mantle demonstrates that sources placed on opposite sulci walls cancel out. As orientation of the neural generators on the gyral crown and sulci bottoms have equal directions, activities from these sites sum up. However, for reasons rooted in MEG physics, radial sources cannot be detected by MEG sensors. It is obvious that cancellation heavily influences MEG characterization of epileptic activities (Ahlfors et al., 2010; Dale et al., 2000; Hämäläinen and Ilmoniemi, 1994).

In conclusion, revising some elementary parts of MEG analysis reveals that interpretation of such results in epilepsy evaluation and research requires understanding of the mathematical underpinnings of MEG source estimations.

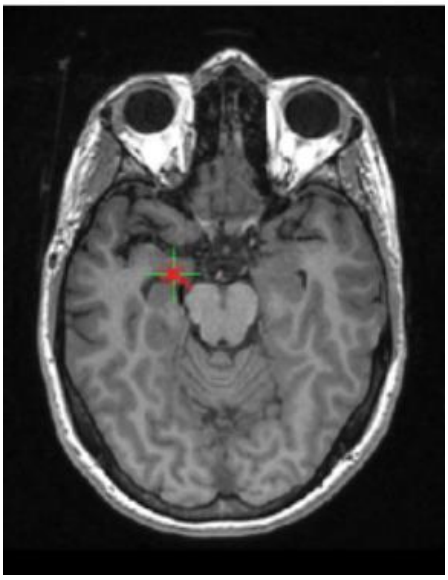


***Figure 7***
***IED source estimation***
*Example of equivalent current dipole calculated from averaged IEDs plotted on the patient's MRI. From study II.*

### 1.3.4 MEG Source Estimations in Epilepsy

MEG has been used in epilepsy evaluations for almost 30 years. Primarily, MEG is used for IED source estimations to guide sEEG recordings to increase the likelihood of successful mapping of the SOZ. (Duez et al., 2016) In a study of 1000 epilepsy surgery cases MEG detected IEDs in 72% of all recordings. In a majority of recordings, MEG pointed towards a more localized findings than other non-invasive modalities. Approximately 50% of all MEG recordings exhibited concordant findings with other investigations, including EEG and MRI as well as descriptions of the clinical semiology. 405 epilepsy surgeries were performed in this case series. The MEG IED source estimations were usable in 92%. Approximately 50% of these patients reached seizure outcome Engel 1 (very favorable surgical outcome). Importantly, resection of the region containing the MEG findings was significantly correlated with seizure freedom. The sensitivity of resecting MEG findings resulting seizure freedom was 66%, and the corresponding specificity was 83%. All IED source estimations in this study was performed using ECDs. (Rampp et al., 2019). Similarly, Duez et al (Duez 2019) analyzed the benefit of source imaging in 141 epilepsy evaluations. This study did analyze the combination of MEG and EEG. The combination could itself improve source estimation accuracy due to the complimentary sensitivity profiles of these modalities. MEG/EEG source estimations changed the management plan in 34% of all patients, and in 80% these changes proved useful to the patient. It is noteworthy that the concordance between source imaging results and intracranial registrations was 53-89% (Duez et al., 2019).

In conclusion, IED source estimations using MEG provides a useful tool in epilepsy surgery evaluations, increasing the likelihood of favorable surgical outcomes.

### 1.3.5 On Scalp MEG and Epilepsy

Although MEG constitutes an important tool in epilepsy research, the convMEG system exhibits some inherent limitations. Due to the cooling of the sensors, the MEG sensors are positioned 2-4 cm from the scalp. As the magnetic signal diminishes rapidly with distance, this negatively affects sensitivity of the signal. The system is also highly sensitive to movement artifacts and requires the patient to sit still, making seizure recordings unfeasible (Boto et al., 2018, 2016). In response to these limitations, osMEG sensors and systems have been developed. All osMEG sensors can be placed on-scalp, improving the signal sensitivity. Commercially available osMEG sensors

(optically pumped magnetometers, OPMs) can also be positioned within an individualized helmet that allows for free head movements, potentially allowing for osMEG seizure recordings. (Boto et al., 2021).

There exist two main types of osMEG sensors, OPMs and high-critical temperature SQUIDS (htc-SQUIDs). It should be noted that these sensors are magnetometers only (Borna et al., 2018; Budker and Romalis, 2007; Zhang et al., 1993).

Similar to convMEG sensors, htc-SQUIDs are based upon superconducting loops with Josephson junctions. While ltc-SQUIDs require cooling to 4 K to function properly, htc-SQUIDs can operate at a higher temperature. These function at a temperature of 77 K and can be cooled with liquid nitrogen. The sensors require very little insulation and can be placed much closer to the brain. There are some severe limitations to htc-SQUID sensors. The sensors exhibit high internal noise levels, and no whole-head montage exists. The sensors can only measure activity from a small cortical region, and the patient has to sit still close to the cryostat containing the sensors (for details on htc-SQUID osMEG sensors, see (Pfeiffer et al., 2020)). OPMs, on the other hand, depend on a different technology. These detect local magnetic fields through measurement of laser transmission through a vapor of spin-polarized rubidium. These sensors function at room temperature, and can be mounted within an individualized helmet. As a consequence, any head movement moves the sensor array as well, why the recording will not be distorted by movements. The snug fit of the helmet also results in an even sampling of all brain regions, which cannot be achieved with the convMEG system (Budker and Romalis, 2007; Riaz et al., 2017).

Both modeling and experimental studies have investigated advantages and limitations of osMEG sensory systems. In an exhaustive mathematical investigation Iivanainen et al compared the sensitivity profiles of convMEG sensors and osMEG sensors. Whole head montages with 306 sensors were simulated for both modalities. The authors demonstrated that osMEG sensors exhibit higher signal power and lower field spread than convMEG. The localization error was comparable for both sensory types. Thus, while the source estimation precision was similar, the spatial resolution was better for osMEG (Iivanainen et al., 2017). Other modeling studies have confirmed these results, consistently demonstrating that osMEG sensors increase signal amplitude, as well as the information content of the signal compared to convMEG sensors (Boto et al., 2016; Schneiderman, 2014).

To date, only a few experimental studies of osMEG have been performed. Also, most such studies have evaluated reduced montages that often do not cover the entire head, while modeling studies often assume an equal number of convMEG and osMEG sensors. Despite these limitations, studies on both htc-SQUIDs and OPMs consistently show an increased signal amplitude, from both superficial and deep sources. However, similar to convMEG sensors, these magnetometers are more sensitive to superficial sources than to deep ones. Interestingly, Andersen et al showed that osMEG sensors could capture radial components that can not be detected by convMEG. This is probably due to a shift in the sensor-scalp angle compared to convMEG (Andersen et al., 2017; Boto et al., 2018; Xie et al., 2017).

A part from the practical advantages of osMEG sensors, the improved sensitivity and spatial resolution opens up potential applications for these sensors in epilepsy research and evaluations. The increased signal power indicates that osMEG might also pick up signals from smaller active cortical patches. Thus, osMEG might detect a larger number of low-amplitude IEDs, as well as initial seizure activity stemming from a small cortical region. Whole head recordings with improved spatial resolution also opens up to improved analyses of large-scale epileptic networks.

***Figure 8***
***An htc-SQUID on scalp MEG sensor system***
*Left: Seven htc-SQUID sensors positioned with in the cryostat.*
*Right: An experimental setup using htc-SQUID sensors.*
*Copyright Christoph Pfeiffer*

***Figure 9***
***An OPM on scalp MEG sensor system***
*Illustration of an OPM whole head montage with individualized fit. From (Hill et al., 2020)*

# 2. RESEARCH AIMS AND THESIS FRAMEWORK

It is evident that characterization of epilepsy has required development of advanced neuroimaging techniques, often based upon quantum mechanical properties. Nonetheless, the understanding of epileptic networks and their generation of epileptic activities remains limited. Furthermore, neurophysiological recordings of human epilepsy fall into one of two categories: Either intracranial registrations with very high spatial resolution but limited coverage, or non-invasive modalities with less spatial resolution but with whole-head coverage. These limitations are reflected both by moderate epilepsy surgery outcomes, as well as by an incomplete understanding of epileptic activity generation. Accordingly, the aim of this thesis is to I) deepen the understanding of development of IEDs as a local network phenomenon; II) analyze a potential role for osMEG sensors within clinical epilepsy evaluations and research. *Part I* especially analyze a role for low-frequency activity in generation of IEDs using both iEEG (**Study I**) and convMEG (**Study II**). In Study I, low-frequency activity is experimentally induced using neurostimulation with subsequent analysis of the effect on IED characteristics. Study II studies the occurrence and dynamics of naturally occurring low-frequency oscillations within the irritative zone prior to IED onset in convMEG recordings of focal epilepsy patients. Study II also investigates the occurrence of gradual development of the low-frequency oscillations that might reflect dynamical cortical processes that end in IED onset. Both of these studies employ a combination of time-frequency analyses with specific applications to both intracranial and MEG neural time series characteristics. Importantly, sbdEEG exhibits less field spread than convMEG, which allows for detailed comparison of time series extracted from the SOZ, and those extracted from adjacent non-SOZ regions While convMEG do not allow for such high-spatial resolution investigations, it does allow for time-frequency analyses of distant cortical regions. Combining these two approaches gives both a detailed and large-scale perspective on the neurophysiological processes that influence IED development. Study I conclude that low-frequency stimulation both reduces IED frequency at the SOZ, and reduces both IED amplitude and duration. Study II clearly shows that IED onset exhibits a gradual up-regulation of low-frequency oscillations only at the irritative zone. As cortical low-frequency activity often mediates inhibition, it is possible that the findings of study II indicate that IED onset emerges once the irritative zone hypersynchronization overcomes cortical inhibition.

*Part 2* constitutes a pioneering investigation of osMEG in epilepsy evaluations with special focus on IED detection and source estimations. With reduced sensor-scalp distance

and possibility for MEG recordings with free head movements, the sensor type could potentially provide substantial improvement to non-invasive epilepsy evaluations. However, any clinical application requires thorough scientific comparison of these sensors, and other well-known neurophysiological modalities. **Study III** was the first ever osMEG recording of an epilepsy patient using htc-SQUID sensors. The paper provides a detailed benchmarking protocol for comparing IED detection by convMEG, osMEG and scalp EEG (21 electrodes). In addition, the study presents a novel machine-learning based algorithm for IEDs detected only by osMEG, and not by convMEG or scalp EEG. The algorithm quantifies statistical properties of such osMEG-unique IEDs and thus opens up for mathematical approaches to extract highly detailed, large-scale epilepsy-associated neural time series features detectable only by osMEG. **Study IV** is a pure modeling study that simulates realistic epilepsy evaluations using osMEG, convMEG, hdEEG and sbdEEG. Both IEDs and propagating seizure activity originating from common epileptogenic focus sites was modeled using a neural mass model. Both source estimations using ECDs and cancellation index were compared across these modalities. In addition, osMEG and sbdEEG was compared using so-called representational similarity analysis (RSA) (Kriegeskorte et al., 2008). RSA aims to quantify and compare results from different neuroimaging modalities. This analysis especially enabled to investigate how osMEG source estimation accuracy depended on source depth and source orientation. Both Study III and Study IV conclude that osMEG detects epileptic activities from smaller cortical regions than do other non-invasive modalities. In fact, osMEG epileptic activity detection and source estimation might approach sbdEEG accuracy.

# 3. MATERIALS & METHODS

## 3.1 PART 1

### 3.1.1 Study I

*Participants*

At total of seven focal epilepsy patients (age range: 14-56 years, median age 20 years) were recruited at the Department of Neurology, Mayo Clinic. Four of these had FCDs, two had post-ischemic epilepsy and one had post-traumatic epilepsy. The patients had epileptic foci at the temporal, frontal and parietal lobe, and four of them at more than one epileptic focus. All patients underwent sbdEEG implantation as a part of clinical epilepsy surgery evaluations. As the SOZ was found to be located within eloquent cortex, surgery was unfeasible and patients were offered neurostimulation with chronic subthreshold cortical stimulation (CSCS) (Lundstrom et al., 2016).

