Functional Connectivity of Oscillatory Neural Networks in Children with Medically-Intractable Localization-Related Epilepsy

by

George M. Ibrahim, BSc(Hon), MD

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Graduate Department of the Institute of Medical Science
University of Toronto

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Abstract

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Doctor of Philosophy
Graduate Department of the Institute of Medical Science
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Epilepsy is the most common serious neurological condition affecting children. Currently, a fraction of children who may be eligible for epilepsy surgery are referred for presurgical evaluation. This lack of access to epilepsy surgery is partially due to an incomplete understanding of the ways in which epilepsy interferes with childhood development and uncertainty in the mapping of epileptogenic cortex. Since neuronal oscillations within the human brain facilitate inter-regional communication and subserve a host of cognitive functions, the study of networks measuring direct (electroencephalographic and electromagnetic signals) and indirect (blood oxygen-dependent neurovascular signals) oscillating neuronal populations in the brain may prove valuable in understanding epilepsy and developing improved strategies for the treatment of affected children.

The first segment of the dissertation explores putative mechanisms by which localization-related epilepsy interferes with typical childhood development. First, intrinsic connectivity networks (ICNs), formed by coherence in spontaneous fluctuations of blood oxygen level dependent signal were measured using resting-state fMRI. The development and functional organization of ICNs in children with epilepsy were compared to matched controls. Clinical heterogeneity in the patient population was also modeled against neuroimaging data to identify patient phenotypes that are associated with specific network impairments. Second, ICNs were reconstructed by measuring phase-locking synchrony in neuromagnetic recordings using magnetoencephalography. The resilience and vulnerability of ICNs to interictal epileptiform discharges (IEDs) was correlated with neurocognitive outcomes. Finally the effects of seizures on the organization of functional networks were tested using electrocorticography (ECoG) data obtained during invasive monitoring from subdural electrodes. This segment provides evidence that epilepsy disrupts
the developmental trajectory of functional networks in affected children and that these effects may be mediated both by ongoing IEDs as well as recurrent seizure activity.

The second segment examines ECoG recordings from children undergoing invasive monitoring for surgical candidacy to study the extent to which normative patterns of connectivity are disrupted during seizure activity. One emerging marker of epileptogenic networks is the expression of pathological high frequency oscillations (pHFOs; >80 Hz). Since the hierarchical coupling of high frequency amplitudes to the phase of slower oscillations is described as a mechanism to regulate neural communication within networks across different spatiotemporal scales, the cross-frequency coupling (CFC) of pHFOs to the phase of slower cortical rhythms was studied. Topographic concentrations of CFC were evaluated as a marker of epileptogenic cortex and breakdowns in the expected normative relations between pHFOs and slower oscillations were identified during seizure initiation and propagation. During these epochs, frequency-specific inter-regional phase synchrony in ECoG recordings was also found to be disturbed, suggesting that aberrant functional interactions within epileptogenic cortex are inextricably related to impaired inter-regional communication during seizures. These findings highlight dynamic changes in neural communication within epileptogenic networks that are associated with seizure activity, and supply novel avenues for seizure mapping and treatment.
Dedication

This thesis is dedicated to the memory of my father, Monir and to my mother Mona. For everything.
Acknowledgements

I would like to foremost thank my thesis supervisors and mentors, Jim Rutka and Carter Snead. Knowing that I can never adequately express my gratitude for your confidence, support and guidance, I can only hope to one day pay this debt forward by being a mentor to others and sharing the lessons you’ve taught me. Undoubtedly, I am a better doctor, scientist and person for having worked with you. I would also be remiss not to thank Jim Drake, and my neurosurgery program director, Abhaya Kulkarni, for their unique insights and encouragement as well as Mark Bernstein, for always showing me a different perspective.

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Nomenclature

AC-PC  Anterior commissure-posterior commissure
ACC  Anterior cingulate cortex
AEDs  Antiepileptic drugs
ANCOVA  Analysis of covariance
ANOVA  Analysis of variance
BLP  Band-limited power
BOLD  Blood oxygen level dependent
CFC  Cross-frequency coupling
CSF  Cerebrospinal fluid
CT  Computed tomography
DAN  Dorsal attention network
DMN  Default mode network
EC  Eigenvector centrality
ECD  Equivalent dipole model
ECoG  Electroencephalography
EDF+  European Data Format Plus
EEG  Electroencephalography
EPSP  Excitatory postynaptic potentials
FCD  Focal cortical dysplasia
FDA  Filter Design and Analysis
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>FEAT</td>
<td>fMRI Expert Analysis Tool</td>
</tr>
<tr>
<td>FEF</td>
<td>Frontal eye fields</td>
</tr>
<tr>
<td>FIR</td>
<td>Finite impulse response</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>FSL</td>
<td>FMRIB Software Library</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full width at half maximum</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GLM</td>
<td>General linear model</td>
</tr>
<tr>
<td>HFOs</td>
<td>High frequency oscillations</td>
</tr>
<tr>
<td>ICA</td>
<td>Independent component analysis</td>
</tr>
<tr>
<td>ICNs</td>
<td>Intrinsic connectivity networks</td>
</tr>
<tr>
<td>IEDs</td>
<td>Interictal epileptiform discharges</td>
</tr>
<tr>
<td>IFG</td>
<td>Inferior frontal gyrus</td>
</tr>
<tr>
<td>IFSECN</td>
<td>International Federation of Societies for Electroencephalography and Clinical Neurophysiology</td>
</tr>
<tr>
<td>ILAE</td>
<td>International League Against Epilepsy</td>
</tr>
<tr>
<td>ING</td>
<td>Interneuron gamma</td>
</tr>
<tr>
<td>IPS</td>
<td>Intraparietal sulcus</td>
</tr>
<tr>
<td>IPSP</td>
<td>Inhibitory postsynaptic potential</td>
</tr>
<tr>
<td>IQ</td>
<td>Full-scale intelligence quotient</td>
</tr>
<tr>
<td>ISI</td>
<td>Interstimulus interval</td>
</tr>
<tr>
<td>LFPs</td>
<td>Local field potentials</td>
</tr>
<tr>
<td>LRE</td>
<td>Localization-related epilepsy</td>
</tr>
<tr>
<td>MEG</td>
<td>Magnetoencephalography</td>
</tr>
<tr>
<td>mGluR</td>
<td>Metabotropic glutamate receptor</td>
</tr>
<tr>
<td>MI</td>
<td>Modulation index</td>
</tr>
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</table>
MN  Motor network
MRI  Magnetic resonance imaging
MSC  Mean-squared coherence
PCC  Posterior cingulate cortex
PET  Positron emission tomography
pHFOs  Pathological high frequency oscillations
PING  Pyramidal-interneuron network gamma
PLI  Phase-lag index
PLV  Phase-locking value
REM  Rapid eye movement
ROI  Region of interest
SAL  Salience network
SMA  Supplementary motor area
SOZ  Seizure onset zone
SPECT  Single-photon emission computed tomography
SPMs  Statistical parameter maps
SQUIDs  Superconducting quantum interference devices
STG  Superior temporal gyrus
TE  Echo time
TLE  Temporal lobe epilepsy
UNG  Unit noise gain
vmPFC  Ventromedial prefrontal cortex
WM  White matter
WMTB-C  Working Memory Test Battery for Children
wPLI  Weighted phase-lag index
Chapter 1

Introduction

Data presented in this chapter have been previously published as follows:


(ii) Ibrahim GM, Rutka JT, Snead OC III. Epilepsy surgery in childhood: No longer the treatment of last resort. CMAJ (Accepted).

Contributions: GMI (conception/design of study, drafting/revising article, final approval); BWB, OCS, JMD, JTR, MB (conception/design of study, final approval)

1.1 Localization-Related Epilepsy in Children

1.1.1 Epilepsy: An Important Neurological Disease of Childhood

Epilepsy is identified by the World Health Organization as the most serious neurological disorder worldwide with 50 million affected individuals in the world today, 85% of whom live in developing countries. This brain disorder is characterized by recurrent, unpredictable seizures that disrupt normal brain function. Epilepsy occurs in 1-2% of children [154] and up to one-third of individuals with epilepsy are refractory to medical therapy, which is generally defined as having failed two appropriately selected antiepileptic drugs (AEDs) at maximally tolerated serum levels for two years [39, 361].

A subset of affected children may be diagnosed with localization-related epilepsy (LRE), where seizures originate from a specific brain region, and secondarily spread to involve other areas [250]. In contrast to adults, nearly half of children may present with extensive extra-temporal or multi-lobar epileptic foci [157, 212]. Furthermore, epileptogenic pathologies are much more heterogeneous in children, with cortical dysplasias (23-78%), and tumors (17-38%) comprising the most common pathologies [365]. Perinatal injury, hemispheric syndromes, neurocutaneous disorders, epileptic encephalopathies, hypothalamic hamartomas and mesial temporal sclerosis are less frequent surgically amenable pathologies encountered in children.
1.1.2 Epilepsy Surgery: A Viable Option for Localization-Related Epilepsy

Individuals who fail to respond to two appropriately selected antiepileptic drugs stand less than a 5% chance of responding to additional medical treatments [222]. It is not surprising, therefore, that surgical intervention is increasingly considered for medically-intractable localization-related epilepsy [100, 101, 250, 165]. While a randomized controlled trial of adults with temporal lobe epilepsy demonstrated the superiority of surgical intervention over continued medical management [433], no such evidence exists for the surgical treatment of childhood epilepsy. Despite this, surgical strategies are gaining popularity with the increasing recognition that uncontrolled epilepsy may lead to increased mortality [369], poor quality-of-life [367], cognitive decline, and high societal costs [430].

The impetus to intervene surgically in children with epilepsy is also guided by increasing concern regarding the detrimental effects of recurrent seizures on the developing brain [21, 167, 266, 409]. Animal models, which have been extensively utilized to study the effects of epilepsy on brain architecture, have highlighted the detrimental effects of seizure activity on development. Such investigations have shown that while the immature brain may be more resistant to cellular loss following prolonged seizure activity than the adult brain [161, 293, 368, 373], recurrent seizures during brain development result in significant changes in synaptic network organization [239] and irreversible alterations in neuronal connectivity. Table 1.1 summarizes animal studies demonstrating the differential effects of the spectrum of seizures (from prolonged status epilepticus to short, recurrent events) on the immature and adult brain (see Holmes, 2005 [166] for review). The effects of the disease on the developing brain are especially important, particularly given the extended period of post-natal brain development in humans, relative to other species.

<table>
<thead>
<tr>
<th></th>
<th>Status Epilepticus</th>
<th>Recurrent Seizures</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Immature</td>
<td>Adult</td>
</tr>
<tr>
<td>Cell loss</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Synaptic reorganization</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Alterations in dendritic morphology</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Dentate neurogenesis</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Changes in inhibitory/excitatory receptor subunits</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Cognitive impairment</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Reduced seizure threshold</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 1.1: Differential effects of prolonged and recurrent seizures on immature and adult animal brains (adapted from Holmes, 2005 [166], with permission; License ID: 3312080669041).
These investigations support clinical findings. In patient populations, uncontrolled epilepsy has also been long thought to interfere with typical childhood development [206]. Nearly half of children with epilepsy have high rates of comorbid learning difficulties, developmental delay, psychiatric and behavioural challenges and psychosocial problems [365]. Children with epilepsy may also fail to progress along expected developmental trajectories [216]. Longer duration of epilepsy, as well as percentage of life with epilepsy, more frequent seizures and younger age at seizure onset are associated with worse cognitive function [177, 407, 409]. These deficits may be multi-factorial, as a result of the epileptogenic substrate, repeated seizure activity, interictal epileptiform discharges as well as the toxicity of antiepileptic medication and the psychosocial stigma associated with the disease.

1.2 Challenges, Questions and Opportunities

Efforts to mitigate the burden of epilepsy through surgical interventions are limited by significant gaps in knowledge. In contrast to other neurological diseases, such as neuro-oncologic and neuro-vascular conditions, epilepsy is an intrinsic disorder of brain function; therefore, advancements in the understanding of brain development, organization and physiology may offer clinically-meaningful insights for its diagnosis and treatment. Ongoing research to understand neural networks specifically holds great promise for informing the surgical treatment of epilepsy, including patient selection, treatment and prognostication of outcomes. For example, while seizures have long been known to recruit distant neural populations into synchronous activity, which disrupts brain function, it is only recently that the enigmatic mechanisms of inter-regional neural communication are becoming understood. Additionally, while developmental difficulties have been diagnosed in children with epilepsy for decades, only now are the neural correlates being investigated using high fidelity non-invasive neuroimaging methods.

Greater knowledge of brain function is necessary, but not sufficient for the development of novel avenues for the treatment of affected patients. Insights gleaned from physiological and normative studies must be tested in patient populations and translated into clinically-meaningful applications. In this regard, medical challenges related to clinical gaps in knowledge of childhood epilepsy are outlined below with a view towards the opportunities available to address them using methods that have recently become available in the neurosciences.

Lack of access to Epilepsy Surgery in Childhood and Incomplete Understanding of the Effects of Uncontrolled Seizures on Development. Despite advances in epilepsy surgery and evidence-based data supporting its role in the management of intractable epilepsy, surgery remains underutilized in children with epilepsy and is often considered a last resort among front-line clinicians, including pediatricians, family practitioners and neurologists [384]. Misconceptions regarding the ability of surgical treatment to cure children with epilepsy and the risk-to-benefit profiles of surgery remain prevalent [384] and constitute barriers to access to
Chapter 1. Introduction

4

Surgical care. Referral rates for surgical assessment of children living with medically-intractable epilepsy are as low as 5%, with a prolonged mean delay between first seizure and referral for evaluation of surgical candidacy of 4.6 years and up to 16 years [235].

A more rigorous understanding of the mechanisms underlying comorbidities of chronic epilepsy would inform patient referral patterns as well as treatment strategies. While animal models are useful to study the effects of seizures on the developing brain, they are limited in that they cannot yet accurately mimic epilepsy syndromes in clinical populations. Studies in patient populations are necessary to validate the findings of experimental models and provide further insights into the effects of epilepsy on typical childhood development. The study of the functional connectivity of oscillatory neural networks in children with epilepsy offers a unique opportunity to study the mechanisms underlying comorbid cognitive decline and developmental delay. As will be discussed in Sections 2.2.5 on page 19 and 2.4.2 on page 32, the maturation of oscillations within the human brain is strongly correlated with the development of cognitive faculties and structural brain growth. As will be reviewed in Sections 2.2.6 on page 20 and 2.4.5 on page 37, such studies have identified an association between functional brain network integrity and cognitive decline in patients with epilepsy. The evaluation of oscillatory networks in children provides a unique perspective on the intersection between epilepsy and development in this vulnerable patient population. By modeling clinical heterogeneity against neuroimaging data, insights may be gleaned regarding neural correlates of cognitive comorbidities throughout the developmental spectrum, thus informing decisions regarding the management of children with intractable epilepsy.

Lack of Adequate Biomarkers While quality-of-life in children is strongly linked to the incidence and frequency of seizures [103], there is conflicting evidence as to whether resective surgery and subsequent seizure-freedom may improve neurocognitive and psychosocial function in children [358]. The strongest evidence for the beneficial effect of surgery on developmental outcomes is derived from the hemispherectomy literature in patients with catastrophic epilepsy or progressive hemispheric disorders. Several studies from this patient cohort reported improvements in developmental quotients [10, 202, 245], while others reported a lack of deterioration [86, 99]. Most of these studies have suggested that improvements are greatest in children who underwent surgical treatment at younger ages and those with better pre-operative cognitive function.

An important challenge in identifying those patients with epilepsy who may be at risk of developing cognitive decline, predicting seizure recurrence after treatment and evaluating post-treatment neurological improvements is the lack of adequate biomarkers in this patient population. Clinically-relevant and robust biomarkers are needed to quantify and measure disease impairment in order to longitudinally assess children following medical and surgical interventions. As described in Sections 2.2.6 on page 20 and 2.4.5 on page 37, network analysis methods are currently being utilized to attempt to predict seizure outcome after epilepsy surgery.
by characterizing the neural correlates of disease-related impairments.