*Stimulation paradigm and data acquisition*

All patients had a 4x4 sbdEEG with 1 cm spacing implanted covering the SOZ as well as adjacent areas surrounding the SOZ. Prior to neurostimulation, baseline sbdEEG was recorded for 6-7 days. The patients received biphasic 2 Hz stimulation through the SOZ-contacts for two days. Stimulation amplitude was set not to elicit motor response.

*Data analysis*

IEDs were visually identified by an experienced clinical neurophysiologist. Initially, IED rate (number of IEDs per minute), IED amplitude and duration were quantified during baseline and during ongoing stimulation. The spectral power of both SOZ and non-SOZ contacts were quantified during both conditions using autospectral density. Pairwise coherence in between all pairs of contacts was also calculated during both conditions. For all analyses, the pre-stimulation and stimulation conditions were statistically compared using ANOVA or T-tests.

### 3.1.2 Study II

*Participants*

The study included 14 patients (age range 7-46 years, median age 23 years) with monofocal epilepsy undergoing both structural MRI and convMEG recordings as a part of their epilepsy

surgery evaluations. Eleven of these patients exhibited temporal lobe epilepsy, while one had seizures starting from the insula, one from the parietal lobe, and one from the occipital lobe. The patients had varying underlying etiologies, including FCDs, stroke, and tuberous sclerosis.

*Data acquisition and analysis*

ConvMEG data was performed using a 306 channel whole head MEG system (Elekta TRIUX, Elekta Neuromag Oy, Helsiniki, Finland). A total of 102 magnetometer and 204 gradiometers, one magnetometer and two magnetometers together, were placed at each position. Data was recorded with 1000 Hz sampling rate and on-line bandpass filtered between 0.1-330 Hz. The data was hereafter stored for off-line analysis. Both horizontal and vertical eye movements, including eye blink artifacts, were recorded using bipolar electrooculography (EOG). Electromagnetic artifacts were suppressed using both a magnetically shielded room, and an internal active shielding. During the patient preparation, the patient head shape was digitized using a Polhemus FASTRAK. Head movement and head position was registered using the recording. All patients had undergone a structural MRI as a part of the clinical epilepsy evaluation. Anatomical T1-weighted sequences were utilized. As MRI protocols were determined by clinical indications, MRI parameters varied between subjects.

The study employed a combination of time-frequency analysis techniques to identify and characterize low-frequency oscillations (defined as 1-8 Hz) during pre-IED epochs (one second prior to IED onset, [-1000 ms, IED-onset]), compared to control epochs (the second preceding IED epochs [-2000 ms, -1000 ms]). Initially, beamforming analysis (Dynamical Imaging of Coherent Sources) (Gross et al., 2001; Hillebrand et al., 2012) of low-frequency oscillations was performed to compare pre-IED conditions to control conditions, both at the irritative zone, and control zones on both the ipsilateral and contralateral hemispheres. Hereafter, IED-event-locked synchronization/desynchronization was calculated using ERD-ERS (event-related desynchronization-synchronization) maps (Pfurtscheller and Lopes Da Silva, 1999). A non-parametric cluster-based permutation test designed for statistical evaluation of EEG/MEG data (Maris and Oostenveld, 2007) was utilized to test if the pre-IED epochs exhibited any significant up regulation of low-frequency oscillations compared to control epochs. Finally, the evolution of irritative zone pre-IED oscillations was characterized. Hilbert transformation was applied to the irritative zone source estimates, and linear fit of the peaks of the envelope was performed. The direction (positive/negative) of the correlation coefficient was determined, both for the pre-IED epoch and for 20 consecutive control epochs. The direction was used as a metric

to quantify if the amplitude of the oscillations were growing or diminishing. The control epochs were used to establish a binomial distribution, and the probability of the directions of pre-IED epoch low-frequency oscillations was calculated.

## 3.2 PART 2

### 3.2.1 Study III

*Participants and data acquisition*

One patient (45 years) with left temporal lobe epilepsy was recruited for both convMEG and an osMEG recording with htc-SQUID sensors. The patient initially underwent a convMEG recording (resting state, 30 minutes with eyes open, 30 minutes with eyes closed) with co-registration of scalp EEG (10-20 montage, 21 electrodes). The convMEG recording was conducted as described in 3.2 Study II. MNE source estimation were performed and the result was projected to the patient's clinical MRI using MNE-Python (Gramfort et al., 2013) and FreeSurfer (Dale et al., 1999; Fischl et al., 1999). Voltage maps of averaged IEDs were projected onto the reconstruction of the patient's scalp. This map was used to guide the positioning of the osMEG htc-SQUID sensors. A system with seven htc-SQUID sensors (for technical details, please see (Pfeiffer et al., 2020) was utilized for the osMEG recording. Two resting-state osMEG recordings with eyes closed, each lasting 30 minutes, were performed: One from the center of the positive magnetic field projected onto the scalp, and one from the corresponding negative maximum peak of the magnetic field. EEG co-registration was also performed during the osMEG recordings.

*Data analysis*

The osMEG data was filtered at 1-40 Hz followed by visual inspection of the data. The initial identification of IEDs was guided by simultaneous inspection of the co-registered EEG data. As the visual appearance of the osMEG data did not resemble that of convMEG, ocular identification of extra IEDs detected only by osMEG was deemed unfeasible. As a consequence, an alternative machine learning-based IED detection algorithm was developed. Fourteen statistical features including standard deviation, skewness and mean was extracted from the osMEG-IEDs that were also visible in the co-registered scalp EEG. A genetic algorithm (Mitchell, 1998) was hereafter utilized to construct synthetic IEDs resembling these verified, true IEDs. These synthetic IEDs

were used to train a support vector machine (SVM) (Pedregosa et al., 2011). The SVM was hereafter utilized to localize potential IEDs in the osMEG data not detected by the co-registered EEG. Finally, only such potential IEDs that could be considered as a time series anomaly were kept. This was defined as IEDs that entailed an equal or larger change in the extracted statistical features compared to a pre-IED baseline than the EEG-positive osMEG IEDs. The approach was validated on the convMEG data.

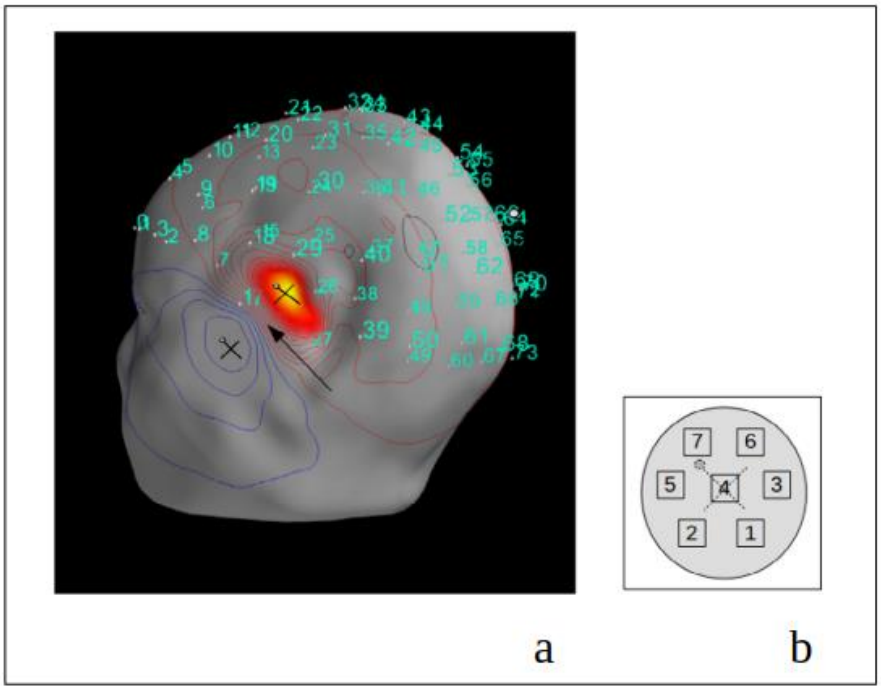



***Figure 10***
***Htc-SQUID osMEG measurement***
*The htc-SQUID osMEG measurements were performed from the maximum (red) and minimum (blue) peak magnetic fields of averaged interictal epileptiform discharges (IEDs) from the convMEG recording (a). Sensor layout and orientation are schematically illustrated in (b).*

### 3.2.2. Study IV

*Data simulations and analyses:*

Both IEDs and seizure activity was simulated using a neural mass model known to produce time series waveforms closely resembling epileptic activity (Wendling et al., 2000). A forward model based upon a template MRI from MNE Python (Gramfort et al., 2013) reconstructed using FreeSurfer (Dale et al., 1999; Fischl et al., 1999) was computed. OsMEG with 128 sensors,

convMEG with 306 sensors, hdEEG with 128 electrodes and sbdEEG with 223 electrodes (0.5 cm center-to-center distance) sensor arrays were modeled using MNE Python, and sensor data for each modality was simulated.

The study consists of four sub studies: In the first study, eight gradually growing epileptic foci (increasing the radius from 1.5 mm to 60 mm, step size 1 mm) were simulated at the mesial temporal lobe, the lateral temporal lobe, the frontopolar region, the lateral frontal lobe, the parietal lobe and the occipital lobe. For each modality, inverse solutions using ECDs where determined. The distances between the ECDs and foci centers were computed to quantify and compare the source estimation accuracies of all modalities. The second sub study characterized the cancellation indices of these epileptic foci.

In the third sub study, propagation seizure activity with SOZs in the mesial temporal lobe and the lateral frontal lobe simulated. ECDs of all modalities were determined and the distances between the ECDs and the SOZs were computed.

In the fourth study, a modified RSA (Kriegeskorte et al., 2008) was applied to compare IED source estimations of osMEG and sbdEEG. RSA is specifically developed to extract and compare features across different neuroimaging modalities. In this application, IEDs from over 4000 sites across the cortical mantle were simulated. Source estimations of these IEDs were calculated for osMEG, and osMEG virtual sensors from the same sites as the sbdEEG electrodes were extracted. Two 223x223-matrices, one with the osMEG virtual sensors with IED source estimation data, and one with sbdEEG IED sensor data were constructed. For both of these, the Euclidean distances between all pairs of entries were computed. The entry (both for osMEG and for sbdEEG) with the longest Euclidean distance to all other entries was defined as the maximum source estimation. For each modality and for each simulated IED, the Euclidean distance between this maximum source estimation and the true IED origin was computed. This allowed us to compare similarities between osMEG and subdural EEG recordings, especially with respect to how osMEG source estimation accuracy varies with source depth and orientation.

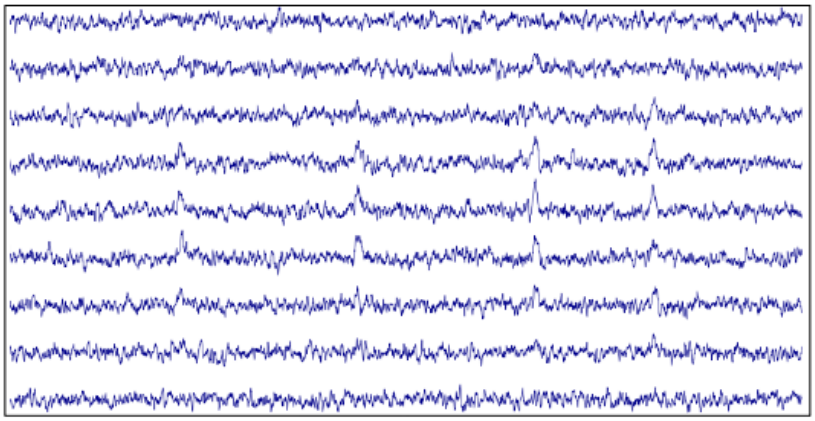


***Figure 11***
***Simulated raw on scalp MEG (osMEG) data***
*Example of interictal epileptiform discharges (IEDs) modeled using a neural mass model (for details see (Wendling et al., 2000)) in simulated raw osMEG sensor data.*

## 3.5 ETHICAL CONSIDERATIONS

All studies involving human study participants were approved by Ethical Review Boards. Study I was approved by Mayo Clinic IRB (Mod15-006530-38), Study II and Study III were approved by the regional Ethical Review Board in Stockholm (dnr 2016/1563-31 and 2018/1337-31). Written informed consents were obtained from all study patients.