**Mapping Epileptogenic Cortex** Increasingly aggressive surgical treatments are being performed in children with intractable epilepsy. At one institution, a 58% increase in the number of resective procedures for epilepsy were reported in recent cohort compared to historical data [157]. A meta-analysis of children undergoing resective procedures for tuberous sclerosis also showed greater numbers of children undergoing surgical treatment over the last 15 years, as well as treatment at significantly younger ages [184]. Furthermore, numerous ethical arguments have been proposed for such aggressive surgical treatments, even in children with relatively modest expected improvements or developmental delay [182]. This increase in acceptance of epilepsy surgery in children is partially due to improved knowledge of surgically remediable epilepsy syndromes in this population, as well as advances in non-invasive diagnostic tools to delineate epileptic foci and eloquent cortex.

While there remains considerable institutional variability in the evaluation of children for epilepsy surgery [152], the Pediatric Epilepsy Surgery Subcommission of the International League Against Epilepsy (ILAE) has proposed guidelines for the evaluation of surgical candidates including interictal and video electroencephalography (EEG), structural imaging with magnetic resonance imaging (MRI) and/or computed tomography (CT), functional imaging with single-photon emission CT (SPECT) or positron emission tomography (PET), and neuropsychological evaluation [195]. In selected patient populations, magnetoencephalography is also highly valuable in non-invasively identifying epileptogenic cortex [183].

In addition to revealing insights into the comorbidities of chronic epilepsy in children, the study of oscillatory networks may also facilitate understanding of how epileptogenic networks are organized and structured in the brain, facilitating the development of novel seizure mapping methods and in turn, surgical strategies. As described in Section 2.2.4 on page 18, communication in the human brain is exquisitely sensitive to synchronized oscillatory activity of neural populations. Similarly, the pathological brain communication that occurs during seizures can be studied in terms of its functional connectivity, supplying new avenues to understand and ultimately treat epilepsy. In this regard, novel network markers, such as pathological high frequency oscillations (reviewed in Section 2.2.6 on page 20) are already being investigated as adjuncts in the conduct of epilepsy surgery. With improvements in the delineation of epileptic networks and greater understanding of neural interactions during seizures, pathological foci may be localized and resected for improved accuracy and reduced morbidity.

### 1.3 Thesis Structure

The dissertation is dedicated to the study of functional connectivity of oscillatory networks in the human brain, chiefly in children with medically-intractable localization-related epilepsy. Two forms of oscillations are studied: (a) direct neuronal oscillations; and (b) oscillatory activity
in the cerebrovascular blood flow, an indirect measure of neural activity. The former is studied largely in electrocorticographic recordings (ECoG) as well as neuromagnetic signal from magnetoencephalography (MEG) from children undergoing resective epilepsy surgery at the Hospital for Sick Children, Toronto Ontario. The latter is studied using resting-state functional magnetic resonance imaging (fMRI) in the patient cohort.

The introductory Chapter 2 will introduce the reader to the basic principles of direct and indirect neural oscillations and their synchronization in physiological conditions, during childhood development and the current state of knowledge regarding their derangement in epilepsy. Methods to study and quantify functional connectivity of oscillatory activity are also presented therein. Chapter 2 will conclude by presenting general and specific hypotheses that will be tested in the first and second parts of the thesis.

**Part I: Epilepsy and the Brain's Developing Networks** The first segment of the thesis explores mechanisms by which localization-related epilepsy interferes with typical childhood development by measuring spontaneous oscillations in direct and indirect neural activity. Evidence will be presented of impaired maturation of intrinsic connectivity networks and both interictal and ictal epileptic dynamics will be implicated in their pathogenesis.

Chapter 3 begins by evaluating developmental differences in intrinsic connectivity networks of children with epilepsy compared with propensity-score matched controls and subsequently, models clinical heterogeneity in the patient population against clinical data to identify phenotypes that are associated with specific network impairments. In the latter parts of this chapter, the same networks are also reconstructed by measuring phase-locking synchrony in neuromagnetic recordings from MEG. Using the high temporal resolution of this modality, combined with the spatial resolution of fMRI, the effects of interictal epileptiform discharges (IEDs) on these intrinsic networks formed by spontaneous neural oscillations are examined. The resilience and vulnerability of intrinsic networks to IEDs are correlated with neurocognitive outcomes and resting-state fMRI analyses.

Finally, in Chapter 4, electrocorticographic recordings are studied to evaluate how ictal events may also disrupt functional networks in children with epilepsy. The effects of ictal activity on the organization of functional networks are tested by measuring coherence within the motor network – as identified by extra-operative mapping – during seizures. The extent of band-limited ictal disturbance of oscillatory functional connectivity is correlated with neuropsychological and motor outcome in these children. Taken together, this segment provides evidence that epilepsy disrupts the developmental trajectory of functional networks in affected children and that these effects may be mediated both by ongoing IEDs as well as recurrent seizure activity. These data, in turn provide strong support for earlier referral and surgical treatment of children with intractable epilepsy.

**Part II: The Network Organization of Epileptogenic Cortex** The second segment is dedicated to the study of the functional connectivity of oscillatory neural networks within epilep-
togenic brain regions using electrocorticographic recordings from children undergoing invasive monitoring for surgical candidacy. Evidence will be presented for impaired oscillatory neural communication within these regions, with applications for seizure mapping and future therapeutic intervention.

Chapter 5 begins by exploring one emerging marker of epileptogenic networks, pathological high frequency oscillations (pHFOs). Recently, it has been described that physiological spontaneous and task-evoked oscillations near this frequency range are regulated by cross-frequency phase-amplitude interactions. In Chapter 5, this mechanism of hierarchical control of neuronal communication is studied in epileptogenic cortex to show that dynamic cross-frequency phase-amplitude coupling involving pHFOs occurs in epileptogenic cortex. Breakdown in the normative regulatory mechanisms are identified during specific seizure epochs, elucidating novel insights into dysfunctional oscillatory processes underlying ictal activity.

Chapter 6 expands on these findings by measuring changing patterns of inter-regional functional connectivity in electrocorticographic recordings associated with the expression of pHFOs. Frequency-dependent changes in functional network topologies within epileptogenic cortex are identified and applied to the mapping of epilepsy foci in children. These findings suggest that functional isolation of epileptogenic cortex from the controlling influence of large-scale networks subserved by cross-frequency coupling and phase synchrony coincides with seizure initiation, and a return to presumably normative patterns of functional connectivity is associated with seizure termination. By using neural oscillations as a framework to understand dynamic changes in neural communication during seizures, novel seizure mapping methods and therapies may be developed.

As a conclusion to the thesis, Chapter 7 presents a summary of the findings and their clinical implications. A discussion of ethical issues in knowledge translation follows, outlining ways to apply these analyses to patient populations.
Chapter 2

Literature Review and Hypotheses

Data presented in this chapter have been previously published as follows:


Contributions: GMI (conception/design of study, drafting/revising article, final approval); OCS, JTR, AML (conception/design of study, final approval)

2.1 Epilepsy as a Network disorder

Penfield and Jasper, epilepsy surgery pioneers, were among the first to view epilepsy as a network phenomenon [305]. Their model of epileptogenicity proposed that a seizure-onset zone (SOZ) was responsible for the ictogenesis. Hypersynchronization of the SOZ with distant brain regions was thought to be a mechanism of seizure propagation, as well as ictal impairment of cognitive function. Talairach and Bancaud subsequently conceptualized an “epileptogenic zone” composed of both the SOZ as well as regions of early seizure propagation, which must be surgically resected in order to achieve seizure freedom [387]. Since then, epileptogenic networks have been classically fractionated into zones, namely to facilitate the conduct of epilepsy surgery (Table 2.1).
Table 2.1: Zones related to epileptogenic networks

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
<th>Method of Determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epileptic Lesion</td>
<td>Cortical region(s) of visible abnormality on structural imaging</td>
<td>Structural imaging (MRI or CT)</td>
</tr>
<tr>
<td>Epileptogenic Zone</td>
<td>Cortical region(s) capable of generating seizures, the removal of which results in seizure freedom</td>
<td>Estimated from all available diagnostic methods</td>
</tr>
<tr>
<td>Functional Deficit Zone</td>
<td>Cortical region(s) demonstrating functional abnormality in the interictal period</td>
<td>Directly (neurological examination, neuropsychological testing); indirectly (EEG, MEG, SPECT, PET, MRS, fMRI)</td>
</tr>
<tr>
<td>Ictal (Seizure) Onset Zone</td>
<td>Cortical region(s) from which clinical seizure originate</td>
<td>Ictal EEG (scalp or intracranial)</td>
</tr>
<tr>
<td>Irritative Zone</td>
<td>Cortical region(s) generating interictal epileptic discharges</td>
<td>Interictal EEG, MEG</td>
</tr>
<tr>
<td>Symptomatogenic Zone</td>
<td>Cortical region(s) generating initial ictal symptomatology</td>
<td>Patient history, ictal video recordings</td>
</tr>
</tbody>
</table>

While the zone model of epileptogenicity is useful, it is important to recognize that the zone, regardless of the nomenclature is only a subset of a larger network paradigm. Spencer was first to explicitly describe a model whereby focal epilepsy is based on an organization of a neural network in which epileptogenicity is distributed throughout a specific network [366]. The axioms of her model were that seizures were a disease of large neural networks and not discrete cortical regions. This hypothesis was supported by numerous studies, which will be explored in greater depth in subsequent sections, that implicate cortical and subcortical structures as well as inter-hemispheric connections in epileptogenicity. The Penfield and Talairach models of epileptogenicity may compliment the themes of Spencer’s network paradigm if one were to consider that functionally defined regions within epileptic networks (such as the seizure-onset or early propagation zones) carry disproportionate weights or have a greater responsibility for seizure generation within a larger network [283]. The zone classifications may therefore inform the network theory by defining regions that may sustain seizure activity but that are themselves insufficient for seizure initiation.

Perhaps most importantly, the view that epilepsy represents impaired neural communication within specific networks allows the application of network analysis methods to the study of epilepsy. The subsequent sections will provide a more detailed understanding of the mechanisms of network formation in the brain and current understanding of epilepsy as a network disorder.
2.1.1 Functional Connectivity of Neural Networks in Epilepsy Research

Greater emphasis has been placed in recent years on the study of network impairments in epilepsy, and a search of the most cited works in epilepsy research revealed an increasing proportion of studies evaluating neural networks in epilepsy [179]. These studies have been conducted on multiple scales, from a molecular and cellular level up to a large-scale global brain network. An example of the former is the study of c-fos expression to map neural activity following seizures in individual neurons, revealing extensive brain networks involved in ictal activity [276]. Examples of the latter will be probed in more detail throughout the dissertation, and include numerous emerging neuroimaging studies.

The current dissertation investigates epileptic networks on a meso- and macro-scale by studying functional connectivity of direct and indirect oscillatory activity in the human brain. The following sections will describe how oscillations of neural populations may be used to study functional connectivity of brain networks and their impairments in children with epilepsy. Of fundamental importance is the definition of functional connectivity, which is the statistical dependencies among remote neurophysiological events [132]. As will be reviewed in more depth in subsequent sections, these dependencies may be measured with correlations, coherence or a number of other metrics. The study of functional connectivity aims to establish predominant patterns of correlation and to predict, classify or understand the endophenotype of the group from which subjects are sampled [132]. Functional connectivity must be distinguished from “effective connectivity”, where the explicit influence of one neural system over another is measured using metrics such as dynamic causal modeling and Granger causality. Effective connectivity, which is not explored in the current dissertation, implies a direct causal influence and is used to test hypotheses concerning coupling architectures that have been probed experimentally.

2.2 Networks and Oscillations

A central topic of the dissertation is oscillatory brain activity. Here, oscillatory neural activity is introduced. A discussion follows of the mechanisms and functional importance of the synchrony of oscillatory activity as well as the specific role of different frequencies of oscillations.

2.2.1 Segregation of Networks Through Oscillations

The brain contains approximately 100 billion neurons with an estimated 200 trillion contacts between them. The most prevalent cell type is the pyramidal cell, which contains 5,000-50,000 postsynaptic receiving sites [58]. A single neuron firing action potentials in a regular, rhythmic manner will generate periodic fluctuations in the intracellular membrane potential of all postsynaptic targets [363]. If several neurons fire action potentials regularly and synchronously, the fluctuating output signal is amplified, defining temporal windows for increased and reduced excitability in a larger population of cells. Neural oscillations can therefore, be viewed as rhythmic changes in cortical excitability [130]. The fluctuating field potential generated by rhythmic
synaptic activation can be easily measured using electrophysiology (EEG). Oscillations may be viewed as a mechanism to control the flow of information within brain networks. As will be discussed subsequently, if high neural excitability is associated with a specific point in an oscillatory cycle (i.e. the trough), inputs that are locked to the trough may be more readily processed than those that are time-locked to the peak. Similarly, long-range communication within- and between- networks is influenced by oscillatory activity, which adjusts the effective gain of communication.

Oscillations cannot be formed exclusively on the basis of excitatory communications between neurons. Synaptic $\gamma$-aminobutyric acid (GABA)-ergic inhibition is needed to balance excitation and control spike timing. With the presence of inhibitory circuitry, oscillatory patterns emerge, which comprise the spectrum of the human brain’s fundamental rhythms. Inhibitory interneurons may be activated quickly to transmit inhibition to all cell-types, effectively resynchronizing the post-synaptic neurons [251]. The benefit of an inhibitory interneuron system in a cortical circuit is its ability to set the network at a state of phase transition, such that populations of neurons can respond to even slight external stimuli, while conversely absorbing large external effects without undergoing functional breakdown [58]. Oscillatory networks are therefore considered the substrate of cognitive flexibility.

A single GABAergic neuron might be sufficient to synchronize the firing of a large population of pyramidal neurons [74] and the duration of the inhibitory postsynaptic potential (IPSP) can determine the dominant frequency of oscillation [416]. Although individual neurons can sustain oscillations, they cannot on their own generate activity that is conspicuous on EEG. Activity in the human brain is therefore, often considered in the context of a network of oscillating neural populations balancing excitation and inhibition. The specific firing patterns of principal cells in a network are depend on the temporal and spatial distribution of inhibition. In response to the same input, the same network can produce different output patterns at different times, depending on the state of inhibition. For example, object perception and salience may be affected by external and internal states of mind. Fast coupling of excitatory and inhibitory influences can bring about sub-millisecond precision of spiking time [313].

### 2.2.2 Coupled Oscillators and The Kuramoto Model

Oscillators also tend to synchronize. Collective synchronization of weakly coupled oscillators is a phenomenon that is present in a gamut of natural occurrences, including within the human brain. This is characterized by the spontaneously locking of a large system of oscillators to a common frequency, despite differences in the natural frequencies of individual oscillators [381, 436]. In the human brain, two distinct mechanisms may lead to synchronous oscillations. The first involves pacing by a common input. For example, the spontaneous firing of neurons in the intralaminar nuclei of the thalamus may generate oscillations in neocortex [378]. Subclasses of pyramidal neurons within the cortex (i.e. “chattering cells”) may also represent local pacemakers [142]. More commonly, however, synchrony emerges from populations of neurons within a network,
through activity of inhibitory interneurons.

In one of the first models to study how networks of independent or weakly coupled oscillators synchronize, Winfree supposed that oscillators were coupled to the collective rhythm generated by the whole population and used numerical simulations to show that these populations may exhibit a phase transition past a certain threshold \([436]\). When the range of natural frequencies of the oscillators was wide, the system behaved incoherently, but as the range decreases past this threshold, a sub-population of oscillators synchronized. The Kuramoto model (Equation 2.1) is one archetypal mathematical formulation to describe the behaviour of weakly coupled oscillators, each with an independent natural oscillatory frequency \([220]\). The model describes the behaviour of a large population of coupled limit-cycle oscillators with natural frequencies that follow a given distribution. The term “limit-cycle” describes a closed trajectory in phase space, into which at least one other trajectory spirals as time approaches infinity or negative infinity. Again, past a certain threshold, the system undergoes a phase transition such that a sub-population of oscillators become coherent.

\[
\dot{\theta}_i = \omega_i + \sum_{j=1}^{N} \Gamma_{ij}(\theta_j - \theta_i), \quad i = 1, ..., N \tag{2.1}
\]

According to the Kuramoto Model, \(\dot{\theta}_i\) represents the phase of oscillator \(i\) and \(\omega_i\) represents its natural frequency. Each oscillator \(j\) exerts a phase-dependent influence on all others, which is related to the interaction function \(\Gamma_{ij}\). This interaction function can be calculated as integrals involving certain terms of the original limit-cycle model. In summary, the phase, \(\dot{\theta}_i\) is pulled towards the mean phase. At a critical value, the model displays phase transition from a desynchronized to partially synchronized state. As the population becomes more coherent, the coupling between the oscillators also increases, recruiting additional oscillators into synchrony. Thus, the phase transition is solely determined by the width of the distribution of the natural frequencies.

### 2.2.3 Rhythms of the Brain and Their Generators

The synchronized activity of oscillators, which may be studied mathematically, serves to segregate functional networks and forms the substrate for cognitive flexibility. Remarkably, the human brain is capable of generating a host of oscillations at multiple frequencies, from slow delta to high frequency activity. This is facilitated by numerous elegant biological features, including the presence of a variety of channel species with different temporal activation kinetics. The complexity of these systems allows the neuron to generate oscillations and resonance at multiple frequencies \([309]\). Numerous bodies of work, particularly the atlas of Niedermeyer and Lopes da Silva \([347]\) review cortical rhythms in great depth. The following section will summarize salient aspects of selected neuronal oscillations that will become relevant to the hypothesis tested in the dissertation.
**Slow Delta Waves**  Delta oscillations are defined by the International Federation of Societies for Electroencephalography and Clinical Neurophysiology (IFSECN) as a wave of duration greater than 0.25 ms (or 0-4 Hz). It is likely that this frequency range encompasses numerous distinct oscillatory patterns with different and specific network properties. There are at least two distinct cellular sources of delta rhythms: thalamic and cortical. Thalamic delta, which is expressed by numerous thalamic nuclei is an intrinsic oscillation that is dependent on inward currents of thalamocortical cells [364]. Interestingly, this clock-like delta is prevented when corticothalamic loops are intact [292]. The existence of a cortical generator of delta activity was suggested by evidence of delta oscillations on EEG in athalamic cats [410]. In the setting of lesions of the subcortical white matter, thalamus or mesencephalic reticular formation, extracellular microphysiological recordings suggested that cortical delta is generated by vertically arranged dipolar generators lying in parallel planes, most likely, the pyramidal neuron [14]. Cortical delta is therefore thought to be generated by the summation of long-lasting afterhyperpolarizations produced by a variety of potassium currents in deeply lying pyramidal neurons [380]. These potassium currents are markedly attenuated by acetylcholine, which may explain suppression of delta waves upon arousal due to cortically projecting cholinergic neurons from the basal forebrain bundle [60]. Highlighting the point that the delta band encompasses a variety of distinct oscillations with unique generators, a novel delta oscillation (<1 Hz) associated with slow-wave sleep and anesthesia has also been described [379], which may be modulated by control of extracellular potassium by spatial buffering of glial cells [7].

**Theta Rhythms**  Theta oscillations are particularly conspicuous in the limbic system and are strongly correlated to processes of memory and learning [54, 204, 388]. These oscillations in the 4-7 Hz range may reflect or subserve hippocampal network engagement in spatial navigation and memory [347]. While theta rhythms have been recorded in animals from the hippocampal formation (stratum lacunosum-moleculare of the hippocampal CA1, dentate gyrus and CA3 region) as well as the subiculum, entorhinal cortex, perirhinal cortex, amygdala and cingulate cortex, they do not occur in isolation from the rest of the brain [347]. It is thought that neurons in limbic structures possess intrinsic membrane properties that facilitate their entrainment into oscillations in the theta range [30].

Initial studies of the generators of theta rhythms proposed that the medial septo/diagonal band-hippocampal cholinergic system, driven by the brainstem reticular core may serve as a pacemaker for theta rhythms. Although this system provides both cholinergic and GABAergic input to the hippocampi [129], electrophysiological and pharmacological studies revealed that this model is too simplistic for the generation of theta. Two types of theta rhythms in the hippocampi have since been characterized, atropine-sensitive and atropine resistant oscillations [404]. The latter is thought to originate from glutamatergic entorhinal inputs to NMDA synapses on distal apical dendrites of CA1 pyramidal neurons [57].
**The Family of Alpha Oscillations**  The family of alpha oscillations (~8-12 Hz) encompasses the alpha rhythms of the visual cortex (the so-called posterior dominant rhythm), the sensorimotor mu rhythm and the tau rhythms of the auditory cortex. Of note, spindles (although encompassing a similar frequency range) are both behaviourally and mechanistically unique from alpha oscillations. Visual alpha occurs during reduced visual attention, while mu rhythms occur in states of muscular relaxation [347]. As such, alpha has historically been labeled a marker of an "idling state" of the brain [2], although task-induced increases in alpha have been documented [322]. These findings can potentially be reconciled in the context of more recent literature derived from a visuo-spatial orienting task, whereby enhanced occipital alpha was associated with active suppression of unattended position to prepare the cortex for optimal target processing [324]. Pulsed alpha inhibition plays an important role in functional networks by exerting a strong inhibitory influence on spike time and firing rates, and therefore, the processing capabilities of a given cortical region [198, 262]. Alpha oscillations reflect fundamental mechanisms of cortical inhibition and idling that may direct information flow within brain networks across different contexts [213]. For example, prestimulus alpha-band phase has been shown to predict suppression of object perception when stimuli were presented at the trough of alpha phase [259, 262].

Studies of the generators of alpha oscillations have been limited by the fact that this rhythm appears only during wakefulness and no valid anesthesia model has been established. Alpha rhythms have been shown in dogs to be generated by the cerebral cortex, although they may also be recorded in the thalamus [80]. In the visual cortex, they are generated by pyramidal neurons in layers IV and V. It was also found that alpha rhythms in the thalamus (lateral geniculate body and pulvinar) moderately affected coherence between cortical alpha rhythms, suggesting that both intracortical and thalamocortical circuitry contribute to the generation of alpha oscillations [81]. In the awake primate, alpha current generators were found in all cortical layers (primary pacer in infragranular layer) in V2 and V4 whereas they were only found in the infragranular and supragranular layers (primary pacer in supragranular layer) in the inferotemporal cortex [36], suggesting that alpha rhythm physiology differs in higher and lower order cortices.

The influence of the thalamus on alpha oscillations has been further characterized by in vitro recordings, although it has been known for some time that thalamic lesions lead to pronounced disorganization of alpha rhythms [297]. Strong activation of metabotropic glutamate receptor (mGluR), mGluR1a, which is located postsynaptically to corticothalamic fibers generates alpha oscillations [175]. Reduction in activation intensity resulted in the occurrence of theta oscillations, suggesting a causal link between mGluR1 antagonism, reduction in alpha waves and emergence of drowsiness-related rhythms. The firing behaviour of single-units recordings from a subset (~20%) of thalamocortical cells during alpha oscillations is termed high-threshold bursting, which occurs repetitively in a frequency range encompassing theta and alpha oscillations (2-13 Hz) [175]. The specific frequency of firing is determined by the extent of neuronal depo-
larization. Furthermore, the coupling between high-threshold bursting thalamocortical neurons and rhythmic alpha field activity is reliant upon gap junction connectivity [174]. Importantly, a variety of diseases may affect thalamocortical high-threshold bursting and lead to a deceleration of the alpha rhythm to the theta band, a phenomenon termed “thalamocortical dysrhythmia” [244].

**Faster Rhythms (Beta and Gamma Oscillations)** Beta (12-32 Hz) and gamma (>40 Hz) oscillations are associated with vigilance states of wakefulness as well as paradoxical (REM) sleep [347]. These rhythms were first reported in response to stimulation of brainstem structures, which resulted in suppression of spindles and slower cortical rhythms [278], coincident with the appearance of faster oscillations. These oscillations are state-dependent and enhanced by arousal, attention and vigilance [326]. These dependencies are mediated by acetylcholine muscarinic receptors. As will be discussed in more detail in Section 2.2.4, the primary function of these oscillations and their spatiotemporal synchronization is the aggregation of multiple cortical regions, creating global and coherent properties of patterns, a so-called “feature binding”. Importantly, while the primary generators of faster rhythms are intracortical, the view that these oscillations occur exclusively in cortical structures and during wakefulness has been shown to be inaccurate as thalamocortical cells have been found to spontaneously oscillate coherently with field potentials from related cortical regions in this frequency range [295]. A 40-Hz response, during sensory input, with a rostrocaudal phase lag, is also thought to be mediated by cortico-thalamo-cortical pathways [243, 242]. A resonant oscillation that is similar in distribution phase and amplitude to those observed during wakefulness is also observed in rapid eye movement sleep, which are not reset by sensory input [240].

While there may be intrinsic properties that render cortical neurons prone to faster oscillations, the manifestation of beta-gamma rhythms is a network phenomenon. The embedding of neurons in complex cortical circuitry is required for short- and long-range synchronization of these rhythms and their manifestation on EEG [347]. Fast oscillations have been shown to be associated with alternating microsinks and microsources throughout the cortex [376] in highly specific synaptic networks, which may be regulated by various processes, including the aforementioned glial spatial buffering. Gamma rhythms have been found in superficial somatosensory cortical layers (II/III), while beta rhythms were generated in deep layer V in association with fast-spiking interneurons [327]. The generation of these rhythms is also reliant on different mechanisms. Whereas the former is attenuated by antagonists of gap junction conductance, the latter is reduced by blockade of chemical synaptic transmission. Furthermore, blockade of GABA(A) receptors abolishes gamma activity, while enhancing beta rhythms, a phenomenon often observed on scalp EEG.

Interestingly, excitatory input is not required for the generation of these oscillations, as networks of interconnected interneurons may generate gamma oscillations under experimental conditions [429]; therefore in the case of gamma, inhibition is both necessary and sufficient.
Chapter 2. Literature Review and Hypotheses

Gamma oscillations may be generated by interactions within interconnected GABAergic interneuron networks (interneuron network gamma, ING) or network interactions between populations of pyramidal cells and local interneuron networks (Pyramidal-interneuron network gamma, PING). Causal interactions between interneurons and pyramidal cells leading to the generation of gamma activity has recently been studied using optogenetic methods [65]. Light-driven activation of fast-spiking interneurons was found to selectively amplify gamma oscillations, whereas pyramidal neuron activation amplified only low frequency oscillations. Since GABA-containing interneurons are a heterogeneous population of cells, it is also important to note that fast-spiking basket cells expressing parvalbumin, which innervate the perisomatic region are likely essential for the expression of gamma oscillations [18]. Interneuron network gamma (ING) has provided a framework to experimentally and computationally study the generators of these rhythms with regards to synaptic, electrical and kinetic regulators.

Physiological High Frequency Oscillations While high frequency oscillations (HFOs; defined broadly as frequencies higher than gamma) are gaining wide interest, there is little agreement regarding definition of appropriate frequency bands. High gamma often refers to frequencies above 70 Hz, whereas ripples may refer to frequencies between 80-160 Hz, 100-200 Hz or 100-250 Hz, and fast-ripples between 160, 200, or 250 Hz and 500 or 600 Hz [347]. Several historical protocols and factual inaccuracies have also, until recently, hindered the ability to detect HFOs. First, the EEG has traditionally been subjected to a low-pass filter around 60 or 70 Hz prior to analysis. Second, it has been thought that the skull itself acts as a low-pass filter, which is in effect only true at a frequency threshold of 10,000 Hz [299]. More accurately, the higher the frequency, the smaller the volume of brain that generates it and the lower its amplitude becomes; therefore it is difficult to detect volumes generating HFOs on scalp EEG and MEG. Intracranial EEG is therefore a robust method to capture HFOs, although spatial sampling may also preclude the recording of small generators and the size of the electrodes relative to the generators may attenuate the signal. The optimal method to record and analyze HFO is perhaps through systematic exploration of microelectrode recordings [41].

The association between HFOs and local processing is evident by the presence of HFO frequency components in event-related potentials. For example, sigma bursts (~600 Hz spike-like wavelets) have been recorded from somatosensory evoked potentials following median nerve stimulation [78]. With an increasing number of studies evaluating intracranial electrodes in patients undergoing epilepsy surgery, the role of high gamma or ripple band (80-150 Hz) oscillations in cognition became more apparent [321]. Power signatures in the ripple band from the motor cortex also correlated with electrocorticographic stimulation for mapping of motor function [77, 270], suggesting that HFOs represent local processing within spatially-restricted and specific cell assemblies. With increases in HFO power there was also concomitant decreases in lower-frequency band power (8-32 Hz) with a more restricted spatial pattern [270].

While the generators of HFOs remain elusive and their identification may be biased by
methods of HFO measurement (as previously described), they are more likely to be recorded in the presence of underlying synchrony among a population of neural generators. In simulations, it was found that while both increases in neuronal firing rate and synchrony increased high-gamma (60-200 Hz) power, HFOs were much more sensitive to increases in neural synchrony than firing rate [321]. In vitro slice recordings of carbacbol-induced fast network oscillations in the rat hippocampus have suggested a critical role for perisomatic-targeting inhibitory interneurons in the generation of these oscillations [254]. Pathological ripple and fast ripple HFOs have also been recorded using micro- and macroelectrodes in patients with epilepsy. The latter will be discussed further in Section 2.2.6 on page 20.

Interactions Between Frequencies Synaptic and axonal conduction delays are an important factor to consider in physiological systems. As a result of these delays, faster oscillations are typically constrained to small spatial extents, whereas slower rhythms may propagate across a larger volume of cortex. Furthermore, the slower the rhythm, the wider the window of opportunity for synchronization because synaptic and axonal conductance delays are less limiting; therefore, low-frequency rhythms modulate activity over large spatial regions and across long temporal windows, whereas high frequencies are restricted to small regions and short temporal windows [64, 413]. The interactions among neural oscillations at different frequency bands may therefore serve to regulate neural processing and inter-regional communication occurring across multiple spatiotemporal scales [64, 226]. A system of mutually interacting oscillators is well-positioned to regulate multi-scale integration within the brain.

Cross-frequency coupling (CFC) describes the embeddedness of one rhythm within another. It is thought that cross-frequency interactions facilitate network integration across distributed cortical regions, thereby supporting cognitive function. The study of these phenomena in physiological conditions has been the subject of considerable recent interest. Slow rhythms have been shown to co-exist with fast, transient oscillations [61] and the phase of low frequency theta rhythms has been reported to modulate the power of high gamma activity (80-150 Hz) [62]. CFC may also generalize to many frequency bands [226, 303, 348, 187, 280]. Two principal forms of cross-frequency interactions have been proposed: (a) amplitude-independent phase-phase synchrony; and (b) nested oscillations reflecting the locking of high frequency amplitude to lower frequency oscillations [405]. Less characterized amplitude-amplitude interactions have also been reported [351, 354]. CFC has been implicated in memory encoding and retrieval [349], short-term memory processing [338], and a variety of other cognitive functions [62, 303, 12, 236, 88, 53]. These findings suggest that CFC represents a physiological process regulating cortical processing. Since CFC occurs between many frequency bands and across large distances, it may allow selective information routing between parallel brain networks and overlapping cortical motifs [400].