*Study I*

The main risks associated with this project are related to the potential, and not negligible, adverse effect of sbdEEG implantation. These include both infections and hemorrhages. Although the electrode implantation was exclusively clinically motivated, the experimental neurostimulation prolonged the procedure and thereby the risk of infection.

*Study II*

All MEG recordings and clinical MRI measurements analyzed in this study were performed as a part of the patients' clinical epilepsy evaluations. Thus, study participation was not in itself associated with any increased risk for the participants. Study inclusion was not associated with any risk of incidental findings. Both MEG and MRI are considered safe procedures associated with minimal risks.

*Study III*

The study utilized an in-house osMEG sensor system with seven htc-SQUID sensors positioned within a thin cryostat filled with liquid nitrogen. The main, and potentially very harmful, risk associated with the study is any damage to the cryostat resulting liquid nitrogen leakage. Consequently, preparation of the ethical permit application involved extensive technical and medical safety evaluation of the device. The sensor system had been used in several cognitive neuroscience experiments in healthy participants prior to this study. Only highly trained and skilled personnel were involved in the recording. The participant also underwent convMEG with minimal to no risks involved. A clinical MRI with no risk of incidental findings was utilized.

# 4 RESULTS AND DISCUSSION

As the thesis contains to main themes – *part 1*) development of IEDs with special focus on low-frequency oscillations, and *part 2*) on-scalp MEG instrumentation for improved detection and source estimations of IEDs – presentation of results and discussion of these is also subdivided into two parts.

## 4.1 PART 1: DEVELOPMENT OF IEDS WITH SPECIAL FOCUS ON LOW-FREQUENCY OSCILLATION

### 4.1.1 Results Part 1

*Study I*

Onset of low-frequency neurostimulation significantly changed the content of the background activity. Neurostimulation entailed a lowering of spectral power for all frequencies 4-40 Hz, especially in the SOZ. Furthermore, functional connectivity quantified by coherence increased within the seizure onset zone during neurostimulation. Analyzing the spike rate demonstrated that number of IEDs per minute was significantly reduced during neurostimulation. During baseline, the patients exhibited on average 4.8 IEDs per minute. This number decreased to 1.5 IEDs/minute during stimulation. Interestingly, the stimulation also modified the IED appearance at the SOZ, but not in regions surrounding the SOZ. IED amplitude was significantly reduced ($p < 0.01$). A reduction in IED duration was noted, but these changes were not statistically significant. There was a significant correlation between the reduction in spike rate and background spectral powers. However, any potential causality between these factors cannot be inferred based upon this study. Thus, in conclusion, the study clearly demonstrates that low-frequency neurostimulation both reduce and modify IED rate and appearance in the SOZ.

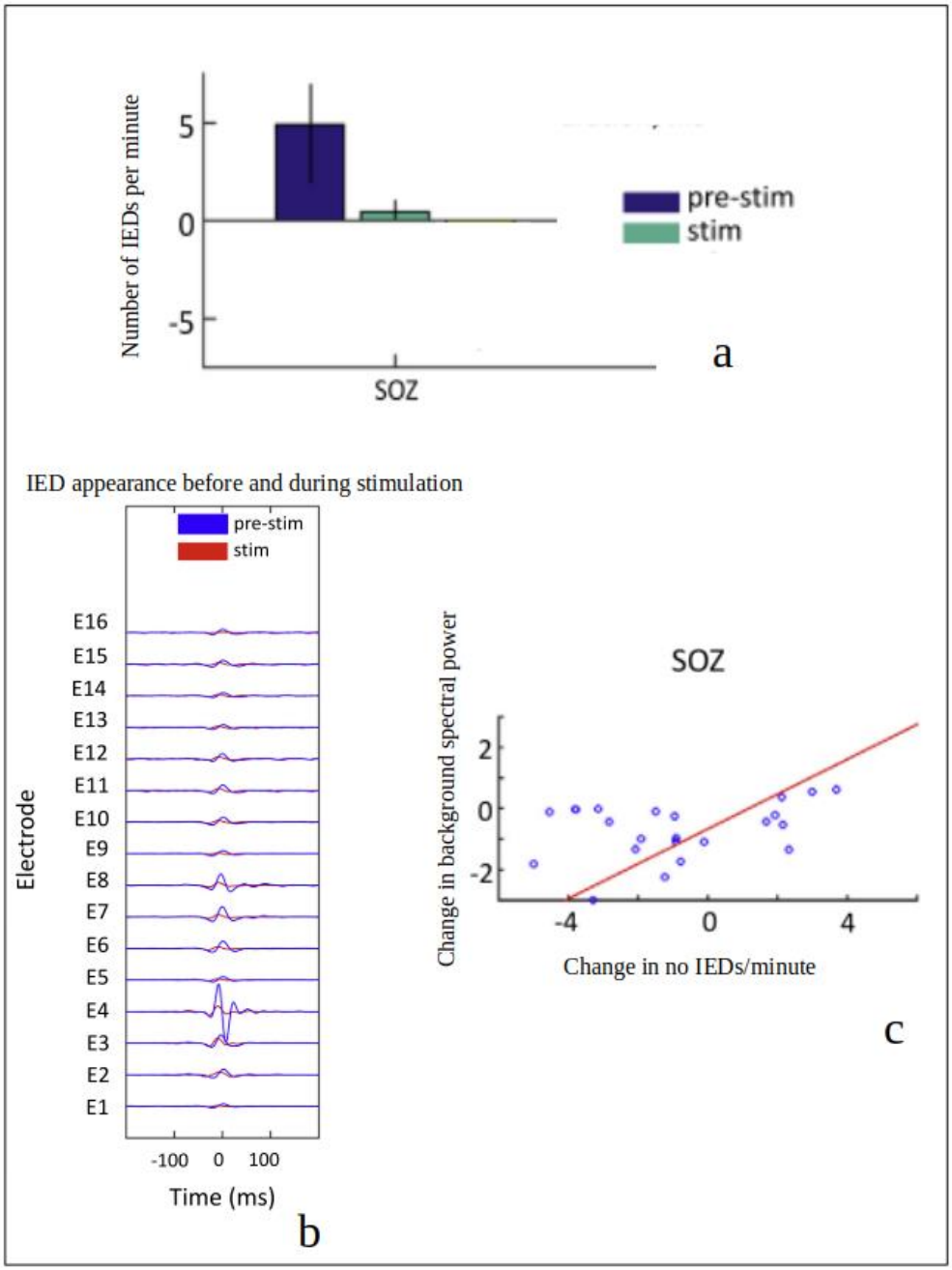
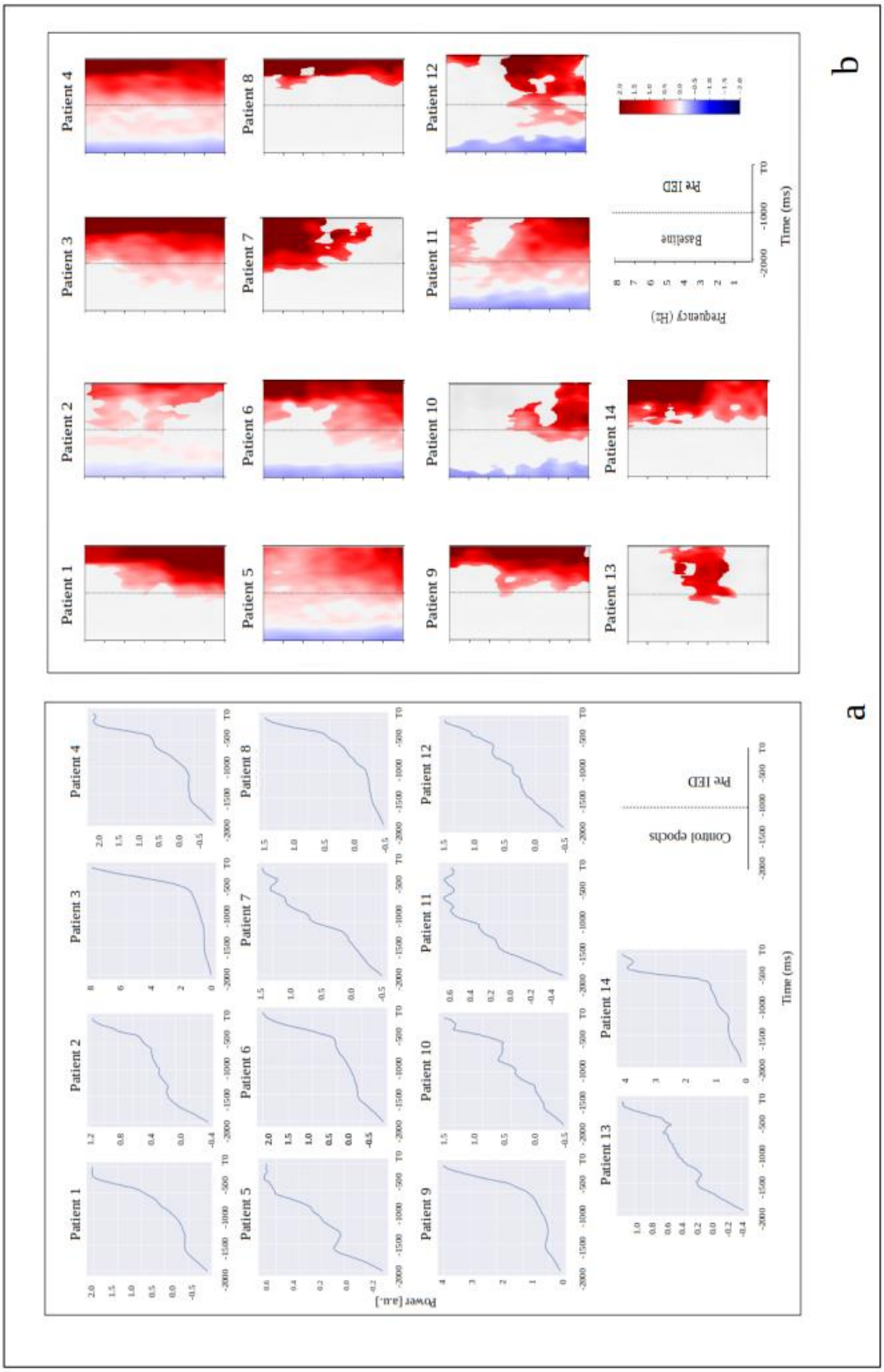



***Figure 12***
***Results from Study I***
*a) Change in IED rate (number of IEDs per minute) from pre-stimulation to stimulation at the seizure onset zone (SOZ) from 4.8 IEDs/minute during baseline to 0.05 IEDs/minute during stimulation.*
*b) Demonstration of the change in average IED appearance before and during stimulation. Stimulation significantly reduces IED amplitude. IED duration was slightly shorter during stimulation compared to baseline, but these changes were not significant.*
*c) Correlation between the change in background spectral power (4-40 Hz) and change in number of IEDs per minute.*

*Study II*

This study investigated naturally occurring low-frequency activity prior to IED onset. Analyzing the occurrence of low-frequency oscillations using beamformer DICS demonstrates that pre-IED epochs exhibit increased synchronization within delta (1-4 Hz) and theta (4-8 Hz) frequency bands compared to control epochs. Furthermore, these changes were seen only at the irritative zone. No significant differences were seen between pre-IED and control epochs around the irritative zone, or at the contralateral hemisphere. Analyzing ERD-ERS maps, we also showed that the significant increase in irritative zone low-frequency oscillations was time-locked to IED onset. Finally, we found evidence indicating the amplitude of these oscillations grew gradually throughout the pre-IED epoch, reaching a maximum at IED onset. In conclusion, the study demonstrated a gradual up-regulation of low-frequency oscillations prior to IED-onset at the irritative zone, but not at other sites on the cortex.


Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
Patient 9
Patient 10
Patient 11
Patient 12
Patient 13
Patient 14
Power [a.u.]
Control epochs
Pre IED
Time (ms)
a
Frequency (Hz)
Baseline
Pre IED
Time (ms)
b

***Previous page:***
***Figure 13***
***Results from Study II***
*a) Increase in low-frequency (1-8 Hz) spectral power during the pre-IED epoch (T0 occurs at IED onset)*
*b) Event-related desynchronization/synchronization (ERD-ERS) maps of control and pre-IED epochs demonstrating a significant pre-IED increase in low-frequency (1-8 Hz) oscillations (T0 occurs at IED onset).*

### 4.1.2 Discussion Part 1

Combining the results from Study I and Study II point to a role of low-frequency activity in IED development. In conjunction with Study I, Lundstrom et al have shown that this CSCS neurostimulation paradigm also reduce seizure frequency (Lundstrom et al., 2016). Interestingly, Study I also demonstrated that the neurostimulation especially modified the seizure onset zone, and the stimulation did not only modify spike rate, but the background activity as well. Mechanisms underlying the effect of neurostimulation still remains largely unknown (Starnes et al., 2019; Torres Diaz et al., 2021). The study of neurostimulation dynamics is also further complicated by the fact that there exists a large range of stimulation paradigms, targeting different sites. However, most stimulation paradigms target subcortical structures, why it is reasonable to assume that these techniques mainly target seizure propagation pathways. Several of these also utilize high-frequency stimulation. On the contrary, this neurostimulation paradigm directly targets the cortical areas. Thus, it is possible that CSCS directly influences the local cortical network that itself generates the seizure activity. It can be speculated that the direct stimulation of the SOZ itself modulates local epileptic network activity, thereby reducing the cortical propensity to generate epileptic activities comprising both IEDs and seizures. The 2 Hz stimulation could affect dendritic input to the pyramidal cells, possibly hyperpolarizing these cells and reducing IED spiking. An inhibitory role for low-frequency stimulation has been corroborated by other authors. For instance, low-frequency TMS has been proven to induce several seconds inhibition, resulting in decreased seizure burden. (Kile et al., 2010).. Furthermore, the study showed a correlation between reduction in spectral power and decrease in spike rate at the SOZ. Although any causality cannot be inferred from this study, it is possible that these changes in overall spectral content reflect local network processes that influence development of epileptic activities. The hypothesis that such factors influence IED generation was further explored in Study II. This paper clearly demonstrates that the development of IEDs do not occur

momentously, but rather out of mesoscopic interactions in the irritative zone. This is further supported by other studies using different neuroimaging modalities. For instance, functional MRI (fMRI) studies have indicated that metabolic changes develop slowly before the IED occurs. (Hawco et al., 2007; Jacobs et al., 2009). As mentioned above, low-frequency mediate inhibition in both cognitive neuroscience and epilepsy (Herring et al., 2019; Schevon et al., 2012b; Trevelyan and Schevon, 2013). We speculate that the low-frequency oscillations seen here reflect local inhibition that eventually is overcome by the hypersynchronization and hyperexcitation associated with the actual IED. Such a role is well described in seizure dynamics. Here, slow activity has been coupled to an ictal inhibitory penumbra which is eventually overcome by a stronger excitation to allow for seizure propagation (Eissa et al., 2016). Furthermore, cellular recordings as well as local field potential recordings have demonstrated that both individual neurons and smaller groups of neurons exhibit inhibition during several seconds prior to eliciting paroxysmal depolarizing shift (Schevon et al., 2012; Trevelyan and Schevon, 2013).

In summary, Study I and Study II together indicate a role for low-frequency activity as a conveyor of inhibition in epilepsy.

## 4.2 PART 2: DEVELOPMENT OF ON-SCALP MEG INSTRUMENTATION WITH SPECIAL FOCUS ON IED DETECTION AND SOURCE ESTIMATIONS

### 4.2.1 Results Part 2

*Study III*

First of all, the study demonstrated that it was feasible to record IEDs with an osMEG system. At the time, this was the first-ever recording from an epilepsy patient. Especially, we could verify recordings of IEDs in the on-scalp MEG data using simultaneous EEG recordings, stressing the importance of utilizing one well-known modality to verify any findings. Furthermore, we found an additional potential 31 IEDs that were visible only in the osMEG data, and not in the EEG data. These were found using the machine learning-based IED detection algorithm that was developed to extract such potential IED activity. The algorithm extracted 14 statistical features from the EEG-positive IEDs found in the on-scalp MEG data. Hereafter, waveforms in the osMEG data that resembled these EEG-positive IEDs were identified. These potential IEDs were inspected by an experienced clinician in order to determine whether these resembled epileptic activities. The algorithm was also tested on convMEG. Here, 20 out of 24 IEDs were correctly identified, and no additional false positive events were localized. The amplitude of these events was slightly lower than the amplitude of the EEG-positive IEDs. Thus, in conclusion, the study both demonstrates a feasible benchmarking protocol for detecting epileptic activity using on-scalp MEG sensors, and for the first time ever, that osMEG probably detect more epileptic activities than convMEG.

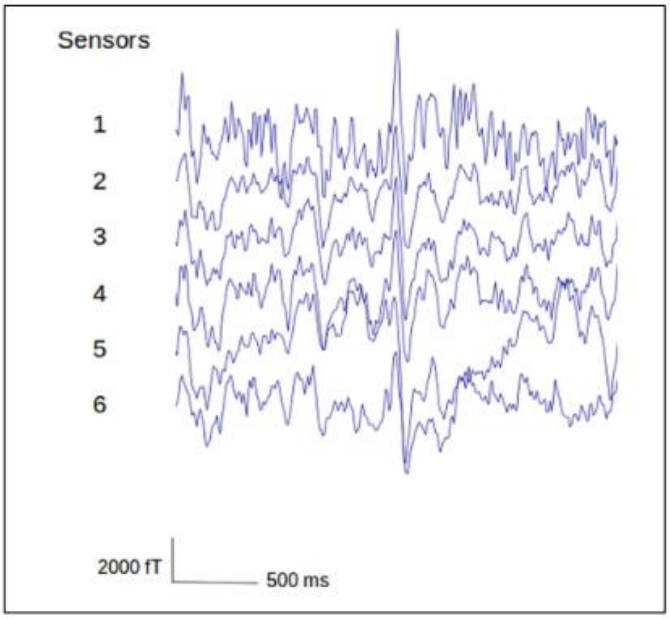



***Figure 14***
***Htc-SQUID OsMEG raw sensor data***
*Illustration of raw sensor data (filtered 1-40 Hz) with a visually identifiable "high-amplitude event" that do correspond to any EEG-positive IED. Thus, only conventional, clinical ocular inspection of the data was not sufficient to identify htc-SQUID osMEG IEDs.*

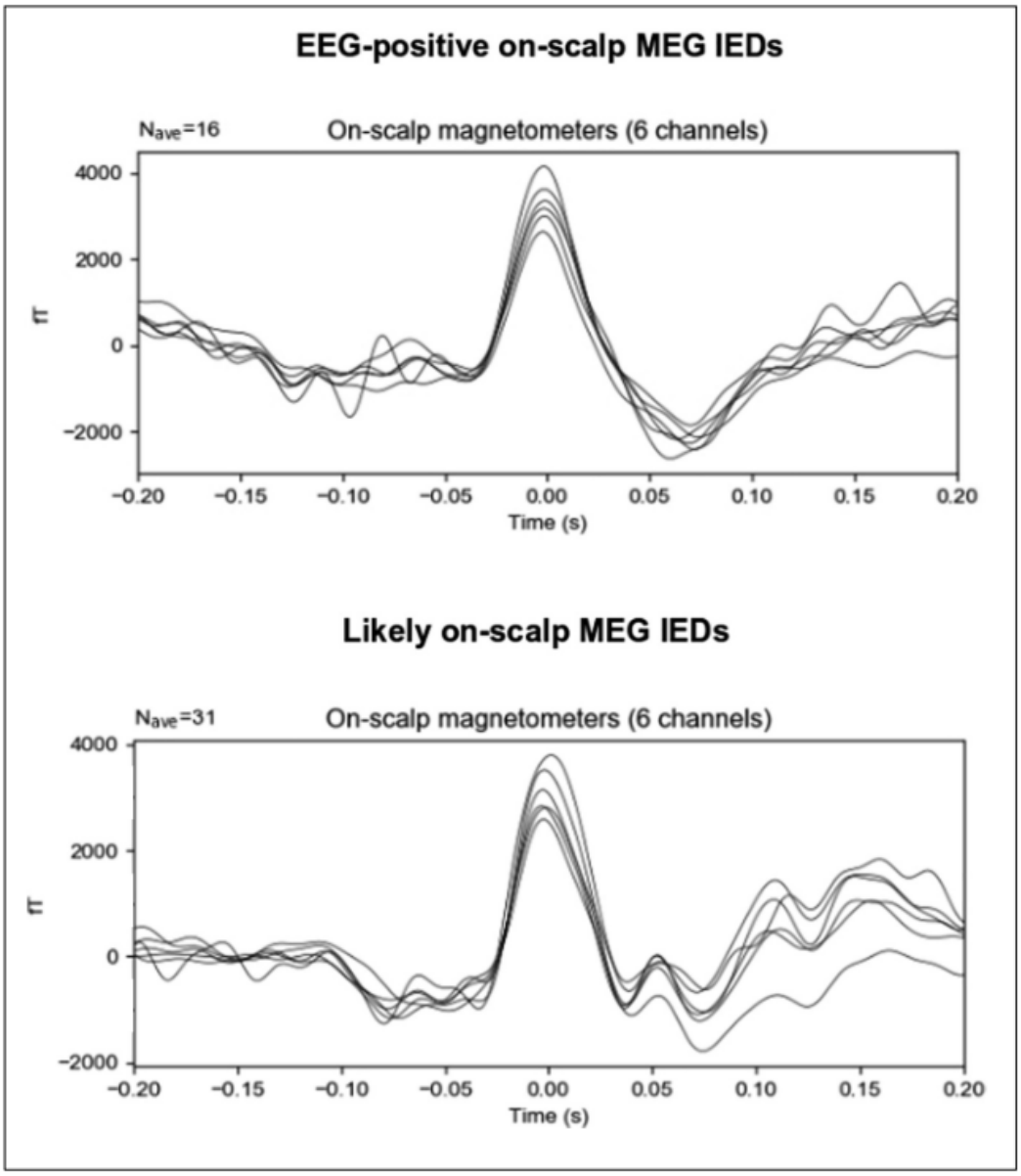



***Figure 15***
***Results from Study III***
*Top: Average of interictal epileptiform discharges (IEDs) seen both in htc-SQUID osMEG, and in the co-registered EEG.*
*Below: Average of IEDs seen only in the htc-SQUID osMEG data found using the IED-detection algorithm developed within the study.*