The phenomenon of phase-amplitude CFC describes the statistical dependence between the phase of a low-frequency rhythm and the amplitude of the high frequency component of electrical
brain activity. A plausible biological significance of CFC relates to the physiology of neural phase and amplitude relations. The former reflects local neuronal excitability, whereas the latter reflects increases in population synaptic activity or an activation of a selected neuronal subnetwork (broad- and narrow-band increases, respectively). The combinations of amplitudes and phases that may interact is likely network and task specific. For example, gamma power was found to be locked to alpha phase in the posterior cortex [302], whereas high gamma amplitude is locked to theta rhythms in auditory cortex [62]. Interestingly, Aymacher and colleagues showed that the modulating phase of CFC during a working memory task, depended on the memory load, where greater load is associated with a shift of the modulating low frequency rhythm toward the lower frequencies [12]. This shift suggests that CFC may in fact represent a substrate of cognitive flexibility, as slower oscillations possess more phase positions to recruit gamma oscillations to maintain working memories. Interestingly, even a common protocol used to induce long-term potentiation consisting of high frequency bursts (~100 Hz) repeated at slower interburst intervals of 3-5 Hz corresponds exactly to gamma-theta phase amplitude coupling [227]. CFC is highly dynamic in the brain, with unique patterns of coupling and temporal modulation within different brain regions and between different frequencies [391].

The cellular mechanisms of CFC remain to be elucidated, although gamma-theta interactions are increasingly viewed as a physiological process in the human brain rather than a statistical epiphenomenon among filtered signals [64]. For instance, parvalbumin-positive interneurons have been shown to be essential for the generation of CFC [440]. Intrinsic resonance properties of neurons may also facilitate selective responses to inputs at specific low-frequencies of preference, a phenomenon termed resonance [176].

2.2.4 Synchronization of Neuronal Oscillations in Physiological Conditions

Having established that neuronal cell assemblies oscillate and that these oscillations tend to synchronize in a manner predictable by mathematical models, it remains important to review some literature on the physiological significance of the synchronization of these oscillations in health and disease. As alluded to previously, an increasing body of work suggests that oscillations play an important role in neuronal communication and information processing in the brain. In several landmark studies, Gray and colleagues showed that neurons in the visual cortex of cats oscillate in the gamma band (defined therein as 40-60 Hz), and these oscillations synchronize both within [143] and between [141] functional columns. Furthermore, synchronization of these oscillations occurred between homologous regions in the two cerebral hemispheres [104]. It was demonstrated, therefore, that oscillations provide a mechanism to temporally coordinate the transient patterns of activity within spatially separate regions of the brain. These synchronous oscillations were generated from cortical rather than subcortical structures [143] and were tightly correlated with local field potentials (LFPs). The synchronization of neuronal oscillations also serves to mediate selective attention, as it may amplify salient signals in the cortex by increasing synaptic gain [131] as well as formation of memory [113] and even corticospinal interactions to
potentially facilitate shortened reaction times [345].

Subthreshold gamma oscillations also produce windows for cortical-cortical communication whereby coherent oscillating cell assemblies interact more effectively because their communication windows are open at the same time [130]. Subthreshold oscillation of membrane potentials impose a precise temporal window for the integration of synaptic inputs. The sensitivity of the neuron to short excitatory postsynaptic potentials (EPSP) is strongly enhanced for a temporal window of a few milliseconds during such oscillations to the extent that a single PSP can be sufficient to generate an action potential [412]. Synchronization also causes spikes to coincide, enhancing their impact on the postsynaptic neuron. Oscillatory membrane potential may therefore facilitate synaptic transmission between similarly synchronized neurons and suppress PSPs received asynchronously. PSP received in synchrony with the membrane oscillatory rate are selectively propagated, while others are suppressed, a phenomenon referred to as “neuronal gating” [188].

While evidence mounted regarding the inter-relations among network integration, cognitive processing and neural oscillations, Womelsdorf and colleagues also showed that synchronization of oscillations modulate neuronal interactions and that neuronal interactions mechanistically depend on phase relationships between rhythmic activities [437]. In an important study, this group demonstrated that precise phase relations precede strong power correlations and that effective connectivity diminishes when synchronization is less precise because synaptic input is more likely to arrive at random phases. These findings were buttressed by neurocognitive experimental data showing that accurate object perception is biased by the phase of ongoing oscillatory activity in the brain. For example, prestimulus alpha-band phase has been shown to predict suppression of object perception and motor inhibition when stimuli were presented at the trough of alpha phase [259, 262].

2.2.5 Synchronization of Neuronal Oscillations in Childhood Development

In children, the synchronization of oscillatory activity is relevant for the development of cortical circuitry. This is evident by two lines of arguments, as summarized by Uhlhaas and colleagues [393]. First, as discussed previously, synchronous neural oscillations are strongly implicated in synaptic plasticity. According to the Hebbian rule, synapses increase their efficiency if the synapse persistently takes part in firing the post synaptic target. Put another way, “neurons that fire together wire together.” The interstimulus synchrony between two connected neurons determines whether the postsynaptic potential will be potentiated, with a greater likelihood of long-term potentiation when presynaptic spikes precede postsynaptic spikes [279]. Similarly, the phase sensitivity of synaptic interactions is evident by findings of long-term potentiation following stimulation at the depolarizing peak of theta oscillations in contrast to stimulation at the troughs, which results in long-term depression[173]. This is particularly relevant as hippocampal theta oscillations are expressed during periods of learning and memory retention [388]. A similar phenomenon been also been observed in the neocortex at higher frequencies, such
as beta and gamma, where long-term potentiation occurring at the peak of membrane potential oscillations and long-term depression at the trough [427]. Oscillations therefore provide a critical framework for the alignment of temporal and amplitude relations between pre- and post-synaptic cells in order to strengthen or weaken synaptic contacts during development [394, 393].

Second, changes in frequency and synchronization of neural oscillations occur throughout stages of development that correspond to molecular and structural brain changes in children. With development, there is a reduction in absolute EEG power across a variety of frequencies that coincides with decreases in gray matter volume and synaptic pruning [428]. Furthermore, with development, there is an increase in higher frequency rhythms, in the alpha and beta range, concurrent with decreases in activity in lower frequencies [428, 386]. The increase in activity in higher frequency ranges is thought to correlate with the development of higher cognitive faculties with healthy childhood development [23], and coincides with critical periods for the maturation of GABAergic neurotransmission [153]. Particularly, the expression of the $K^{+}/\text{Cl}^{-}$ co-transporter, KCC2 increases with development, which contributes to the negative resting potential of the mature cortical pyramidal neuron. Shifts in the expression of GABA subunits (from $\alpha_2$ in the immature brain to $\alpha_1$ in the adult brain) may also facilitate the generation of gamma oscillations as they are expressed in synapses of parvalbumin-positive basket cells, that are essential for the generation of these rhythms [362].

The developmental changes in the amplitude of oscillations is also associated with changes in inter-regional synchrony, with more precise coupling among different frequencies, reflecting the development of long-range connectivity [371]. There is also maturation of neural synchrony during cognitively-demanding tasks, which may represent an increasing role for perceptual binding with development. Uhlhaas and colleagues also demonstrated increased neural synchrony during visual perception and cognitive performance in the beta and gamma bands in young children. The patterns of neural synchrony they observed were dynamic with age and decreased during early adolescence, only to increase in the theta and beta bands thereafter [394]. Furthermore, long-range synchrony may be facilitated by myelination throughout development, which may increase conduction velocity therefore facilitating synchrony among higher frequencies [335].

Similar developmental changes have been observed in indirect oscillatory activity (in blood oxygen level dependent hemodynamic responses). The coherence, synchronization and development of these oscillations are outlined in Sections 2.4.2 on page 32 and 3.1 on page 49.

### 2.2.6 Synchronization of Neuronal Oscillations in Epilepsy

Abnormalities in the synchronization of neuronal oscillations have been documented in a host of neurological and psychiatric conditions. The study of synchronization in epilepsy offers the opportunity to study epileptogenic networks as well as networks associated with the cognitive and behavioural morbidities of epilepsy. As previously stated, epilepsy has historically been assumed to result from neuronal synchronization that is pathologically increased and prolonged [305]. This view is an intuitive extension of the finding that high voltages recorded from epileptic
cortex reflect synchronous neural activity. Importantly, abnormal synchrony in epilepsy is the result of interactions among cortical and subcortical generators. For instance, synchronous oscillations in epilepsy are unlikely to be the exclusive byproduct of thalamic mechanisms, as it has been shown that cortical spike-wave seizures can be recorded after ipsilateral thalamectomy [380]. Furthermore, although bilateral synchronous spike-and-wave discharges (SWD) reflect highly synchronized oscillations in thalamocortical networks, spike and wave discharges originate in the cortex and initiate oscillations in the thalamo-cortical-thalamic loop [267].

The Ictal Period

Several studies examined oscillatory synchrony in the interictal, preictal and ictal periods. These studies challenge the prevailing notion that neural synchrony is uniformly increased in epilepsy. Put another way, although visual interpretations of EEG recordings suggest highly synchronized patterns of activity, more detailed analysis has revealed that voltage recordings may not be indeed synchronous. Quiroga and colleagues applied several linear and nonlinear measures of synchronization to typical EEG signals from rats during seizures to show that mathematical modeling of synchrony provided information that would not have been predicted by visual inspection and in some circumstances, synchrony was much lower than appreciated visually [316]. Similarly, using neuromagnetic recordings, Garcia Domínguez and colleagues showed that local synchrony was increased in small neighbouring cortical regions during seizures, but distant synchrony did not increase during most of the seizures, and was on occasion lower than during the interictal period [93]. At baseline, this study also found no difference in inter-regional synchrony between controls and patients with epilepsy. In agreement, van Putten found that enhanced local synchrony was associated with onset of seizure activity [402]. These findings are buttressed by in vitro studies showing that bursts within CA1 pyramidal neuronal activity are synchronized with high precision in the beta band, but during seizures, activity is no longer synchronous [286]. Emerging understanding is, therefore, that epileptic networks are characterized by abnormal synchrony rather than hypersynchrony.

Taken together, these data would suggest that hypersynchrony remains an important hallmark of epileptic seizures in specific regions and at distinct times. It is important to appreciate that ictal events are dynamic and during a seizure, multiple different networks and subnetworks constantly fragment and coalesce [218]. Indeed, it has been shown that while the overall synchronization during seizures changes modestly, the network topology changes dramatically [218]. Interestingly, a single subnetwork dominated the dynamic pattern of connectivity, which may reflect decreased centrality of hubs and altered patterns of excitation and inhibition.

The Preictal Period

During the preictal period, epileptogenic areas demonstrate decreased interactions with surrounding cortex, as demonstrated by decreased synchrony in the beta-band in the majority of seizures [317]. This “phase scattering” relative to the interictal occurs prior to seizures on a large time scale, sometimes hours before the onset of ictal activity. Velazquez and colleagues noted increases in the fluctuations of phase differences before seizure onset in all subjects studied using MEG and intracranial EEG [408]. Such “phase slips” are associated
with decreased phase synchrony in the preictal period. In agreement, Kramer and colleagues determined that seizure onset is characterized by a global breakdown in inter-regional coupling across the cerebral cortex with a decrease in degree and closeness centrality [217] (Refer to Section 2.5 on page 40 for a thorough overview of these network properties). Desynchronizations may functionally isolate the epileptogenic regions from the influence of large-scale brain networks, creating an idle population of neurons that may be more susceptible to recruitment into seizure. Such desynchronizations may also represent depression of synaptic inhibition in areas around epileptogenic cortex, isolating it from the controlling influence of the embedded network [395].

**The Interictal Period**  
Data from interictal periods have also shown that the epileptogenic zone is functionally disconnected [434], with greater disconnection associated with greater epileptogenicity [401]. Furthermore, synchrony is expressed in discrete clusters, which were identified by examining phase relationships [301]. This pathological isolation of the epileptogenic tissue is also supported by resting-state fMRI studies (for a more thorough review of resting-state fMRI abnormalities in patients with epilepsy refer to Section 2.4.5 on page 37).

**Pathological High Frequency Oscillations**  
One manifestation of abnormal synchrony in epilepsy is the expression of pathological HFOs (pHFOs). As discussed in Section 2.2.3 on page 12, HFOs occur in physiological conditions and are associated with local processing within discrete and spatially restricted cortical regions. pHFOs were first recorded in the hippocampal formation of epileptic intrahippocampal kainic acid-treated rats [40]. It has been shown in animal models that the presence of electroencephalographic HFOs precedes clinical seizures following an epileptogenic injury [44] and that the number of electrodes expressing pHFOs is associated with higher seizure frequency [43]. While physiological network properties, including the action of the inhibitory interneurons, as described in Section 2.2.4 on page 18, likely play a critical role in the generation of these oscillations, it is important to recognize that they remain pathological. For instance, ripples are not normally seen in the dentate gyrus, but appear therein prior to seizures in kainic acid-treated rats [42].

The generators of pHFOs are not fully characterized, but are thought to be at the crossroads of synaptic interactions and intrinsic membrane properties [197]. Pathological ripples may represent the synchronous firing of abnormally bursting principal cells localized to a discrete neuronal cluster (pathologically interconnected neuron clusters) [41]. Fast ripple frequencies, however, (250-800 Hz) are well above the maximal firing frequency of glutamatergic neurons. In hippocampal slices from pilocarpine-treated epileptic rats, generating pHFOs > 300 Hz, single pyramidal cells were found to fire similarly but less precisely than cells recorded from slices from prepared normal animals [119]. Furthermore, manipulations that improve spike-timing reliability, particularly the reduction of membrane potential fluctuations, deteriorated fast ripples and restored ripple pattern in the epileptic tissue. This suggests that less precise bursting (desynchronization, rather than hypersynchronization of the normal ripple pattern, so-called “ripple shuffling”)
correlate with more disorganized pHFO power spectra and the expression of fast ripples. Each fast-ripple cycle represents emergent oscillations from slower-discharging cells with 1/2 to 3/4 intraburst firing delays [178]. Since not all neurons contribute to consecutive cycles, an emergent character develops whereby very fast oscillations may be generated. Very recently, these findings were supported by findings that channelopathies, namely the functional reduction of the delayed rectifier potassium channel $\alpha$–subunit Kv1.1 reduced spike-timing precision of neurons, promoted the emergence of fast ripples and resulted in epilepsy [355].

In addition to synaptic conduction, electrical transmission and ephaptic interactions may enhance and amplify pHFOs. Such mechanisms could facilitate the organization of out-of-phase firing clusters that express pHFOs. Simulations show that neurons firing with lags shorter than 2 ms contribute to the same population spike, whereas longer delays may generate an independent cycle [178]. Functional clustering of neural populations with jittered firing may then occur based on the preferential phase of adjacent clusters. It has even been suggested that neocortical ripples (during normal network activity) may participate in initiating seizures at a certain threshold amplitude through a mechanism involving a “vicious feedback loop” in which bursts of population spikes generate a local field potential (LFP), which in turn generates and synchronizes action potentials in adjacent neurons through gap junctions [146]. Other mechanisms that may lead to clusters of neurons with jittered firing include heterogeneities in recurrent connectivity or recruitment delays between pools or neurons or patchy neuronal loss in epileptogenic brain regions [197].

Recordings of pHFOs in humans have been performed using micro- and macroelectrodes. LFPs from microelectrodes can be measured by filtering out action potentials (frequencies above 600 Hz). pHFOs are most frequent in patients with epilepsy during non-REM sleep, and REM sleep suppresses ripple activity [372]. Very recently, pHFOs recorded on microelectrode recordings were shown by Weiss and colleague to differentiate two types of epileptogenic territories, the ictal core and ictal penumbra [423]. The former was characterized by synchronized neural burst firing with the amplitude of pHFOs phase-locked to the low-frequency (1-25 Hz) ictal rhythm, whereas the latter exhibited sparse and asynchronous neural activity with no pHFO activity in surrounding macroelectrodes. Since weak electric fields contribute to neuronal synchronization (i.e. ephaptic transmission) [125, 196], it would expected that neuronal synchrony would be observed during large electric fields generated by ictal events. This suggests a pattern of “inhibitory restraint” whereby field effects are negated in the presence of strong synaptic inhibition in the penumbra [340]. In the core, neural synchrony may be enhanced by field effect interactions that synergistically pace and entrain rhythmic paroxysmal depolarizing shifts.

The majority of studies of pHFOs have utilized macroelectrode recordings, which first revealed a temporal association between pHFOs and ictal activity [117]. More recently, numerous studies have linked ictal and interictal pHFO activity to underlying epileptogenicity, and suggested a role for their utility in guiding neocortical resections [192, 193, 296, 5]. Identification of pHFOs involves any number of time-frequency analyses, including short-time Fourier trans-
form or multiple band filter analysis of electroencephalographic recordings. Interictal pHFOs (defined by the authors as 60-100 Hz) were found to occur in the seizure onset and become more frequent 20 minutes prior to neocortical seizure activity. To facilitate their clinical application, Akiyama and colleagues have presented a method to easily visualize pHFO activity from intracranial recordings [4]. Higher pHFO frequencies (fast ripples; >200 Hz) have been recorded from macro-electrodes, although the signal becomes attenuated given the size of the recording electrode relative to the volume of the generator [200]. In the interictal period, these pHFOs may occur on top of traditional EEG spikes, independent of spikes, or as a result of filtering sharp spikes. Since the irritative zone where spikes occur is presumably larger than the epileptogenic zone, it is important to appreciate that pHFO activity was shown to be a stronger indicator of the seizure onset zone independent of spike activity [192]. pHFOs are also more strongly linked to seizure activity than IED, evident by the fact that medication weaning leads to increased seizure activity and pHFOs, but not IEDs [449]. Interestingly, after IEDs, pHFO power is attenuated, suggesting a role for postspike depression in neural activity [396].

An important area of ongoing research is the study of how pHFOs differ from physiological high frequency activity that may encompass overlapping frequency bands (see Section 2.2.3 on page 12 for an overview of physiological oscillations and their generators). They may encompass higher frequencies and possess longer durations. Furthermore, in contrast to physiological HFOs, it is unlikely that pHFOs observed in close temporal relationship with seizure activity and/or sleep states are influenced by cognitive activity. Furthermore, while pathological and physiological ripples may share similar frequency bands, the power increases of pHFOs at the onset of, and during seizures may be greater than the physiological activity [147].

**Synchrony and the Comorbidities of Epilepsy** In addition to providing novel insights into seizure onset, propagation and termination, the study of oscillations has led to better understanding of the neural correlates of comorbidities of chronic epilepsy, such as behavioural problems and cognitive decline. Patients with epilepsy demonstrate more random brain topologies when synchronization was used as a measure of connectivity [98]. Increased theta band connectivity in particular was suggested to be a hallmark of epileptic brains [98, 97]. Patients with epilepsy also exhibited different global brain network characteristics compared to controls, with more regular (less efficient) patterns of synchrony-based connectivity in patients with epilepsy [172]. The literature is divided as to whether brain networks in patients with seizures have less synchronous brain regions (or decreased connectivity when using synchrony as an index) or greater synchrony [16, 398, 172]. While this likely reflects heterogeneity in pathologies and patient demographics, it was found that individuals with epilepsy generally demonstrate lower synchronizability, a measure of the stability of the synchronous state in a network [398]. Lower synchronizability was associated with worse cognitive decline and greater seizure frequency, suggesting that the lack of precise physiological synchrony is related to the comorbidities of epilepsy.
2.3 Functional Connectivity in Direct Neural Oscillations

2.3.1 Measurement of Direct Neural Oscillations

The previous sections have reviewed evidence for the critical role of neural oscillations and their synchronization in normative development and their abnormal function in epilepsy. In the current section, the mathematical methods to infer functional connectivity from direct neural oscillations are reviewed. In the current dissertation, direct neural oscillations are measured using electrocorticography (ECoG) and MEG.

Electrocorticographic (ECoG) signals are synchronized post synaptic potentials measured directly from the cortical surface in patients undergoing invasive electrographic monitoring from subdural electrodes to determine candidacy for epilepsy surgery. In contrast to scalp EEG, which requires cortical activation of 2.5 to 3 cm$^2$ in order to detect activity, ECoG offers a temporal resolution of approximately 5 ms and a cortical activation of 1 cm [347]. Together with direct cortical stimulation, ECoG may be used to identify motor, language and somatosensory cortex during epilepsy surgery. The relevant methods used to collect, filter and analyze these data are presented in the germane sections throughout the dissertation.

Magnetoencephalography (MEG) measures magnetic fields induced by neural current flows in the brain. Sensitive magnetometers, superconducting quantum interference devices (SQUIDs) are used to measure change in magnetic flux detected by the induction of a current in a wire. Since magnetic fields, unlike electrical fields, are not distorted by inhomogeneous conductivity in the head, MEG provides a more accurate projection of neural activity in source space, with improved spatial resolution relative to electroencephalography (EEG). To identify intracranial neural current generators from MEG data (to solve the “inverse problem”), various solutions have been proposed, including parametric and imaging models. The former account for measured fields in terms of a small number of sources (i.e. equivalent dipole model; ECD), whereas the latter estimate source activity at specific points of interest (i.e. local linear estimators). Imaging methods are discussed in further detail and applied in Section 3.5.1 on page 66.

2.3.2 Measurement of Instantaneous Amplitude and Phase

There are various methods from which functional connectivity may be inferred from direct neural activity. When measuring synchrony in neural oscillations, amplitude-independent measures that exclusively evaluate the phase information of an oscillations may be helpful. The evaluation of amplitude interactions may also be relevant for the study of functional connectivity, for instance when measuring cross-frequency phase-amplitude coupling, as described in Section 2.2.3 on page 12. The current section will review methods for the derivation of phase and amplitude information from a physiological signal and the use of this information to infer functional connectivity between two or more oscillators.

Numerous mathematical approaches may be used to calculate the instantaneous amplitude or phase of a real signal, $f(t)$. The amplitude (or envelope) is a function that interpolates from
peak to peak for an oscillatory waveform, whereas the phase is a measurement of the position within a full cycle of an oscillatory waveform, measured in radians. These methods may involve convolutions with complex wavelets, or Hilbert transformation. In the current dissertation, the instantaneous amplitude, \( A(t) \) and instantaneous phase \( \theta(t) \) were derived from the real signal \( f(t) \) and its Hilbert transform, \( \tilde{f}(t) \), as shown in Equation 2.2.

\[
\varsigma(t) = f(t) + i\tilde{f}(t) = A(t)e^{i\theta(t)}
\] (2.2)

In this equation, \( i = \sqrt{-1} \). The Hilbert transform is related to the original signal by a \( \frac{1}{2}\pi \) phase shift that does not alter the spectral distribution. The Hilbert transform of \( f(t) \) is defined as:

\[
HT(f(t)) = \tilde{f}(t) = \frac{1}{\pi} P.V. \int_{-\infty}^{\infty} \frac{f(t)}{t-\tau} d\tau
\] (2.3)

where P.V. denotes Cauchy principal value. From Equation 2.2, the instantaneous amplitude and instantaneous phase can be computed by:

\[
A(t) = \sqrt{\tilde{f}(t)^2 + f(t)^2}
\] (2.4)

\[
\theta(t) = \arctan \frac{\tilde{f}(t)}{f(t)}
\] (2.5)

Example time series of the instantaneous amplitude and phase may be found in Figure 5.1 on page 95.

### 2.3.3 Measures of Connectivity

As mentioned, there are a variety of methods available to index connectivity, each with advantages and disadvantages. Earlier measures used to evaluate functional connectivity largely involved linear correlations between two signals as a function of the frequency [45]. Measures that have attempted to directly study synchrony between neural oscillators have largely relied on frequency coherence (or mean-squared coherence, MSC). MSC is the frequency-domain analog of the correlation coefficient. This is a measure of linear covariance between two spectra by subdividing the whole data into segments, computing approximations of spectra of each segments using the Discrete Fourier Transform and averaging the subspectra across all segments [73]. This method requires an assumption of stationarity, whereby each segment of data corresponds to the same process with the same spectral properties. Furthermore, coherence, as indexed by this measure does not quantify phase-relations. Measures of phase-locking, which are described below do not require stationarity and directly measure phase relations between oscillators, therefore providing a more robust approximation.

The question remains, however, which of these methods is “the best”? That is to say, which of the methods of measuring connectivity is most likely to yield the most accurate measure
of the relationship between two oscillators. Wendling and colleagues addressed this question in a recent study and determined that no single method was superior to others [425]. They found that results often depended on the signal parameters, as well as the specific hypothesis, or relationship that was being investigated.

**Amplitude-Independent Measures** There are measures that are independent of amplitude, involving phase synchronization. Phase synchronization between two narrow-band signals may be characterized by several related values, including the phase-locking value (PLV), phase-lag index (PLI) and weighted phase-lag index (wPLI). It is important to appreciate that all of these measures represent average vector directions for differences in instantaneous phase, $\theta(t)$ between two signals and may be calculated across time or across trials in an event-related experimental design.

**Phase-Locking Values (PLV)** The Phase-Locking Value (PLV) [225] is one amplitude-independent measure that may be used to index functional connectivity. Given the instantaneous phase time courses of oscillators, $\theta_j(t)$ and $\theta_k(t)$, the difference between these two time series ($\Delta \theta$) represents the locking between the phases of the different oscillators. If the oscillations in two discrete signals rise and fall in synchrony, or with a certain time lag, $\Delta \theta$ will be consistent between trials or across time. The PLV, therefore, only measures the variability in $\Delta \theta$ either over time (Equation 2.6) or across trials (Equation 2.7). This measure possesses a value between 0 and 1, with 0 signifying random rise and fall in $\Delta \theta$ with no phase synchrony between the oscillators, whereas 1 signifies that one signal is in perfect phase synchrony with the other.

$$PLV_{j,k} = \frac{1}{T} \left| \sum_{t=1}^{T} e^{i[\theta_j(t) - \theta_k(t)]} \right|$$  \hspace{1cm} (2.6)

$$PLV_{j,k,t} = \frac{1}{N} \left| \sum_{n=1}^{N} e^{i[\theta_j(t,n) - \theta_k(t,n)]} \right|$$  \hspace{1cm} (2.7)

In these equations, $PLV_{j,k}$ quantifies the phase-locking between two oscillators, $\theta_j(t)$ and $\theta_k(t)$, over time, $t$, while $PLV_{j,k,t}$ quantifies the phase-locking between the two oscillators at time point $t$ across $N$ trials. For simplicity, the remainder of the equations displayed will take the form of synchrony over trials at a given time point.

**Phase-Lag Index (PLI)** One challenge with the PLV is that time series with zero-phase lag may show artefactual high connectivity due to volume conduction from a single strong source [294]. True interaction between recorded sites may be characterized by a certain time delay, whereas volume conduction effects do not give rise to time delay. The Phase-Lag Index (PLI) [374] rejects zero phase lag synchrony, and therefore may be beneficial in circumstances
where invariance against the presence of common sources (volume conduction and/or active reference electrodes in the case of EEG) is desirable. The way in which this is achieved is by defining an asymmetry index for the distribution of phase differences, when the distribution is centered around a phase difference of zero. If no phase coupling exists between the two time series, this distribution would be expected to be flat, whereas deviations from a flat distribution would indicate phase synchronization.

If the phase distribution is asymmetric, there is a different likelihood that the phase difference $\Delta \theta$ will be in the interval $-\pi < \Delta \theta < 0$ rather than the interval $0 < \Delta \theta < \pi$. Such an asymmetry implies the presence of a consistent, non-zero phase lag between the two time series. Conversely, symmetry in the distribution of phase differences would imply no coupling between the two signals, or phase differences with zero-lag. An index of the asymmetry of the phase difference distribution (the PLI, shown in Equation 2.8) would therefore provide a means to reject zero-lag phase synchrony. The PLI across $N$ trials can be measured from the time series of phase differences $\Delta \theta_{j,k}(t, n)$.

$$PLI_{j,k,t} = \left| \frac{1}{N} \sum_{n=1}^{N} sgn [\Delta \theta_{j,k}(t, n)] \right|$$

(2.8)

Where the sign (or signum) function is defined as:

$$sgn(x) = \begin{cases} -1 & \text{if } x < 0 \\ 0 & \text{if } x = 0 \\ 1 & \text{if } x > 0 \end{cases}$$

(2.9)

**Weighted Phase-Lag Index (wPLI)** The PLI may be biased and lose ability to detect changes in phase synchrony in circumstance where weak coupling exists or noisy data is acquired. This is because PLI, by definition does not distinguish whether the value of phase difference is close to zero or not, but rather whether it is positive or negative. It is therefore said to be discontinuous. In noisy data, when the phase differences may be close to zero, the value of the relative phase may change from lead (+1) to lag (-1) only due to the presence of noise. The Weighted Phase-Lag Index (wPLI) was more recently described to address the issue of the discontinuity of the PLI [411] by considering not only the phase but also the the imaginary component of the cross-spectrum (Equation 2.10).

$$c_{j,k} = A_j A_k e^{i \Delta \theta_{j,k}} = A_j A_k [\cos (\Delta \theta_{j,k}) + i \sin (\Delta \theta_{j,k})]$$

(2.10)

In this equation, the imaginary component of the cross-spectrum, $\Im \{X\}$ is given by $A_j A_k \sin (\Delta \theta_{j,k})$.

$$wPLI_{j,k} = \frac{|\langle \Im \{X\} \rangle|}{\langle |\Im \{X\}| \rangle} = \frac{|\langle |\Im \{X\}| \cdot sgn (\Im \{X\}) \rangle|}{\langle |\Im \{X\}| \rangle}$$

(2.11)

In Equation 2.11, $\langle x \rangle$ denotes the expectation value of $x$. From the second part of Equa-
tion 2.11, the relationship between the PLI and wPLI becomes more apparent. The difference in angles $\Delta \theta_{j,k}$ in the PLI measurement is based exclusively on the imaginary component of the cross-spectrum. The sign of the change in phase will match the sign of the imaginary component of the cross-spectrum (i.e. if one is positive, the other will also be positive) and therefore, the signum of the two will be identical. The wPLI therefore weights the $\text{sgn}(\Im\{X\})$ (or $\text{sgn}[\Delta \theta_{j,k}(t,n)]$ in Equation 2.8) by $|\Im\{X\}|$, which is the magnitude of the imaginary component.

Amplitude-Dependent Measures

**Pearson’s Correlation Coefficient** Although synchronous activity may be measured exclusively based on phase relations, there are also important measures that may incorporate information from the amplitude of the oscillations. Perhaps the simplest amplitude-dependent measure of dependency is the Pearson’s Correlation Coefficient, described in Equation 2.12.

$$R_{j,k} = \frac{\text{COV}(j,k)}{\sigma_j \sigma_k}$$  \hspace{1cm} (2.12)

This measure, between -1 and +1 is a measure of the degree of linear dependence between two variables and is defined as the covariance of two variables divided by the product of their standard deviation. A related approach involves partial correlation, where the general dependence of two variables is measured after adjusting for the influence of third-party effects. As will be discussed in subsequent sections, it has been shown that both Pearson correlation and partial correlation provide excellent performance at estimating functional connections in fMRI data, although Pearson correlation is superior when the number of nodes in brain networks increased significantly [356]. There are, however, disadvantages in using this metric when evaluating neural oscillations, particularly direct neural activity. First, under heavy noise conditions, extracting correlation is non trivial. Second, these correlation methods assume linearity, which may not be a valid assumption.