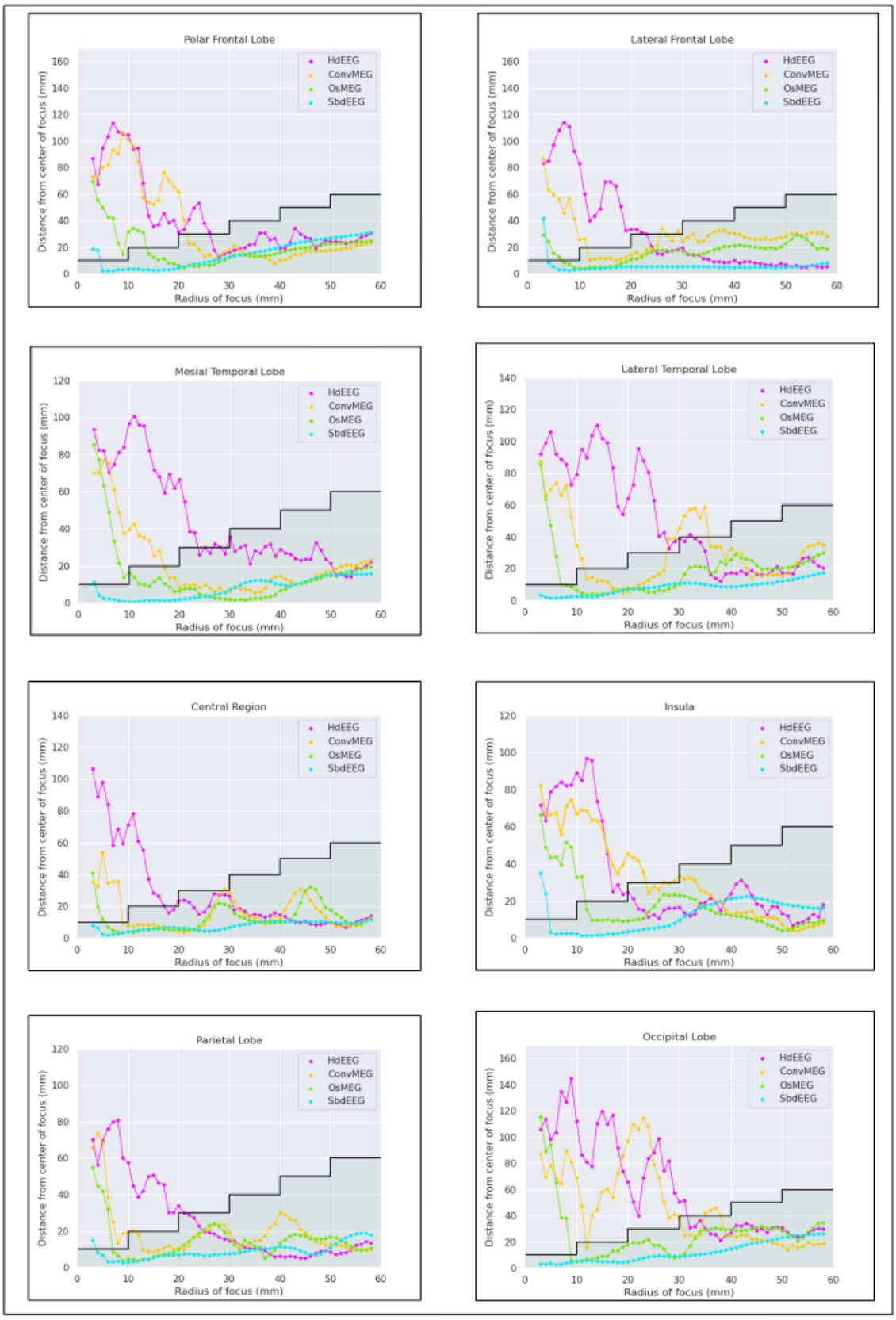
*Study IV*

The modeling study compared detection and source estimations of IEDs as well as epileptic seizure activity of the three non-invasive modalities convMEG, osMEG, hdEEG and sbdEEG.

Analyzing how the modalities localized IEDs in the polar frontal region, the lateral frontal region, the mesial temporal region, the lateral temporal region, the central region, insula, the parietal lobe and the occipital lobe demonstrated, as suspected, that sbdEEG was superior to all other modalities. SbdEEG could accurately localize IEDs originating from regions as small as approximately 3 $cm^2$. However, for foci sizes between 3 and 23 $cm^2$, osMEG ECD dipoles were significantly closer to the source of the epileptogenic foci than the other non-invasive modalities.

Investigating how the four modalities localized a simulated SOZ demonstrated that only sbdEEG and osMEG could localize mesial temporal lobe onset to the correct lobe, while both convMEG and hdEEG localized the SOZ to other lobes. However, in localization of a dorsal frontal lobe seizure, all modalities performed relatively equal.

Finally, using RSA, we compared the performance of osMEG and sbdEEG for localization of IEDs originating from over 4000 sites across the entire cortical mantle. This study showed that osMEG source estimations were never more than 4.4 cm away from the source estimation derived from the sbdEEG data. In addition, this comparison gave us a tool to analyze how IED source estimation depended on source depth and source orientation. Very interestingly, osMEG source estimation accuracy did not depend on source orientation. Source depth, however, did reduce the accuracy of osMEG source estimations.


Polar Frontal Lobe
Lateral Frontal Lobe
Mesial Temporal Lobe
Lateral Temporal Lobe
Central Region
Insula
Parietal Lobe
Occipital Lobe
HdEEG
ConvMEG
OsMEG
SbdEEG
Distance from center of focus (mm)
Radius of focus (mm)

***Previous page:***
***Figure 16***
***Preliminary results from Study IV***
*Comparison of equivalent current dipoles (ECDs) determined for on scalp MEG (osMEG), high density EEG (hdEEG), conventional MEG (convMEG) and subdural EEG (sbdEEG) computed for gradually growing epileptic foci at eight different sites across the cortical mantle.*

### 4.2.2 Discussion Part 2

Both studies clearly demonstrate that osMEG potentially has an important role to play in epilepsy evaluations. Study III experimentally showed that osMEG might detect more epileptic activity than convMEG and clinical EEG (21 electrodes). Of course, we cannot be certain that these events are true IEDs. However, both modeling studies as well as other experimental studies demonstrated that osMEG detect cortical activity with higher amplitude than convMEG (Andersen et al., 2017; Xie et al., 2017). It is reasonable to assume that osMEG thus picks up activity of lower amplitude than convMEG. As amplitude directly corresponds to the size of the cortical area activated, it implies that osMEG should capture IEDs originating from smaller patches of the irritative zone than do convMEG. Correspondingly, the amplitude of the potential IEDs detected only by osMEG did have a smaller amplitude than those detected by convMEG, indicating that they could indeed be IEDs originating from smaller patches not identifiable on convMEG, or EEG. Of course, definitive verification whether this is the case would require simultaneous intracranial registrations. This is true not only for Study III, but rather to all future studies of osMEG in epilepsy evaluations. Comparison of iEEG and scalp EEG reveals that iEEG picks up much more small amplitude spikes from separate regions of the irritative zone, that cannot be seen on scalp EEG. (Ray et al., 2007; Tao et al., 2007). It is probable, that osMEG will pick up at least some of these. However, no other non-invasive functional neuroimaging modality can be used to verify these findings. Any characterization of how well osMEG detects, localizes and separates epileptogenic foci, will require simultaneous intracranial EEG. Nonetheless, both Study III and Study IV indicates a clear role for osMEG within epilepsy evaluations. It is probable that osMEG will be better at capturing epileptic activity from sites that are quite inaccessible to other non-invasive techniques. The technique might drastically improve non-invasive surgery evaluations with better sEEG planning potentials. However, equally interesting, osMEG will entail a first-ever possibility to characterize whole-head epileptic networks. Today, such a characterization falls in between the seats of highly detailed, but limited, intracranial recordings,

and less detailed whole head non-invasive techniques. Study IV also investigated how sensitive osMEG source estimations are to both source depth, and source orientation. We found that osMEG, as well as convMEG, is less sensitive to deep sources than shallow one. This is in accordance with other studies. However, we demonstrated that osMEG was equally sensitive to source orientation than subdural EEG. Similar findings have been reported before with osMEG detecting more radial components of evoked potentials than do conventional MEG (Andersen et al., 2017). This is probably due to these sources not being truly radial, so that the shift in orientation of the sensors results in detection additional signal components.

In conclusion, osMEG do not only open up for improved ictal MEG recordings, but also to for radical improvement of both clinical evaluations as well as epileptic network research.

# 5. LIMITATIONS

*Study I*

The study only included a very small patient population which, of course, is a major limitation. There are also some methodological limitations to the study. The raw sbdEEG data contains a strong 2 Hz stimulation artifact. Only visual inspection of the data was performed to determine the temporal extent of stimulation artifact data contamination. No additional quantification of the spectral content was performed to investigate any frequency leakage into the analyzed time series. As the coherence between all pairs of electrodes increased during stimulation, investigation of whether these findings resulted from stimulation artifacts should have been performed. Similarly, the study reports that stimulation onset changed the spectral power for all frequencies 4-40 Hz. Such findings could also be influenced by stimulation frequency leakage. The discussion section point out a correlation between background power and IED rate, and speculates that the background might influence SOZ microcircuit propensity to develop IEDs. Inclusion of any causality analysis, for instance Granger causality, could have been included to better support such assumptions. Although it is well known that sbdEEG electrodes exhibit less field spread than non-invasive modalities, approximation of electrode field spread should have been included as adjacent electrode time series are compared. An easily accessible field spread metric could have been achieve by comparing the number of contacts delivering stimulation, with the number of electrodes that pick up the stimulation artifacts.

*Study II*

Similarly to Study I, a relatively small patient population was analyzed. The study aimed to analyze pre-IED changes both within and outside of the irritative zone. The majority of sites outside of the irritative zone were chosen only based upon the distance from this region. As epilepsy is a network disorder, distant areas might exhibit both structural and functional abnormalities. Thus, the choice of extrafocal sites constitute a study limitation. A better approach would have been to first characterize epileptic networks of the patient's, possibly using investigation of global functional connectivity changes associated with IED onset. Hereafter, both nodes within and outside the epileptic network could have been analyzed to better characterize pre-IED changes.

The study demonstrates that the patients exhibit changes within either the delta (1-4 Hz) or theta (4-8 Hz) bands, or both. These bands are collectively referred to as low-frequency

oscillations. It is not discussed whether up-regulations in one of the frequency bands in one patient, can be compared to up-regulations in the other frequency band in another patient. A more exhaustive evaluation of pre-IED changes should have included analyses of higher frequencies as well.

ERD-ERS maps utilize sensor data. In the study, the sensors that covered the irritative zone were chosen for analysis of pre-IED changes. This is unfortunate, since this sensor data might contain data from sources outside of the irritative zone as well.

As a concluding remark, it can note that the analysis of gradual development using Hilbert transformation is based upon a linear fit of peak amplitude of the signal envelope. However, the linear fit might exhibit a positive correlation coefficient, even though the amplitude of the original time series drops right before IED onset. Thus, the methodological might not be suitable for evaluation of temporal evolution.

*Study III*

OsMEG recordings of epileptic activities is the art of detecting patient-specific brain signals potentially not seen by any other non-invasive neuroimaging modality. Indeed, understanding the sensor output would require simultaneous iEEG recordings. This constitutes the major limitation to Study III. Only seven osMEG sensors were used here. This makes interpretation of any potential IED findings difficult. A larger number of sensors could have revealed whether the findings are localized to the irritative zone or not. Furthermore, source imaging could have been performed further supporting the results.

There are also several limitations to the IED detection algorithm developed. Of course, the data sample size from which EEG-positive osMEG IED statistical features were extracted was too small. Furthermore, parameter exploration should have been performed in order to determine statistically which features that best captured the characteristics of EEG-positive osMEG IEDs. Finally, the detection algorithm probably suffers from severe overfitting problems. A large number of synthetic IEDs were created using a genetic algorithm. These were used to train a SVM to classify the same osMEG data from which the original statistical features were extracted. Obviously, this is not an optimal approach, but a result of having only one recording to analyze.

*Study IV*

This manuscript should be considered as an unfinished study requiring much additional revisions. There are several limitations in the methodology. The major limitation concerns simulation of so-called brain noise in the osMEG recordings. That is, all other cortical activities, except epileptic activities. The current manuscript utilizes a very low and maybe too optimistic OPM sensor noise level, and no modeling of additional sources except for the epileptic activity. These limitations might render the results to be unrealistically in favor of osMEG sensors. A more realistic background activity simulation might potentially obscure the epileptic activity more in the osMEG data than in convMEG. Epilepsy patients often exhibit pathological slow, high amplitude activity. Such activity will also have higher signal amplitude in osMEG than in convMEG. It might be that epileptic activities might be more difficult to identify in osMEG data, than in convMEG data. It is theoretically possible that detection of such pathological epilepsy-associated activity made visual inspection the htc-SQUID recordings of Study III unfeasible.