**Mutual Information** More complex measures may also be used to index amplitude-dependent functional connectivity, although their superiority over traditional measures of dependence has not been established [356]. One emerging metric is mutual information, which is a dimensionless quantity that describes the reduction in uncertainty about one random variable given knowledge of another. A value of zero suggests no mutual information meaning that the variables are statistically independent, whereas higher mutual information suggests a greater reduction in the uncertainty. The mutual information between two random variables is described as:

$$I(X;Y) = \sum_{x \in X} \sum_{y \in Y} P(x,y) \log \frac{P(x,y)}{P(x)P(y)}$$  \hspace{1cm} (2.13)
In this equation, \( P(x) \) and \( P(y) \) are the marginal distributions of random variables \( X \) and \( Y \) and \( P(x,y) \) is the joint probability distribution.

**Cross-Frequency Coupling and the Modulation Index**  As described in Section 2.2.3 on page 12, phase-amplitude coupling between different frequencies comprises a neural syntax that represents hierarchical regulation of cortical processing. Measuring the combined influence of signal amplitude and phase may therefore reveal additional information regarding functional interactions between brain regions. There are many mathematical ways of measuring cross-frequency coupling (see Canolty and colleagues for review [64]), and the specific methods used in the current dissertation are described in further detail in Section 5.2.4 on page 95.

### 2.4 Functional Connectivity in BOLD fMRI

Thus far, this chapter has reviewed topics relevant to oscillations that can be measured directly through electrophysiology. germane literature was reviewed on neural oscillations and the means by which they facilitate the segregation of networks. Spontaneous oscillations can, however, also be observed indirectly by studying the metabolic and hemodynamic activity associated with neural activity. One challenge in studying local and global networks structure is that the spatial coverage afforded by methods to directly measure neural activity is inherently limited by the positioning of the electrodes and sensors or the inverse problem (which will be addressed in Section 3.5.1 on page 66), which is, in turn, constrained by the prior hypotheses of the investigator. In contrast, a great strength of functional brain imaging, in particular functional magnetic resonance imaging (fMRI), is that it can provide a global view of activity throughout the brain during tasks and at rest. These studies provide highly complementary information on the segregation, function and organization of large-scale neuronal networks. The following segment will discuss methods to study functional connectivity using indirect measures of neuronal activity (i.e. BOLD-fMRI). The relationship between functional connectivity measured indirectly through cerebrovascular coupling and direct neural oscillations is explored subsequently in Section 2.4.6 on page 39.

#### 2.4.1 Basic Principles and Neurophysiology

It has been recognized for a long time that brain activity is associated with concomitant changes in cerebrovascular circulation. As early as 1928, John Fulton, an American neurosurgeon noted a cranial bruit over the occiput of a patient with an occipital arteriovenous malformation, which increased in intensity with visual tasks [134]. The advent of magnetic resonance imaging (MRI) and the realization that the oxygenation status of hemoglobin alters its ability to disrupt a magnetic field allowed for the visualization of task-related and task-independent changes in cerebral blood flow [304]. Deoxyhemoglobin is paramagnetic, becoming slightly magnetized in the presence of a magnetic field, thereby generating its own, weak, magnetic field and resulting in
more local field inhomogeneities and thus a shorter duration of T2* (spin-spin coupling). In the presence of highly oxygenated tissue, there is a decrease in local magnetic field inhomogeneity and an increase in T2*, which in turn results in a brighter signal. During brain activation, there is a local increase in blood flow and glucose consumption that exceeds the local increase in oxygen consumption [124]. Because of the rise in blood oxygen level in activated tissue, there is a T2* signal increase on the order of 1-5% for prolonged activation and as little as 0.1-0.2% for transient activations [15, 52]. This is the basis for blood oxygen level dependent (BOLD) signal.

The relationship between BOLD signal observed in fMRI studies and underlying neuronal activity has been the subject of controversy. It has been shown that increases in cerebral blood flow are not due to increased metabolic demands for oxygen [272] or glucose [314]. Furthermore, increases in cerebral blood flow far exceed the oxygen metabolic rate (so-called “overshoot” or functional hyperemia) [55]. The most convincing study of the underlying neurophysiology of BOLD signal is derived from experiments with simultaneous fMRI and microelectrode recordings in primates [248]. In this study, checkerboard visual stimuli were presented to primates in an MRI scanner. Using direct neural recordings, various aspects of neuronal firing were examined and correlated with hemodynamic response. These recordings distinguished between local field potentials (LFPs) and action potentials (all-or-nothing firing rates of individual neurons [single-unit firing] and groups of neurons [multi-unit firing]). LFPs are more slowly varying and arise from input to, and processing within the dendrites of neurons [318]. The BOLD signal was found to be more closely related to LFP than action potentials. Furthermore, BOLD signal was tightly coupled to gamma oscillations in anesthetized cats [290]. Interestingly, brain regions that exhibit significant neuronal activity were not consistently found to generate reliable fMRI statistical maps due to the signal-to-noise profile of fMRI, implying that the absence of a BOLD response may not correlate to a lack of brain activity.

Furthermore, BOLD signal is likely a result of changes in LFP in postsynaptic neurons that occurs as a result of changes in excitatory neurotransmission [318]. Excitatory neurotransmission is mediated largely by the release and detection of glutamate by pre- and post-synaptic neurons. This neurotransmitter is then rapidly removed from the synapse and broken down by astrocytes, which requires energy consumption and necessitates a rise in blood oxygen levels and therefore, BOLD signal. While inhibitory processes, mediated through GABA neurotransmission also utilize glucose [1], it has been shown through transcranial magnetic stimulation that inhibition evokes no measurable change in BOLD signal, suggesting that it may be metabolically less active [414]. The BOLD signal in fMRI studies is therefore believed to be the result of excitation rather than inhibition, and likely reflects neuronal signaling rather than loci of increased energy utilization [11]. More recently, optogenetic methods are being exploited to better understand the cell types and network circuitry associated with BOLD signal [231].
2.4.2 Intrinsic Connectivity Networks

While the human brain has traditionally been studied with respect to the localization of its physiological functions (i.e. task-dependent activations), at rest, the brain consumes 20% of the oxygen needed by the body, despite accounting for only 2% of its mass [318]. Neuronal responses to external stimuli account for less than 5% of the brain’s overall energy budget, devoting the majority of its capacity to intrinsic neuronal signaling [319]. In the absence of overt perceptual input or behavioural output, spontaneous fluctuations in BOLD signal occur and have provided remarkable insight into the brain’s functional organization and connectivity at rest. At low frequencies (approximately less than 0.1 Hz), these spontaneous BOLD oscillations exhibit a pattern of temporal coherence within specific brain regions that are also co-activated during task performance [32] and are reflected in the brain’s structural connectivity [145, 399].

Coherence in low frequency spontaneous BOLD oscillations across different brain regions have revealed multiple functional intrinsic connectivity networks (ICNs). These include primary input-output networks, such as the visual, sensorimotor and auditory networks, as well as higher integrative networks, including language, attention and salience networks, as well as the default mode network (DMN; see Zhang and Raichle for review [445]). Of these, perhaps the most studied is the DMN. This networks is comprised of brain regions that demonstrate a canonical pattern of coactivation both during tasks and at rest. The network is termed task-negative, since these regions are more active at rest than during task engagement, likely reflecting baseline levels of neuronal and metabolic activity [109, 318]. The core regions that comprise the DMN are the posterior cingulate cortex/precuneus/retrosplenial cortex (PCC) and the ventromedial prefrontal cortex (vmPFC) and lateral parietal cortices.

Fractionating the DMN into functional and anatomical subregions reveals a more complex inter-play between critical cortical hubs and subsystems that may underlie the importance of this network in health and disease. The PCC is highly anatomically connected and forms a structural core within large-scale brain networks[149], thereby supporting functional integration. Furthermore, there is a dissociation between the dorsal and ventral PCC, which may facilitate internally-directed thought through differential interactions with attention networks [232]. The vmPFC node of the DMN overlaps with brain regions that are activated during self-reflective thoughts and judgments that depend on inferred social and emotional context [148]. This may therefore implicate the DMN in in Theory of Mind, the ability to attribute mental states to oneself and others [273]. The PCC, vmPFC and lateral parietal cortices form anatomical hubs to which all other regions of the DMN are correlated. Peripheral nodes of the DMN include the medial temporal lobes, including the hippocampi and parahippocampal gyri, which are activated during episodic memory. The importance of these regions for construction of mental simulations supports the hypothesis that the DMN is critical for internal mentation. Indeed, a meta-analysis of 24 studies of autobiographical memories revealed regions of activations that that were remarkably similar to the DMN [383]. Interesting the medial temporal and prefrontal subsystems of the DMN do not demonstrate a high degree of intrinsic correlation with each
other, suggesting some extent of functional separation (See Buckner and colleagues for review [51]).

The nodes of the DMN are also anti-correlated to networks that are preferentially activated during task-performance, so-called task-positive networks. These primarily include attention networks (dorsal and ventral), executive control networks and the salience network [123, 126]. While, there is a mathematical bias towards negative correlations introduced by regression of the global signal [8, 282, 334], considerable evidence suggest that anti-correlations have a biological basis. For example, by using simultaneous recordings of the cat homologue of task-positive and task-negative brain regions, Popa and colleagues showed that a phase difference in LFP was present between anti-correlated regions [312]. They also found that attentional demands increased LFP power of task-positive regions, while decreased the power of task-negative areas.

The higher integrative task-positive networks have been identified across a variety of tasks [75]. One of the most robust of these, the dorsal attention network, is involved in voluntary top-down orienting and demonstrates increased activity when cues are presented to direct a subject’s attention. Important nodes of the dorsal attention network include the intraparietal sulci bilaterally as well as the bilateral frontal eye fields [122]. This system is distinguished from the right-lateralized ventral attention network (particularly the right temporal-parietal junction and right ventral frontal cortex), involved in reorienting attention in response to salient sensory stimuli [122]. The salience network, which is preferentially activated by behaviourally-salient stimuli, consists of the dorsal anterior cingulate cortex (ACC) and bilateral insulae [350]. This network, which is important for the initiation of cognitive control, is thought to be driven the right insula (a “cortical outflow hub”), which provides an early cognitive control signal [150]. The salience network is activated by conditions, which require a change in behaviour, particularly errors and signals the need for behavior adaptation [68]. In the resting-state, the salience network is dissociable from a second ICN responsible for adaptive behavior, the executive-control network linking the dorsolateral frontal and parietal neocortices [350]. Characterization of the gamut of task-positive ICNs, provides evidence for neural architectures supporting fundamental aspects of human cognition and behaviour in the resting human brain.

Functional network connectivity is constrained by the brain’s anatomical circuitry. Honey and colleagues have shown that structure-function relations exist across multiple time scales [169, 170, 171]. By simulating nonlinear neural dynamics in the macaque cortex, they found that functional hubs correspond to structural hubs over long time windows of neural activity [169]. At shorter time intervals, functional hubs exhibit much more dynamic inter-regional interactions (“functional microstates”), deviating somewhat from structural hubs, but remain linked by integral regions in prefrontal and parietal cortices. These regions are functional hubs that are have been traditionally as polysensory or multimodality [370]). Slow variations of connectivity within even shorter time-windows simulate BOLD correlation patterns seen across longer time scales.
2.4.3 Measuring Intrinsic Connectivity Networks

Local activations of discrete brain regions from BOLD-fMRI have traditionally been measured using task paradigms, namely through block and event-related designs. A block experimental design is based on the principle of maintaining cognitive engagement on a given task or in a specific state by presenting sequential stimuli that are relevant to that task/state. Epochs of such a state are alternated with contrast epochs of a different task/state [203]. Its advantages include the relatively large BOLD signal change relative to baseline [133] and powerful statistical ability to detect an task/state effect [56, 137]. Conversely, event-related fMRI designs have the advantage of evaluating transient variations in the hemodynamic response and allowing analyses related to individual responses to trials [203]. The technique permits the randomization of the order of presented conditions [329], as well as variation in the time interval between presentation (the interstimulus interval [ISI]). This may reduce the subject’s ability to predict the course of the experimental design and mitigate the habituation phenomenon [89]. Such experimental designs are also highly effective to study emotional responses to specific stimuli [50, 435] and to evaluate neuroanatomical substrates of learned behaviour over the course of the experiment [249].

As stated, however, in the absence of overt stimuli, the brain exhibits intrinsic neuronal signaling, which is thought to represent over 95% of its overall energy consumption [319]. Understanding the development, organization and impairment of spontaneous BOLD oscillations subserving ICNs has revealed a wealth of information about the human brain and its dysfunction in disease states. There are several methods that have been introduced to measure temporal coherence of BOLD signals between brain regions at rest. The current section reviews the analysis of resting-state fMRI data, which will be performed subsequently, from the preprocessing of raw fMRI data to the derivation of statistical parametric maps.

Data Preprocessing  Prior to the analysis of any fMRI experiment, data are preprocessed for correction of artifacts and spatial normalization. The first correction is typically for slice-time acquisition. Since the interslice time difference is equal to twice the echo time (TE; ~40ms), the last slice acquired in a given brain volume is temporally later than the first. Functional MRI analysis is, however, based on the premise that all slices in a given brain volume are acquired simultaneously and interpolation techniques are used to approximate the slice timing. The second correction is for subject motion, which is achieved by coregistering image volumes using a rigid-body process to a target image, usually the first volume. Motion time courses can then be derived from the coregistration process and used as predictors in the statistical (general linear) modeling. Noise may also be introduced in the fMRI study from low-frequency drifts from various nuisance sources, including physiological functions (respiration and heartbeat) or variations in scanner performance over the imaging period. Time courses for cerebrospinal fluid (CSF) and white matter (WM) may also be extracted and included in a general linear model. A high-pass filter is typically applied to remove low-frequency content from the Fourier transform
of the signal, followed by a reverse Fourier transform to transform the filtered signal back to the time domain.

The individual subject images may then be transformed to a common standard space. In the current dissertation, the MNI152 common reference (Montreal Neurological Institute, Montreal, Canada) is used exclusively as a common template for all data. Normalization to standard stereotactic space is performed by aligning the high resolution T1-weighted anatomical images of each subject to the anterior commissure-posterior commissure (AC-PC) and inter-hemispheric lines, and transforming the functional data (which is registered to the anatomical data) using the same matrix. Finally, a Gaussian filter is applied to smooth the data prior to analysis to increase signal-to-noise ratio and accommodate inter-subject anatomical variation. The resolution of the filter is expressed in terms of the full width at half maximum (FWHM) of the Gaussian distribution. Statistical parameter maps (SPMs) of brain activation and/or functional connectivity may then be calculated on the preprocessed data.

Hypothesis-Driven Approaches (Seed-Based Analyses) A common practice is to identify a brain region of interest and perform seed-based whole brain connectivity analysis. This approach involves extracting the mean time series of the seed, and correlating it with the time series of all other voxels in the brain. The general linear model (GLM, shown in Equation 2.14) is generally applied, where the signal $Y$ in each voxel is modeled on $N$ predictors, $X_N$.

$$Y = B_0 + B_1X_1 + B_2X_2 + \ldots + B_NX_N + E \tag{2.14}$$

Where $B_0$ is the intercept, $B_N$ is the regression coefficient for each predictor $N$, and $E$ is the residual error term. GLM may be applied to task-related fMRI designs (such as block and event-related designs), in which case, the explanatory variable of interest is the task condition or event. Conversely, in resting-state data, the predictors are mean time series of spontaneous BOLD oscillations of the seed region-of-interest (ROI). In a higher-level analysis, a separate set of predictors that define group classification or subject-specific variables allows for inter-individual comparisons, group analyses or subgroup analyses. The time series of nuisance signals, such as those arising from white matter and cerebrospinal fluid are also removed prior to, or during general linear modeling. Non-neuronal physiological noise may also be removed, but its presence does not significantly impact the spatial distribution of networks [31, 353]. This approach is hypothesis-driven in that it requires an a priori hypothesis regarding the connectivity of given brain regions and a pre-defined ROI.

Data-Driven Approaches (Independent Component Analyses) Hypothesis-driven analyses are agnostic to unforeseen patterns of intrinsic correlations within the data. A second, data-driven method that may be useful in the analysis of resting-state data involves independent component analysis (ICA) [20]. This approach involves decomposing the data into statistically independent components, resulting in maps that combine correlated regions into a single com-
ponent. ICA does not require a priori hypotheses and can typically separate nuisance signals into independent components. Assignment of components to known networks may be subsequently performed by visual inspection or comparison of components to network templates. Interestingly, the results derived from ICA and seed-based approaches are often comparable [20, 144, 350].

**Multiple Comparisons** An important consideration in the analysis of fMRI data pertains to multiple comparisons. Given that a brain volume may contain well over 10,000 voxels, depending on the sequence and scanner protocols, a high number of false positive findings are expected by chance alone. Excessively stringent methods for correction in fMRI include the Bonferroni adjustment [246]. Other approaches may take advantage of spatial relationships in statistical parameter maps. Since voxels are highly dependent (i.e. voxels that are adjacent to each other are must more likely to be highly correlated), cluster-based thresholding may be applied. This method capitalizes on this dependence in that spatially extended activations are less likely than spatially restricted activations to be spurious findings. Such methods smooth the statistical parametric maps and identify suprathreshold voxels and calculate the significance of each cluster of contiguous voxels based on its size using Gaussian field theory or permutation testing [359]. One limitation of this technique, however is the need to define an arbitrary threshold, above which the voxels within statistical parametric maps are considered significant. Emerging methods such as threshold-free cluster enhancement method [360] may, however, circumvent this challenge by augmenting the height of spatially distributed signals without altering the location of their maxima.

**2.4.4 ICNs in the Developing Brain**

With task-based fMRI, it has been shown that with development, functional activation becomes more localizable to specific functionally-specialized brain regions [102]. Similarly, spontaneous BOLD oscillatory activity exhibits maturation with development. It has been established that dissociable ICNs are evident even in infants [128]. At such young ages, cortical hubs are restricted to primary sensory and motor regions [127], with the maturation of higher integrative ICN nodes as hub regions with age [95]. As will be discussed in more detail in Chapter 3 on page 49, typical development is characterized by increases in the strength of long-range connections between brain regions associated with the same ICN, together with increasing segregation of cortical and subcortical structures associated with different ICNs [95]. These connectivity gradients reflect patterns of gray matter growth and subsequent synaptic pruning with age. Disruption of the organization of ICNs is an emerging area of interest in the study of neurodevelopmental conditions [392, 420].
2.4.5 ICNs in Patients with Epilepsy

Impairments in TLE and Generalized Epilepsies Various studies have also recently emerged to describe impairments in ICNs in different patient populations with epilepsy. The most commonly investigated group of patients is adults with temporal lobe epilepsy (TLE). In general, these individuals possess a decrease in functional connectivity within the DMN and between the DMN and epileptogenic regions [263, 234, 252, 307]. In some studies, a correlation was identified between decreased functional connectivity and epilepsy duration, suggesting that prolonged illness may exert a detrimental effect on cognitively-relevant networks [252]. Decreased connectivity is typically lateralized to the epileptogenic hemisphere, and contralateral compensatory mechanisms may develop [263, 27, 310, 277]. For example, McCormick and colleagues determined that connectivity between the posterior cingulate cortex and epileptogenic and non-epileptogenic hippocampi were decreased and increased, respectively [263]. The extent of contralateral compensation in this study was associated with improved post-surgical memory outcome.

Studies of patients with generalized epilepsies have also demonstrated reduced functional connectivity, as well as increased interhemispheric resting-state connectivity [13]. Different patient phenotypes were also established on the basis of fMRI statistical maps. For example, Carney and colleagues demonstrated that patients with absence seizures may be grouped into different populations based on the activation and deactivation of the dorsolateral prefrontal cortex [67]. The former had greater activations in more widespread cortical and subcortical networks, suggesting a phenotypic and biological difference between the two. Studies in these patients have also demonstrated extensive involvement of cortical and subcortical (thalamic) circuitry [223, 385]. Patients with absence seizures were found to have decreased connectivity in the thalamus and basal ganglia and increases in the medial occipital cortex [258], as well as decreased functional connectivity with resting-state attention networks [210].

Impairments in Children with Localization-Related Epilepsy The number of studies that have characterized ICN network impairments in children with localization-related epilepsy is limited and none have evaluated the effect of epilepsy on the developmental trajectory of these intrinsic networks. Using ICA, it was found that the DMN component in affected children was less functionally connected than controls, although this was not significantly correlated with clinical features, including epilepsy duration and intelligence quotients [432]. Pair-wise combinations of ICA maps from children with frontal lobe epilepsy also revealed altered (both reduced and increased) functional connectivity within numerous ICNs compared to controls [431]. This study also identified both increases and decreases in the functional connectivity between different pairs of independent components representing combinations of task-positive and task-negative networks. Children with frontal lobe epilepsy also demonstrated an increase in the number of functionally isolated and dissociable brain modules within the frontal lobe compared to controls, suggesting that subnetworks are less inter-connected in the patient population [397].
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children with temporal lobe epilepsy, again ICA demonstrated reduced functional connectivity compared to controls [253]. Interestingly, children with abnormal EEGs (the presence of spikes or sharp and/or slow waves on EEG performed outside of the scanner) had greater alterations in connectivity, particularly involving the thalamus, compared to those who did not.

The Role of Interictal Epileptiform Discharges  While the electrophysiological basis for decreased functional connectivity in epilepsy is not understood, they may be partially attributable to the presence of interictal discharges (IEDs). Simultaneous EEG-fMRI has allowed multimodal mapping to study network changes during IEDs. Event-related simultaneous EEG-fMRI studies have shown that lesional cortex may demonstrate BOLD activations or deactivations during IEDs, the latter being more common in children [191, 215]. Cortex demonstrating IED-related hemodynamic BOLD changes is associated with epileptic networks, and resection of regions showing hemodynamic changes has been associated with improved post-surgical outcomes [390]. These studies have also shown that the thalamus plays a central role in propagation of spikes, particularly of bilateral origin [3].

It has long been suspected that IEDs may be partially responsible for impaired neurocognitive function in patients with epilepsy and the interaction between IEDs and cognitively-salient networks such as the DMN are an area of increasing scientific inquiry. This is particularly important, since electric fields generated during interictal discharges are strong enough in intensity to influence action potential firing threshold and synchronization [147]. Several studies have found that spike-related decreases in DMN activation were evident, which are perhaps more conspicuous in temporal lobe epilepsy compared to extra-temporal epilepsy [214, 228]. It should be emphasized, however, that DMN deactivations were also found to accompany generalized spike and wave discharges (GSWD) or focal interictal discharges of temporal, frontal and posterior quadrant origins [107]. In patients with idiopathic generalized epilepsy, thalamocortical activation and suspension of regions of the default mode network were hypothesized to contribute to reduced responsiveness during GSWDs [140]. Discharges with slow waves on EEG were more likely to cause DMN deactivation than those lacking slow waves [214]; however, abnormalities in network organization were also reported in periods without interictal discharges, and therefore, these network impairments cannot be exclusively responsible for impairments in ICNs [252].

The neural correlates of IED impairment of the DMN are similarly elusive. Kobayashi and colleagues found that DMN deactivations were negatively correlated with the power in the alpha band in the EEG, providing some insight into the electrophysiological underpinnings of IED-DMN interactions [214]. Very recently, data from combined fMRI-intracranial EEG revealed that runs of IEDs and short electroencephalographic seizures were associated with decreased gamma power and increases in the power of lower frequency oscillations [108]. As will be discussed in Section 3.5.1 on page 66, further multi-modality studies combining BOLD recordings and direct neural oscillations are needed to characterize these relationships.
2.4.6 The Relationship Between Spontaneous BOLD Oscillations and Direct Neural Oscillations

Section 2.4.1 on page 30 provided an overview of the neurophysiological processes that underlie the hemodynamic response, and thus, the BOLD signal. There is however ongoing uncertainty regarding the electrophysiological signatures that underlie coherence in low-frequency BOLD fluctuations that form the hemodynamic footprint of ICNs. The majority of studies thus-far have attempted to study the correlation between EEG and resting-state BOLD signal [275, 339, 229, 230]. These investigations suggested that specific EEG bands provided signatures of activity within specific networks. With alpha EEG power increases, BOLD connectivity decreased within visual and occipital networks [339, 138, 111] and beta EEG activity (17-23 Hz) was positively correlated with fMRI signal changes in the posterior cingulate, precuneus and dorsomedial prefrontal cortex and temporoparietal junction [230]. Power fluctuations at higher beta bands (24-30 Hz) were also positively correlated with BOLD signal in the anterior cingulate and negatively correlated with signal in retrosplenial (posterior cingulate), temporoparietal, and prefrontal areas. In the default mode network, it was found that theta power showed negative correlations with BOLD signal, whereas beta and gamma activity showed significant positive correlations to BOLD activity [268].

Several recent studies also attempted to use electrocorticography to understand the the electrophysiological underpinnings of ICNs. Recordings from the posterior cingulate cortex and medial prefrontal cortex showed gamma-band power suppression during task engagement, as would be expected in the DMN [199]. Furthermore, the regions within the posteromedial cortex showed high broadband gamma activity (30-180 Hz) during rest, which was attenuated by attention demanding tasks [83]. He and colleagues have also shown that while gamma power demonstrates a similar correlation structure to spontaneous BOLD fluctuations during wakefulness and REM sleep, slow cortical potentials (fluctuations in electrophysiological signals at 0.01-0.1 Hz) exhibit similar patterns during wakefulness, slow-wave sleep and REM sleep [155]. Such slow cortical potentials are an attractive putative electrophysiological correlate of spontaneous BOLD fluctuations, since they occupy the same timescale. Alternatively, temporal variations in band-limited power (BLP; the power of envelope modulation of a relatively narrow range of LFP frequencies) over seconds and minutes have also been studied as electrophysiological correlates of spontaneous BOLD signal. Gamma BLP from depth electrodes in epileptic patients demonstrated significant interhemispheric correlations, suggesting that slow spontaneous modulations in firing rate and gamma LFP may be correlates of spontaneous BOLD fluctuations [291]. More recently, the idea that cross-frequency phase-amplitude coupling (discussed in Section 2.2.3 on page 12) may be related to underlying BOLD signals has also gained attention [121].

Another line of investigations involved neuromagnetic recordings with magnetoencephalography (MEG). BLP was also shown to demonstrate significant interhemispheric correlations, as would be expected from spontaneous fluctuations in BOLD signal across multiple frequencies [238]. Source space localization in MEG demonstrates long range temporal correlation between
spontaneous BLP signals [160, 48]. Correlations of BLP were strongest in these studies across alpha and beta frequency ranges, depending on the underlying oscillation frequency. Further MEG research also employed spontaneous data to derive the spatial structure of several MEG networks based on BLP [84, 49, 85]. These studies again demonstrated that ICNs are best captured by fluctuations in theta, alpha and beta oscillations, although this may be partly attributable to the increased signal-to-noise ratio at these frequencies. Furthermore, independent component analysis of BLP signals from MEG revealed a stronger spatial pattern of correlation to BOLD fMRI networks compared to matched surrogate data [49].

Perhaps the most compelling evidence is derived from non-human primates where fMRI data was simultaneously acquired with direct neural recordings. These studies revealed that BLP signals from LFPs possess large amplitude fluctuations over very long time-scales (>10s) and that these fluctuations are coherent between recording sites during various behavioural states, including at rest [233]. Interestingly, slow, shared fluctuations in gamma-power were most coherent over long cortical distances, which is contrasted with spatially restricted coherence of the raw gamma voltage signals. Furthermore, BOLD signal fluctuations were often correlated with these spontaneous neuronal oscillations in BLP, which occur over a similar timescale. BOLD fluctuations reportedly showed significant correlations with slow fluctuations in LFP activity and fMRI signal both within close proximity to the recording electrode and within distant brain regions. Interestingly, the global component of fMRI BOLD fluctuations is also tightly coupled to with slow modulation of neural events by high and low frequency LFPs [346]. It was proposed that BOLD signals may lag behind neuronal oscillations by a physiological lag for the hemodynamic response [352]. However, the findings of this report were questioned by methodological concerns regarding whether spontaneous activity was indeed recorded from the primates [247].

2.5 Network Analysis and Graph Theory

After measuring functional connectivity amongst a group of oscillating brain regions (be it through direct or indirect measures of neural activity), network analysis may be applied to gain unique insights into their functional organization and architecture. While the investigation of neural oscillations and their synchrony has been established for some time, the application of network analysis to these studies is gaining increasing attention as it offers a framework within which to characterize the organization of brain networks. Network analysis, which is based on graph theory, is an emerging tool for the study of complex systems, and is applied to the study of functional connectivity throughout the dissertation. The term "complex" implies a process defined by perpetual change and characterized by nonlinear relationships among different components and with respect to historical dependence [58]. Neural networks are the prime example of complex systems and network theory has been increasingly applied to characterize their dynamics [332]. Network analysis has specifically shown promise in elucidating abnormal
patterns of cortical connectivity in children with epilepsy [179, 397, 434]. The approach involves decomposing complex interactions into mathematical structures (graphs), which may be used to model system relations and process dynamics. Evaluating the topology of these graphs provides informative data on the structure and organization of networks.

The current section will demonstrate the application of network analysis and graph theory by calculating various metrics for a network comprised of different combinations of antiepileptic drugs (AEDs) administered to children included in the subsequent chapters. AED polytherapy in children with epilepsy may be considered a complex system given the numerous combinations of medications that are often coadministered and changed due to clinical indications. The complex system created by the changing combinations of AEDs creates a conceptual challenge for understanding drug use in this patient population. Traditional inferential statistical approaches lack the discriminatory power to consider the many combinations that may exist as very large sample sizes are required to evaluate patterns of drug administration. The success of network analysis in this regard is a reflection of the fact that graph theory provides a mathematical representation of data, rather than a statistical description of relationships. Furthermore, network analysis may uniquely capture dynamic processes and characterize the value of individual components (i.e. AEDs) within the system (i.e. polytherapy combinations).

The Graph: A Mathematical Representation  The fundamental mathematical relations in network analysis are contained in graphs, which are structures that describe the connectivity among elements within a system. Graphs contain nodes, which are the elements of interest as well as edges, which are pairwise relationships between any two nodes. The latter may be binary or weighted and directed or undirected. Edge weights may represent a variety of indices. For example, in oscillatory neural networks defined by quantitative electroencephalography, phase synchrony indices (such as phase-locking values or the phase lag index) may be appropriate to quantify the relation between two sensors (See Section 2.3.3 on page 26). Alternatively, the correlation coefficient of BOLD signals between two brain regions revealed by functional magnetic resonance imaging may be an appropriate edge weight.

In the current example, each AED may be viewed as a node (or vertex) within a complex system defined by a network graph containing 15 nodes (the total number of unique AEDs). First undirected binary graphs of the children’s current AED regimens may be constructed by assigning an edge (i.e. connection) to medications that were coadministered. Binary connectivity matrices may then created to describe the network structure of the AED administration.