# 6. CONCLUSION

The overall aim of the thesis is to improve the characterization of epileptic activity, and increase the understanding of how it arises from synchronized brain activity. The thesis tries to provide an insight into these complex questions through a dual approach. The first part of the study is concerned with neurophysiological studies of IED development. Study I utilized a neurostimulation paradigm was analyzed to investigate the effect of SOZ low-frequency (2 Hz) stimulation on IED dynamics and behavior. It is concluded that this stimulation not only reduces IED rate, but lower IED amplitude and changes the background activity. Study II explores the natural occurrence of low-frequency (1-8 Hz) oscillations prior to IED onset. It is demonstrated that the irritative zone is characterized by a gradual up-regulation of low-frequency amplitude, reaching a maximum at IED onset. We propose that this might result from a local cortical inhibition that is overcome at IED onset. Taken together, this first part of the thesis indicates that low-frequency activity might play an important role in epilepsy as a reflection of cortical mesoscopic inhibitory processes.

The second part of the thesis deals the utilization of osMEG within epilepsy evaluations. Study III constitutes the first ever osMEG recording of an epilepsy patient. The results demonstrates that osMEG can detect at least as many IEDs as scalp EEG, and probably many more. Thus, the study is a clear indication that osMEG might play an important role in epilepsy evaluations and can improve non-invasive epileptic activity source imaging. However, the study also demonstrates some of the difficulties that come with novel neuroimaging techniques. Understanding the detected, potentially epileptic, activity requires simultaneous iEEG recordings to clarify the nature of spiky activity seen only in osMEG, and no other non-invasive investigation. We also discovered that visual inspection of the osMEG, similar to clinical visual inspection of both EEG and convMEG was highly unfeasible. This might be due to internal sensor noise, but it might also be that the higher signal power of osMEG results in amplification of the background activity as well. Thus, it might be that osMEG brain noise obscures the epileptic activity. The, at the time of writing, unfinished, Study IV compares epilepsy evaluations using convMEG, hdEEG, osMEG and sbdEEG. The current manuscript version simulates only IEDs, and no other cortical background activity. In this setting, osMEG outperforms other non-invasive modalities. However, it is possible that adding more realistic brain noise simulations will in fact demonstrate limitations to the system.

As a conclusion, the thesis clearly demonstrates that understanding the cortical processes by which epileptic activity develops, and can be understood requires a combined understanding for both neurophysiology, and neuroimaging.

# 7. REFERENCES


Ahlfors SP, Han J, Belliveau JW, Hämäläinen MS. Sensitivity of MEG and EEG to source orientation. Brain Topogr 2010;23:227–32. https://doi.org/10.1007/s10548-010-0154-x.

An S, Bartolomei F, Guye M, Jirsa V. Optimization of surgical intervention outside the epileptogenic zone in the virtual epileptic patient (VEP). PLoS Comput Biol 2019;15:1–25. https://doi.org/10.1371/journal.pcbi.1007051.

Andersen LM, Oostenveld R, Pfeiffer C, Ruffieux S, Jousmäki V, Hämäläinen M, et al. Similarities and differences between on-scalp and conventional in-helmet magnetoencephalography recordings. PLoS One 2017;12:1–19. https://doi.org/10.1371/journal.pone.0178602.

Arévalo-Astrada M, McLachlan RS, Suller-Marti A, Parrent AG, MacDougall KW, Mirsattari SM, et al. All that glitters: Contribution of stereo-EEG in patients with lesional epilepsy. Epilepsy Res 2021;170. https://doi.org/10.1016/j.eplepsyres.2020.106546.

Aronica E, Crino PB. Epilepsy Related to Developmental Tumors and Malformations of Cortical Development. Neurotherapeutics 2014;11:251–68. https://doi.org/10.1007/s13311-013-0251-0.

Avoli M. Inhibition, oscillations and focal seizures: An overview inspired by some historical notes. Neurobiol Dis 2019;130:2018–20. https://doi.org/10.1016/j.nbd.2019.104478.

Ayala GF, Matsumoto H, Gumnit RJ. Excitability changes and inhibitory mechanisms in neocortical neurons during seizures. J Neurophysiol 1970;33:73–85. https://doi.org/10.1152/jn.1970.33.1.73.

Baybis M, Yu J, Lee A, Golden JA, Weiner H, McKhann G, et al. mTOR cascade activation distinguishes tubers from focal cortical dysplasia. Ann Neurol 2004;56:478–87. https://doi.org/10.1002/ana.20211.

Becker AJ, Blümcke I, Urbach H, Hans V, Majores M. Molecular neuropathology of epilepsy-associated glioneuronal malformations. J Neuropathol Exp Neurol 2006;65:99–108. https://doi.org/10.1097/01.jnen.0000199570.19344.33.

Beghi E. The Epidemiology of Epilepsy. Neuroepidemiology 2020;54:185–91. https://doi.org/10.1159/000503831.

Benbadis SR, Beniczky S, Bertram E, MacIver S, Moshé SL. The role of EEG in patients with suspected epilepsy. Epileptic Disord 2020;22:143–55. https://doi.org/10.1684/epd.2020.1151.

Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, Van Emde Boas W, et al. Revised terminology and concepts for organization of seizures and epilepsies: Report of the ILAE Commission on Classification and Terminology, 2005-2009. Epilepsia 2010;51:676–85. https://doi.org/10.1111/j.1528-1167.2010.02522.x.

Blair RDG. Temporal Lobe Epilepsy Semiology. Epilepsy Res Treat 2012;2012:1–10. https://doi.org/10.1155/2012/751510.

Blümcke I, Vinters H V., Armstrong D, Aronica E, Thom M, Spreafico R. Malformations of cortical development and epilepsies: Neuropathological findings with emphasis on focal cortical dysplasia. Epileptic Disord 2009;11:181–93. https://doi.org/10.1684/epd.2009.0261.

Boer K, Troost D, Timmermans W, Van Rijen PC, Spliet WGM, Aronica E. Pi3K-mTOR signaling and AMOG expression in epilepsy-associated glioneuronal tumors. Brain Pathol 2010;20:234–44. https://doi.org/10.1111/j.1750-3639.2009.00268.x.

Borna A, Carter TR, Goldberg JD, Colombo AP, Jau YY, Berry C, et al. A 20-Channel Magnetoencephalography System Based on Optically Pumped Magnetometers. Phys Med Biol 2018;62:8909–23. https://doi.org/10.1016/j.physbeh.2017.03.040.

Boto E, Bowtell R, Krüger P, Fromhold TM, Morris PG, Meyer SS, et al. On the potential of a new generation of magnetometers for MEG: A beamformer simulation study. PLoS One 2016;11. https://doi.org/10.1371/journal.pone.0157655.

Boto E, Hill RM, Rea M, Holmes N, Seedat ZA, Leggett J, et al. Measuring functional connectivity with wearable MEG. Neuroimage 2021;230:117815. https://doi.org/10.1016/j.neuroimage.2021.117815.

Boto E, Holmes N, Leggett J, Roberts G, Shah V, Meyer SS, et al. Moving magnetoencephalography towards real-world applications with a wearable system. Nature 2018;555:657–61. https://doi.org/10.1038/nature26147.

Bourel-Ponchel E, Mahmoudzadeh M, Berquin P, Wallois F. Local and distant dysregulation of synchronization around interictal spikes in BECTS. Front Neurosci 2017;11:1–17. https://doi.org/10.3389/fnins.2017.00059.

Bressler SL, Menon V. Large-scale brain networks in cognition: emerging methods and principles. Trends Cogn Sci 2010;14:277–90. https://doi.org/10.1016/j.tics.2010.04.004.

Budker D, Romalis M. Optical magnetometry. Nat Phys 2007;3:227–34. https://doi.org/10.1017/CBO9780511846380.

Cohen D. Magnetoencephalography: Evidence of Magnetic Fields Produced by Alpha-Rhythm Currents. Science 161(3843):784–6.

Cohen JR, D'Esposito M. The segregation and integration of distinct brain networks and their relationship to cognition. J Neurosci 2016;36:12083–94. https://doi.org/10.1523/JNEUROSCI.2965-15.2016.

Coito A, Plomp G, Genetti M, Abela E, Wiest R, Seeck M, et al. Dynamic directed interictal connectivity in left and right temporal lobe epilepsy. Epilepsia 2015;56:207–17. https://doi.org/10.1111/epi.12904.

Dale AM, Fischl B, Sereno MI. Cortical Surface-Based Analysis I. Segmentation and Surface Reconstruction. Neuroimage 1999;9:179–94. https://doi.org/10.1006/nimg.1998.0395.

Dale AM, Liu AK, Fischl BR, Buckner RL, Belliveau JW, Lewine JD, et al. Dynamic Statistical Parametric Mapping: Combining fMRI and MEG for High-Resolution Imaging of Cortical Activity. Vol. 26. 2000. https://doi.org/10.1016/S0896-6273(00)81138-1

Destexhe A. Spike-and-wave oscillations based on the properties of GABA(B) receptors. J Neurosci 1998;18:9099–111. https://doi.org/10.1523/JNEUROSCI.18-21-09099.1998

Dichter M, Spencer WA. Pencillin-Induced Interictal Discharges from the Cat Hippocampus. I. Characteristics and Topographical Features. J Neurophysiol 1969;32:649–62. https://doi.org/10.1152/jn.1969.32.5.649

Dichter M, Spencer WA. Penicillin-induced interictal discharges from the cat hippocampus. II. Mechanisms underlying origin and restriction. J Neurophysiol 1969;32:663–87. https://doi.org/10.1152/jn.1969.32.5.663.

Duez L, Beniczky S, Tankisi H, Hansen PO, Sidenius P, Sabers A, et al. Added diagnostic value of magnetoencephalography (MEG) in patients suspected for epilepsy, where previous, extensive EEG workup was unrevealing. Clin Neurophysiol 2016;127:3301–5. https://doi.org/10.1016/j.clinph.2016.08.006.

Duez L, Tankisi H, Hansen PO, Sidenius P, Sabers A, Pinborg LH, et al. Electromagnetic source imaging in presurgical workup of patients with epilepsy: A prospective study. Neurology 2019;92:e576–86. https://doi.org/10.1212/WNL.0000000000006877.

Eissa TL, Tryba AK, Marcuccilli CJ, Ben-Mabrouk F, Smith EH, Lew SM, et al. Multiscale aspects of generation of high-gamma activity during seizures in human neocortex. ENeuro 2016;3:281–90. https://doi.org/10.1523/ENEURO.0141-15.2016.

Fahoum F, Lopes R, Pittau F, Dubeau F, Gotman J. Widespread epileptic networks in focal epilepsies: EEG-fMRI study. Epilepsia 2012;53:1618–27. https://doi.org/10.1111/j.1528-1167.2012.03533.x.

Fischl B, Sereno MI, Dale AM. Cortical surface-based analysis: II. Inflation, flattening, and a surface-based coordinate system. Neuroimage 1999;9:195–207.https://doi.org/10.1006/nimg.1998.0396.

Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, et al. ILAE Official Report: A practical clinical definition of epilepsy. Epilepsia 2014;55:475–82. https://doi.org/10.1111/epi.12550.

Fisher RS, Cross JH, French JA, Higurashi N, Hirsch E, Jansen FE, et al. Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017;58:522–30. https://doi.org/10.1111/epi.13670.

Foci E, Prince DA, Joe Wilder B, Alto P. Control Mechanisms in Cortical “Surround” Inhibition. 1967. Arch Neurol. Feb;16(2):194-202. Doi: 10.1001/archneur.1967.00470200082007

Gaillard WD, Chiron C, Helen Cross J, Simon Harvey A, Kuzniecky R, Hertz-Pannier L, et al. Guidelines for imaging infants and children with recent-onset epilepsy. Epilepsia 2009;50:2147–53. https://doi.org/10.1111/j.1528-1167.2009.02075.x.

Garcés P, López-Sanz D, Maestú F, Pereda E. Choice of magnetometers and gradiometers after signal space separation. Sensors (Switzerland) 2017;17:1–13. https://doi.org/10.3390/s17122926.

Gramfort A, Luessi M, Larson E, Engemann DA, Strohmeier D, Brodbeck C, et al. MEG and EEG data analysis with MNE-Python. Front Neurosci 2013;7:1–13. https://doi.org/10.3389/fnins.2013.00267.

Grenier F, Timofeev I, Crochet S, Steriade M. Spontaneous field potentials influence the activity of neocortical neurons during paroxysmal activities in vivo. Neuroscience 2003;119:277–91. https://doi.org/10.1016/S0306-4522(03)00101-5.

Gross J, Kujala J, Hämäläinen M, Timmermann L, Schnitzler A, Salmelin R. Dynamic imaging of coherent sources: Studying neural interactions in the human brain. Proc Natl Acad Sci U S A 2001;98:694–9. https://doi.org/10.1073/pnas.98.2.694.

Hagen E, Næss S, Ness T V., Einevoll GT. Multimodal modeling of neural network activity: Computing LFP, ECoG, EEG, and MEG signals with LFPy 2.0. Front Neuroinform 2018;12. https://doi.org/10.3389/fninf.2018.00092.

Hämäläinen M, Hari R, Ilmoniemi RJ, Knuutila J, Lounasmaa O V. Magnetoencephalography theory, instrumentation, and applications to noninvasive studies of the working human brain. Rev Mod Phys 1993;65:413–97. https://doi.org/10.1103/RevModPhys.65.413.

Hämäläinen MS, Ilmoniemi RJ. Interpreting magnetic fields of the brain: minimum norm estimates. Med Biol Eng Comput 1994;32:35–42. https://doi.org/https://doi.org/10.1007/BF02512476.

Hawco CS, Bagshaw AP, Lu Y, Dubeau F, Gotman J. BOLD changes occur prior to epileptic spikes seen on scalp EEG. Neuroimage 2007;35:1450–8. https://doi.org/10.1016/j.neuroimage.2006.12.042.

Heiden C. SQUID and SQUID system developments for biomagnetic applications. Clin Phys Physiol Meas 1991;12:67–73. https://doi.org/10.1088/0143-0815/12/B/009.

Herring JD, Esterer S, Marshall TR, Jensen O, Bergmann TO. Low-frequency alternating current stimulation rhythmically suppresses gamma-band oscillations and impairs perceptual performance. Neuroimage 2019;184:440–9. https://doi.org/10.1016/j.neuroimage.2018.09.047.

Hill RM, Boto E, Rea M, Holmes N, Leggett J, Coles LA, et al. Multi-channel whole-head OPM-MEG: Helmet design and a comparison with a conventional system. Neuroimage 2020;219. https://doi.org/10.1016/j.neuroimage.2020.116995.

Hillebrand A, Barnes GR, Bosboom JL, Berendse HW, Stam CJ. Frequency-dependent functional connectivity within resting-state networks: An atlas-based MEG beamformer solution. Neuroimage 2012;59:3909–21. https://doi.org/10.1016/j.neuroimage.2011.11.005.

Horn A, Ostwald D, Reisert M, Blankenburg F. The structural-functional connectome and the default mode network of the human brain. Neuroimage 2014;102:142–51. https://doi.org/10.1016/j.neuroimage.2013.09.069.

Hwang G, Nair VA, Mathis J, Cook CJ, Mohanty R, Zhao G, et al. Using low-frequency oscillations to detect temporal lobe epilepsy with machine learning. Brain Connect 2019;9:184–93. https://doi.org/10.1089/brain.2018.0601.

Iivanainen J, Stenroos M, Parkkonen L. Measuring MEG closer to the brain: Performance of on-scalp sensor arrays. Neuroimage 2017;147:542–53. https://doi.org/10.1016/j.neuroimage.2016.12.048.

Jabran Y, Mahmoudzadeh M, Martinez N, Heberlé C, Wallois F, Bourel-Ponchel E. Temporal and Spatial Dynamics of Different Interictal Epileptic Discharges: A Time-Frequency EEG Approach in Pediatric Focal Refractory Epilepsy. Front Neurol 2020;11:1–13. https://doi.org/10.3389/fneur.2020.00941.

Jacobs J, LeVan P, Moeller F, Boor R, Stephani U, Gotman J, et al. Hemodynamic changes preceding the interictal EEG spike in patients with focal epilepsy investigated using simultaneous EEG-fMRI. Neuroimage 2009;45:1220–31. https://doi.org/10.1016/j.neuroimage.2009.01.014.

Janca R, Krsek P, Jezdik P, Cmejla R, Tomasek M, Komarek V, et al. The sub-regional functional organization of neocortical irritative epileptic networks in pediatric epilepsy. Front Neurol 2018;9:1–11. https://doi.org/10.3389/fneur.2018.00184.

Javidan M. Electroencephalography in Mesial Temporal Lobe Epilepsy: A Review. Epilepsy Res Treat 2012;2012:1–17. https://doi.org/10.1155/2012/637430.

Jayakar P, Dunoyer C, Dean P, Ragheb J, Resnick T, Morrison G, et al. Epilepsy surgery in patients with normal or nonfocal MRI scans: Integrative strategies offer long-term seizure relief. Epilepsia 2008;49:758–64. https://doi.org/10.1111/j.1528-1167.2007.01428.x.

Jayakar P, Gaillard WD, Tripathi M, Libenson MH, Mathern GW, Cross JH. Diagnostic test utilization in evaluation for resective epilepsy surgery in children. Epilepsia 2014;55:507–18. https://doi.org/10.1111/epi.12544.

Jayakar P, Gotman J, Harvey AS, Palmini A, Tassi L, Schomer D, et al. Diagnostic utility of invasive EEG for epilepsy surgery: Indications, modalities, and techniques. Epilepsia 2016;57:1735–47. https://doi.org/10.1111/epi.13515.

Jehi L. The epileptogenic zone: Concept and definition. Epilepsy Curr 2018;18:12–6. https://doi.org/10.5698/1535-7597.18.1.12.

Jensen O, Mazaheri A. Shaping functional architecture by oscillatory alpha activity: Gating by inhibition. Front Hum Neurosci 2010;4:1–8. https://doi.org/10.3389/fnhum.2010.00186.

Jeong W, Jin SH, Kim M, Kim JS, Chung CK. Abnormal functional brain network in epilepsy patients with focal cortical dysplasia. Epilepsy Res 2014;108:1618–26. https://doi.org/10.1016/j.eplepsyres.2014.09.006.

Jerbi K, Mosher JC, Baillet S, Leahy RM. On MEG forward modelling using multipolar expansions. Phys Med Biol 2002;47:523–55. https://doi.org/10.1088/0031-9155/47/4/301.

Kane N, Acharya J, Benickzy S, Caboclo L, Finnigan S, Kaplan PW, et al. A revised glossary of terms most commonly used by clinical electroencephalographers and updated proposal for the report format of the EEG findings. Revision 2017. Clin Neurophysiol Pract 2017;2:170–85. https://doi.org/10.1016/j.cnp.2017.07.002.

Kappen P, Eltze C, Tisdall M, Cross JH, Thornton R, Moeller F. Stereo-EEG exploration in the insula/operculum in paediatric patients with refractory epilepsy. Seizure 2020;78:63–70. https://doi.org/10.1016/j.seizure.2020.02.011.

Katsarou AM, Galanopoulou AS, Moshé SL. Epileptogenesis in neonatal brain. Semin Fetal Neonatal Med 2018;23:159–67. https://doi.org/10.1016/j.siny.2017.12.004.

Keezer MR, Sisodiya SM, Sander JW. Comorbidities of epilepsy: Current concepts and future perspectives. Lancet Neurol 2016;15:106–15. https://doi.org/10.1016/S1474-4422(15)00225-2.

Keller CJ, Truccolo W, Gale JT, Eskandar E, Thesen T, Carlson C, et al. Heterogeneous neuronal firing patterns during interictal epileptiform discharges in the human cortex. Brain 2010;133:1668–81. https://doi.org/10.1093/brain/awq112.

Kile KB, Tian N, Durand DM. Low Frequency Stimulation Decreases Seizure Activity in a Mutation Model of Epilepsy. Epilepsia 2010;51:1745–53. https://doi.org/10.1111/j.1528-1167.2010.02679.x.Low.

Kleinfeld D, Xiao D, Vanni MP, Mitelut CC, Chan AW, Ledue JM, et al. Mapping cortical mesoscopic networks of single spiking cortical or sub-cortical neurons 2017. https://doi.org/10.7554/eLife.19976.001.

Kramer MA, Cash SS. Epilepsy as a disorder of cortical network organization. Neuroscientist 2012;18:360–72. https://doi.org/10.1177/1073858411422754.

Kriegeskorte N, Mur M, Bandettini P. Representational similarity analysis - connecting the branches of systems neuroscience. Front Syst Neurosci 2008;2:1–28. https://doi.org/10.3389/neuro.06.004.2008.

Kubista H, Boehm S, Hotka M. The Paroxysmal Depolarization Shift: Reconsidering Its Role in Epilepsy, Epileptogenesis and Beyond. Int J Mol Sci 2019;20:1–18. https://doi.org/10.1155/2012/637430.

Kwan P, Schachter SC, Brodie MJ. Drug-resistant epilepsy. New Engl J Med2011 Sep 8;365(10):919–26. https://doi.org/10.1056/NEJMra1004418.

Lee DA, Kim BJ, Lee HJ, Kim SE, Park KM. Network characteristics of genetic generalized epilepsy: Are the syndromes distinct? Seizure 2020;82:91–8. https://doi.org/10.1016/j.seizure.2020.09.022.

Lesser RP, Crone NE, Webber WRS. Subdural electrodes. Clin Neurophysiol 2010;121:1376–92. https://doi.org/10.1016/j.clinph.2010.04.037.

Lundstrom BN, Van Gompel J, Britton J. Chronic subtreshold cortical stimulation to treat focal epilepsy. JAMA Neurol 2016;73:1370–2. https://doi.org/10.1001/jamaneurol.2016.0795.

Malmivuo J. On the detection of the magnetic heart vector - An application of the reciprocity theorem. Acta Polytech Scand 1976;39.

Maris E, Oostenveld R. Nonparametric statistical testing of EEG- and MEG-data. J Neurosci Methods 2007;164:177–90. https://doi.org/10.1016/j.jneumeth.2007.03.024.

Matsumoto H, Marsan CA. Cortical cellular phenomena in experimental epilepsy: Ictal manifestations. Exp Neurol 1964;9:305–26. https://doi.org/10.1016/0014-4886(64)90026-3.

McGonigal A, Bartolomei F, Chauvel P. On seizure semiology. Epilepsia 2021;62:2019–35. https://doi.org/10.1111/epi.16994.

Meijer HGE, Eissa TL, Kiewiet B, Neuman JF, Schevon CA, Emerson RG, et al. Modeling focal epileptic activity in the Wilson-Cowan model with depolarization block. J Math Neurosci 2015;5. https://doi.org/10.1186/s13408-015-0019-4.

Mitchell M. An introduction to Genetic Algorithms 1998:158.

Noachtar S, Peters AS. Semiology of epileptic seizures: A critical review. Epilepsy Behav 2009;15:2–9. https://doi.org/10.1016/j.yebeh.2009.02.029.

Grenier F, Timofeev I, Steriade M. Focal synchronization of ripples (80-200 Hz) in neocortex and their neuronal correlates. J Neurophysiol 2001;86:1884–98. https://doi.org/10.1152/jn.2001.86.4.1884.

Oishi M, Otsubo H, Kameyama S, Morota N, Masuda H, Kitayama M, et al. Epileptic spikes: Magnetoencephalography versus simultaneous electrocorticography. Epilepsia 2002;43:1390–5. https://doi.org/10.1046/j.1528-1157.2002.10702.x.

Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, et al. Scikit-learn: Machine Learning in Python. J Mach Learn Res 2011:12:2825-30.

Perucca P, Bahlo M, Berkovic SF. The Genetics of Epilepsy. Annu Rev Genomics Hum Genet 2020;21:205–30. https://doi.org/10.1146/annurev-genom-120219-074937.

Pfeiffer C, Ruffieux S, Jönsson L, Churkharin ML, Kalaboukhov A, Xie M, et al. A 7-Channel High-Tc SQUID-Based On-Scalp MEG System. IEEE Trans Biomed Eng 2020;67:1483–9.

Pfurtscheller, G., and Lopes Da Silva, F. H. (1999). Event-related EEG/MEG synchronization and desynchronization: basic principles. *Clin Neurophysiol.* 1999;110:1842-57

Rampp S, Stefan H, Wu X, Kaltenhäuser M, Maess B, Schmitt FC, et al. Magnetoencephalography for epileptic focus localization in a series of 1000 cases. Brain 2019;0:1–13. https://doi.org/10.1093/cercor/bhw393.

Rasia-Filho AA, Guerra KTK, Vásquez CE, Dall'Oglio A, Reberger R, Jung CR, et al. The Subcortical-Allocortical- Neocortical continuum for the Emergence and Morphological Heterogeneity of Pyramidal Neurons in the Human Brain. Front Synaptic Neurosci 2021;13:1–35. https://doi.org/10.3389/fnsyn.2021.616607.

Ray A, Tao JX, Hawes-Ebersole SM, Ebersole JS. Localizing value of scalp EEG spikes: A simultaneous scalp and intracranial study. Clin Neurophysiol 2007;118:69–79. https://doi.org/10.1016/j.clinph.2006.09.010.

Riaz B, Pfeiffer C, Schneiderman JF. Evaluation of realistic layouts for next generation on-scalp MEG: Spatial information density maps. Sci Rep 2017;7. https://doi.org/10.1038/s41598-017-07046-6.

Rossi Sebastiano D, Tassi L, Duran D, Visani E, Gozzo F, Cardinale F, et al. Identifying the epileptogenic zone by four non-invasive imaging techniques versus stereo-EEG in MRI-negative pre-surgery epilepsy patients. Clin Neurophysiol 2020;131:1815–23. https://doi.org/10.1016/j.clinph.2020.05.015.

Ryvlin P, Cross JH, Rheims S. Epilepsy surgery in children and adults. Lancet Neurol 2014. https://doi.org/10.1016/S1474-4422(14)70156-5.

Sander JW. The epidemiology of epilepsy revisited. Curr Opin Neurol 2003;16:165–70. https://doi.org/10.1097/01.wco.0000063766.15877.8e.

Sarvas J. Basic mathematical and electromagnetic concepts of the biomagnetic inverse problem. Phys Med Biol 1987;32:11–22. https://doi.org/10.1088/0031-9155/32/1/004.

Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, et al. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017;58:512–21. https://doi.org/10.1111/epi.13709.

Schevon CA, Weiss SA, McKhann G, Goodman RR, Yuste R, Emerson RG, et al. Evidence of an inhibitory restraint of seizure activity in humans. Nat Commun 2012a;3. https://doi.org/10.1038/ncomms2056.

Schevon CA, Weiss SA, McKhann G, Goodman RR, Yuste R, Emerson RG, et al. Evidence of an inhibitory restraint of seizure activity in humans. Nat Commun 2012b;3:1011–60. https://doi.org/10.1038/ncomms2056.

Schneiderman JF. Information content with low- vs. high-Tc SQUID arrays in MEG recordings: The case for high-Tc SQUID-based MEG. J Neurosci Methods 2014. https://doi.org/10.1016/j.jneumeth.2013.10.007.

Schwartzkroin PA, Prince DA. Changes in excitatory and inhibitory synaptic potentials leading to epileptogenic activity. Brain Res 1979;183:61–77. https://doi.org/10.1016/0006-8993(80)90119-5.

Seeck M, Koessler L, Bast T, Leijten F, Michel C, Baumgartner C, et al. The standardized EEG electrode array of the IFCN. Clin Neurophysiol 2017;128:2070–7. https://doi.org/10.1016/j.clinph.2017.06.254.

Seidenberg M, Kelly KG, Parrish J, Geary E, Dow C, Rutecki P, et al. Ipsilateral and contralateral MRI volumetric abnormalities in chronic unilateral temporal lobe epilepsy and their clinical correlates. Epilepsia 2005;46:420–30. https://doi.org/10.1111/j.0013-9580.2005.27004.x.

Shields WD. Catastrophic epilepsy in childhood. Epilepsia 2000;41. https://doi.org/10.1111/j.1528-1157.2000.tb01518.x.

Sidhu MK, Duncan JS, Sander JW. Neuroimaging in epilepsy. Curr Opin Neurol 2018;31:371–8. https://doi.org/10.1097/WCO.0000000000000568.

Smith EH, Liou JY, Davis TS, Merricks EM, Kellis SS, Weiss SA, et al. The ictal wavefront is the spatiotemporal source of discharges during spontaneous human seizures. Nat Commun 2016;7:1–12. https://doi.org/10.1038/ncomms11098.

Staley K. Molecular mechanisms of epilepsy. Nat Neurosci 2015;18:367–72. https://doi.org/10.1038/nn.3947.

Starnes K, Miller K, Wong-Kisiel L, Lundstrom BN. A review of neurostimulation for epilepsy in pediatrics. Brain Sci 2019;9. https://doi.org/10.3390/brainsci9100283.

Stefan H, da Silva FHL. Epileptic neuronal networks: Methods of identification and clinical relevance. Front Neurol 2013;4 MAR:1–15. https://doi.org/10.3389/fneur.2013.00008.

Steriade M, Contreras D. Relations between cortical and thalamic cellular events during transition from sleep patterns to paroxysmal activity. J Neurosci 1995;15:623–42. https://doi.org/10.1523/jneurosci.15-01-00623.1995.

Stoyell SM, Wilmskoetter J, Dobrota MA, Chinappen DM, Bonilha L, Mintz M, et al. High-Density EEG in Current Clinical Practice and Opportunities for the Future. J Clin Neurophysiol 2021;38:112–23. https://doi.org/10.1097/WNP.0000000000000807.

Tao JX, Baldwin M, Hawes-Ebersole S, Ebersole JS. Cortical substrates of scalp EEG epileptiform discharges. J Clin Neurophysiol 2007;24:96–100. https://doi.org/10.1097/WNP.0b013e31803ecdaf.

Tassi L, Colombo N, Garbelli R, Francione S, Lo Russo G, Mai R, et al. Focal cortical dysplasia: Neuropathological subtypes, EEG, neuroimaging and surgical outcome. Brain 2002;125:1719–32. https://doi.org/10.1093/brain/awf175.

Tatum WO, Mani J, Jin K, Halford JJ, Gloss D, Fahoum F, et al. Minimum standards for inpatient long-term video-EEG monitoring: A clinical practice guideline of the international league against epilepsy and international federation of clinical neurophysiology. Clin Neurophysiol 2022;134:111–28. https://doi.org/10.1016/j.clinph.2021.07.016.

Taulu S, Simola J. Spatiotemporal signal space separation method for rejecting nearby interference in MEG measurements. Phys Med Biol 2006;51:1759–68. https://doi.org/10.1088/0031-9155/51/7/008.

Téllez-Zenteno JF, Hernández-Ronquillo L. A Review of the Epidemiology of Temporal Lobe Epilepsy. Epilepsy Res Treat 2012;2012:1–5. https://doi.org/10.1155/2012/630853.

Thom M, Blümcke I, Aronica E. Long-term epilepsy-associated tumors. Brain Pathol 2012;22:350–79. https://doi.org/10.1111/j.1750-3639.2012.00582.x.

Thornton R, Vulliemoz S, Rodionov R, Carmichael DW, Chaudhary UJ, Diehl B, et al. Epileptic networks in focal cortical dysplasia revealed using electroencephalography-functional magnetic resonance imaging. Ann Neurol 2011;70:822–37. https://doi.org/10.1002/ana.22535.

Torres Diaz C V., González-Escamilla G, Ciolac D, Navas García M, Pulido Rivas P, Sola RG, et al. Network Substrates of Centromedian Nucleus Deep Brain Stimulation in Generalized Pharmacoresistant Epilepsy. Neurotherapeutics 2021;18:1665–77. https://doi.org/10.1007/s13311-021-01057-y.

Trevelyan AJ, Schevon CA. How inhibition influences seizure propagation. Neuropharmacology 2013;69:45–54. https://doi.org/10.1016/j.neuropharm.2012.06.015.

Tryba AK, Merricks EM, Lee S, Pham T, Cho S, Nordli DR, et al. Role of paroxysmal depolarization in focal seizure activity. J Neurophysiol 2019;122:1861–73. https://doi.org/10.1152/jn.00392.2019.-We.

Varotto G, Tassi L, Franceschetti S, Spreafico R, Panzica F. Epileptogenic networks of type II focal cortical dysplasia: A stereo-EEG study. Neuroimage 2012;61:591–8. https://doi.org/10.1016/j.neuroimage.2012.03.090.

Vrba J, Robinson SE. SQUID sensor array configurations for magnetoencephalography applications. Supercond Sci Technol 2002;15. https://doi.org/10.1088/0953-2048/15/9/201.

Wang X-J, Kennedy H. Brain structure and dynamics across scales: In search of rules. Curr Opin Neurobiol 2016;37:92–8. https://doi.org/10.1016/j.conb.2015.12.010.Brain.

Wehner DT, Hämäläinen MS, Mody M, Ahlfors SP. Head movements of children in MEG: Quantification, effects on source estimation, and compensation. Neuroimage 2008;40:541–50. https://doi.org/10.1016/j.neuroimage.2007.12.026.

Wendling F, Bellanger JJ, Bartolomei F, Chauvel P. Relevance of nonlinear lumped-parameter models in the analysis of depth-EEG epileptic signals. Biol Cybern 2000;83:367–78. https://doi.org/10.1007/s004220000160.

Wendling F, Bartolomei F, Senhadji L. Spatial analysis of intracerebral electroencephalographic signals in the time and frequency domain: Identification of epileptogenic networks in partial epilepsy. Philos Trans R Soc A Math Phys Eng Sci 2009;367:297–316. https://doi.org/10.1098/rsta.2008.0220.

Wendling F, Benquet P, Bartolomei F, Jirsa V, Wendling F, Benquet P, et al. Computational models of epileptiform activity . J Neurosci Methods 2016;260:233–51.Wilson HR, Cowan JD. Excitatory and Inhibitory Interactions in Localized Populations of Model Neurons. Biophys J 1972;12:1–24. https://doi.org/10.1016/S0006-3495(72)86068-5.

Xie M, Schneiderman JF, Chukharkin ML, Kalabukhov A, Riaz B, Lundqvist D, et al. Benchmarking for on-scalp MEG sensors. IEEE Trans Biomed Eng 2017;64:1270–6. https://doi.org/10.1109/TBME.2016.2599177.

Yuen AWC, Keezer MR, Sander JW. Epilepsy is a neurological and a systemic disorder. Epilepsy Behav 2018;78:57–61. https://doi.org/10.1016/j.yebeh.2017.10.010.

Zhang L, Liang Y, Li F, Sun H, Peng W, Du P, et al. Time-Varying Networks of Inter-Ictal Discharging Reveal Epileptogenic Zone. Front Comput Neurosci 2017;11:1–9. https://doi.org/10.3389/fncom.2017.00077.

Zhang Y, Tavrin Y, Mück M, Braginski AI, Heiden C, Hampson S, et al. Magnetoencephalography using high temperature rf SQUIDs. Brain Topogr 1993;5:379–82. https://doi.org/10.1007/BF01128694.

Zhang ZJ, Valiante TA, Carlen PL. Transition to seizure: From “ macro” - to “ micro” - mysteries. Epilepsy Res 2011;97:290–9. https://doi.org/10.1016/j.eplepsyres.2011.09.025.