Adjacency Matrices and Selected Graph Topologies  An adjacency matrix can thereafter be constructed from the graph. This structure describes which nodes of a graph are adjacent to other nodes. A graph with \( n \) vertices (or nodes) will result in an \( n \times n \) adjacency matrix. In the current example, 15 unique AEDs were prescribed, and therefore, a 15 element \( \times 15 \) element adjacency matrix was plotted showing the AEDs as rows and columns, where the elements of the matrix describe connections between different AEDs.
Graph theoretical properties can subsequently be derived from mathematical relationships in adjacency matrices. Graph properties may describe either the network itself or individual components (nodes) within the network. Numerous graph measures have been previously described in detail [332]. The current section will focus on measures germane to the topics of the dissertation, including: degree, clustering coefficient and betweenness centrality. Degree is, simply, the number of edges that connect with a certain node. The clustering coefficient is calculated by dividing the number of connections between a node and its neighbors by the number of possible connections between them. Conceptually, it is a measure of the number of closed triads surrounding a given node. A high clustering coefficient indicates that the neighbors of a node are also often directly connected (therefore, clustered).

The mean of clustering coefficient for all nodes within the network, $C$, describes the network clustering coefficient. Networks with “small-world” properties are characterized by increases in clustering coefficient and decreases in characteristic path length, $L$. The latter describes the average number of steps along the shortest paths for all possible pairs of network nodes. Small-world networks are specific types of graphs whereby most nodes can be reached from every other node by a small number of steps. A network with small world properties has the characteristics: $C >> C_r$ and $L \geq L_r$, where $C_r$ and $L_r$ describe the clustering coefficient and characteristic path length for random networks with the same number of nodes and edges as the original graph [421]. Small-worldness is a common feature of numerous networks, including social, transportation, and neural networks.

Betweenness centrality is a metric that facilitates the identification of hubs within a network. Nodes that are considered to be hubs play a disproportionately important role within a given network. Betweenness centrality can be described as:

$$C_b(i) = \sum_{j < k} \frac{g_{jk}(i)}{g_{jk}}$$

(2.15)

In Equation 2.15, $g_{jk}$ is the total number of shortest paths from nodes $j$ and $k$ and $g_{jk}(i)$ is the number of those paths that pass through node $i$. The higher the betweenness centrality, the more shortest paths must pass through that node (i.e. the more of a hub it is within a network). Nodes with high betweenness centrality can be viewed as those that are at the intersection of many short paths, meaning in the current example that the AED has the potential to participate in multiple different AED combinations. Furthermore, communities (subnetworks) may be identified within the graph by defining unique modules [72, 71], as shown in Equation 2.16.

$$Q_m^w = \sum_{s=1}^{m} \left[ \frac{W_s}{W_{total}} - \left( \frac{d_{w,s}}{2W_{total}} \right)^2 \right]$$

(2.16)

The modularity $Q$ quantifies the degree to which the network may be decomposed into non-
overlapping communities. In Equation 2.16, \( W_s \) is the sum of weights of all links in the module \( s \). \( W_{\text{total}} \) is the total sum of all weights in the network and \( d_{w,s} \) is the sum of the weighted degrees of the vertices in \( s \). Modularity optimizes the number of observed links relative to the number of edges expected by chance; therefore a random network would have \( Q = 0 \).

Of note, binary graphs with directed edges describing the sequence of AEDs attempted and discontinued may also be created. The in-degree and out-degree of each node (i.e. AED) may be determined, quantifying the extent to which it has been initiated and discontinued respectively. The former refers to the number of edges that are oriented towards a node, while the latter refers to the number of edges that oriented away from the node. The analyses for the following applications of network analysis were performed in MATLAB (The MathWorks, Natick, MA, USA) and Gephi [19].

**Network Analysis of Antiepileptic Drug Polytherapy in Children with Medically-Intractable Localization-Related Epilepsy** To demonstrate the application of network analysis, data were collected on fifteen unique AEDs (carbamazepine, carnitior, clobazam, clonazepam, gabapentin, lacosamide, lamotrigine, levetiracetam, oxcarbazepine, phenobarbital, phenytin, rufinamide, topiramate, valproate, vigabatrin) that were administered to 27 children. The median number of AED on which children were currently placed was 2 (range 1-6). Binary undirected graphs were created of the children’s current AEDs (Figure 2.1). The AED with the greatest degree (i.e. the agent most often co-administered for anticonvulsive therapy) was levetiracetam \( (k = 7) \) followed by clobazam \( (k = 6) \), lamotrigine \( (k = 4) \), carbamazepine \( (k = 4) \), oxcarbazepine \( (k = 4) \), topiramate \( (k = 3) \) and valproate \( (k = 3) \). Gabapentin and lacosamide had degrees less than 2. The AEDs with the largest clustering coefficient were gabapentin and topiramate \( (c = 1) \). This indicates that the medications were never prescribed in isolation.
Betweenness centrality, which is the ratio of the total number of shortest paths (AED combinations) that pass through a given node (a given AED) was greatest for levetiracetam ($C_k = 14$), suggesting that this drug plays a central role in anticonvulsive regimens administered to children with medically-intractable epilepsy that were included in the analysis. This was followed by oxcarbazepine ($C_k = 8$), clobazam ($C_k = 6.8$), valproate ($C_k = 2.2$) and lamotrigine ($C_k = 1.7$).

The AEDs connectivity networks were subdivided into non-overlapping modules, which resulted in the identification of three subnetworks consisting of AEDs that were often co-administered. The first module was comprised of lamotrigine, topiramate, clobazam and gabapentin. The second was comprised of oxcarbazepine and lacosamide and the third, carbamazepine, levetiracetam and valproate.

Binary directed networks were then created, including AEDs that had been previously discontinued to characterize causal relationships in the administration of AEDs. As these edges have a directional component, the in-degree and the out-degree of each AED were quantified. AEDs with high in-degree are those that are more likely to be initiated, whereas those with high out-degree are those that are more likely to be discontinued (Figure 2.2). Lacosamide ($k_{in} = 7$) and topiramate ($k_{in} = 7$) possessed the highest in-degrees, whereas carbamazepine ($k_{out} = 9$), phenobarbital ($k_{out} = 8$) and phenytoin ($k_{out} = 6$) possessed high out-degrees. Interestingly, levetiracetam possessed both high in- and out-degrees (6 and 7, respectively), suggesting that it is both commonly initiated and discontinued.
Figure 2.2: Directed graphs of current and previous AED usage in children with medically-intractable localization-related epilepsy. Nodes are colored by (A) in-degree and (B) out-degree, reflecting chronological sequence of AED trial. Node and text size reflects total node degree.

Through the illustrative example of a network formed by AED polytherapy, salient aspects of network analysis are highlighted as a robust method to characterize and quantify complex systems. Numerous relations, indexed by a variety of functional connectivity measures may be modeled to explore valuable topographic features of graphs and elucidate their structural and architectural dynamics. That is, functional relationships may be deconstructed into a mathematical model, and the dynamic interrelationships between the individual components may be quantified.

2.6 Hypotheses

2.6.1 General Hypothesis

The general hypothesis of this dissertation is that medically-intractable localization-related epilepsy is associated with reorganization of neural networks that may be identified by measuring direct and indirect oscillatory activity. Disorganization of oscillatory activity in the brain is associated with clinical features of epilepsy and is specifically associated with the comorbidities of chronic epilepsy, including the cognitive and neurological deficits that are exhibited by affected children. It is hypothesized that abnormal patterns of oscillatory activity are conspicuous within epileptogenic brain regions. The measurement of functional connectivity within these regions across different temporal windows will provide insight into pathological underlying neural communication, supplying novel avenues for future therapeutics and mapping strategies. The
convergence of abnormal brain development, disorganized synchrony of oscillatory activity and impaired network functional connectivity within cortical regions in proximity to and distant from the epileptogenic regions serves to demonstrate that these phenomena are tightly linked and crucial to the study of childhood epilepsy.

2.6.2 Specific Hypotheses

Specific Hypothesis H1: Disorganized Oscillatory Network Functional Connectivity is Associated with Chronic Co-Morbidities in Children with Localization-Related Epilepsy

Sub-Hypothesis H1.1 The development of brain’s intrinsic connectivity networks (ICNs), as measured using resting-state fMRI, is affected by localization-related epilepsy. The typical developmental processes of network segregation and integration are impaired and this impairment is related to the burden of epilepsy. Furthermore, specific network impairments correlate with heterogeneity in the patient population, particularly clinical phenotypes such as the location of the seizure focus, seizure semiology and neuropsychological outcomes. Measurement of network connectivity in ICNs may, therefore, serve as a biomarker of epilepsy severity and potentially the success of future medical and surgical treatments.

Sub-Hypothesis H1.2 Both ictal and interictal events affect the development of oscillatory networks in the brain. Using a combined fMRI-MEG approach to capitalize on the spatial and temporal resolution of the two modalities, respectively, it can be shown that interictal epileptiform discharges (IEDs) affect the network topology of ICNs. The extent of vulnerability or conversely, resilience of ICNs to IED, is related to neurocognitive outcomes. Disorganized functional connectivity as a result of ictal events also contributes to the chronic morbidity of epilepsy. By studying ECoG recordings from the motor network during seizures, it can be shown that the extent of ictal disruption of cortical functional connectivity is related to neurological deficits in children.

Specific Hypothesis H2: Disorganized Oscillatory Network Functional Connectivity is Involved in Ictogenesis within Epileptogenic Brain Regions, Allowing the Mapping and Study of Epileptic Dynamics

Sub-Hypothesis H2.1 The hierarchical process that regulates neural communication, cross-frequency phase-amplitude coupling, is disrupted in epileptogenic cortex. pHFOs may be decoupled from the regulatory control of slower oscillations at specific times during seizure initiation and propagation and conversely, the restoration of hierarchical control is involved in seizure termination. The study of the disruption of hierarchical regulation may be useful for mapping brain regions involved in the epileptogenic zone and understanding seizure dynamics.
Sub-Hypothesis H2.2  The extent of disruption of inter-regional communication within epileptogenic networks is associated with the expression of pHFOs, which is correlated with functional isolation of the seizure-onset zone. Furthermore, changes in network topology over the course of seizure activity may provide benefit in mapping epileptogenic cortex.
Part I

Epilepsy and the Brain’s Developing Networks
Chapter 3

Intrinsic Connectivity Networks in Children with LRE

The findings presented in this chapter have been submitted for publication as follows:


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(a) American Association of Neurological Surgeons Pediatric Section (Toronto ON, December 3-6, 2013)

(b) Society for Neuroscience (San Diego, California, USA, November 9-13, 2013)

Contributions: GMI (conception/design of study, drafting/revising article, analysis/interpretation of data, final approval); BRM, WL, FW, CAB (design of study, analysis/interpretation of data, final approval); MLS, EJD, PF, MJT, SMD, JTR, OCS (conception/design of study, analysis/interpretation of data, final approval)

3.1 Introduction and Background

Distributed brain networks demonstrate synchronized low-frequency fluctuations in blood oxygen level-dependent (BOLD) signals at rest [32, 318, 320] that are correlated with underlying structural connectivity patterns [145, 399]. These fluctuations of BOLD signal may reflect large-scale coherence of neurophysiological oscillations [48, 49, 84, 291] and/or the cross-frequency coupling of oscillations among cortical regions [121]. Such intrinsic connectivity networks (ICNs) are comprised of groups of brain regions exhibiting temporally correlated activity. Certain ICNs may be relatively quiescent during task performance, whereas other ICNs may express task-dependent increases in connectivity. The former includes the default mode network (DMN) [318], which may support self-referential thought, internally-directed processing and mind-wandering
Task-negative networks are also anti-correlated with regions that demonstrate increased activity during attention-demanding tasks [123], such as the dorsal and ventral attention networks [122], as well as the cingulo-insular or salience network [350].

Typical development is characterized by increases in the strength of long-range connections between brain regions associated with the same ICN, together with increasing segregation of cortical and subcortical structures associated with different ICNs [95]. It has been shown previously that “connectivity gradients” between correlated and anti-correlated regions in major hubs of ICNs become stronger during adolescence and early adulthood with sharpening of the boundaries of the DMN and decreased correlation between the DMN and attention control networks [9]. The fractionation of brain regions into specific regional hubs also represents a transition from a local to distributed network organization, which is a hallmark of typical development [110]. This intra-network integration and inter-network segregation contributes to the formation of mature distributed, functionally-specialized networks seen in adults. Disruption of normative ICN organization is associated with numerous neurological and neuropsychiatric conditions, including neurodevelopmental disorders [392, 420].

Childhood epilepsy has long been known to interfere with typical childhood development [206]. Whereas the adult-onset equivalent may result in a loss or interference with previously acquired function, epilepsy in children may manifest as a failure to progress along the expected developmental trajectory [216]. Furthermore, in contrast to adults, focal seizures in children may result in less specific and more global impairments [158, 357, 358], implicating dysfunction of the development of large-scale networks as a putative underlying process. Knowledge is limited, however, whether or to what extent childhood epilepsy affects the development of ICNs. Here, it was hypothesized that the cumulative effects of prolonged, intractable epilepsy adversely interferes with the development of ICNs, and that disruption of ICN development would be associated with poor neuropsychological outcomes in children with epilepsy (Hypothesis H1.1, as stated in Section 2.6 on page 45). This study investigates whether differing childhood epilepsy phenotypes (seizure semiology, epilepsy duration and epileptic focus location) are associated with impairments in specific ICNs. To test these hypotheses, whole brain graph theoretical analysis of resting-state functional magnetic resonance imaging (fMRI) were performed to identify brain regions that demonstrate atypical development in children with epilepsy. The functional connectivity of specific ICNs that were identified as atypical was subsequently interrogated through seed-based analyses. The organizational structure of ICNs in the children studied were further characterized through agglomerative hierarchical clustering. Network disturbances were then correlated with the children’s neuropsychological function. These findings provide a first demonstration of the effects of epilepsy on the brain’s developing ICNs, emphasize the importance of early surgical treatment to mitigate these effects, and following validation, may provide a benchmark to assess the efficacy of therapeutic interventions.
3.2 Methodology

3.2.1 Study Population

Forty-seven children with localization related-epilepsy were recruited into the study from two separate institutions (Hospital for Children, Toronto, Canada and the Foothills Medical Centre, Calgary, Canada). Of these, 26 children met the stringent inclusion criteria (refer to Appendix 1 (Chapter 9 on page 150) for details of subject demographics). The children ranged in age from 8-17 years with mean epilepsy duration (± standard deviation) of 4.34±3.54 years. Eleven children (42%) had epileptic foci localized to the temporal lobe, whereas 15 children (58%) had extra-temporal epilepsy and 14 children (54%) presented with secondarily generalized seizures.

Control fMRI data were collected from a large cohort of typically developing children who underwent similar acquisitions at The Hospital for Sick Children. To identify appropriate controls, propensity score matching was performed [331] using a multivariate logistic regression with group classification (epilepsy versus controls) as the dependent variable. This approach balances selected covariates (age and sex) between the two study cohorts. Propensity score matching was performed using R statistical software [162, 163].

3.2.2 Resting-state fMRI Acquisition and Pre-Processing

Structural and functional MRI data were collected using 3T scanners. Continuous fMRI data were collected, during which subjects were instructed to focus on a projected fixation cross. The protocols and sequences used are presented in Appendix 1 (Chapter 8). Several validation studies were performed to ensure that these findings were not attributable to differences in scanners or protocols (presented in Appendix 1). The research was approved by the Hospital for Sick Children and the University of Calgary Research Ethics Boards.

FMRI data were preprocessed using standard AFNI and FMRIB Software Library (FSL) tools, as discussed in Section 2.4.3 on page 34. Slice-timing and motion correction were performed before aligning the data to the MNI152 T1 2 mm atlas via the subject’s high-resolution anatomical T1-weighted images. Subjects with maximum head displacement greater than 2 mm from a reference volume for more than one-third of their volumes or those with a total mean head displacement greater than 2 mm were excluded from the analysis. Of the 47 children with epilepsy initially recruited, 8 were excluded because of excessive motion parameters (see Supplementary Figure 8.1 on page 135 for details). The mean head displacement for all subjects included in the analysis was less than 1 mm for both children with epilepsy and propensity-matched controls. Next, data underwent spatial smoothing using a 6.6 mm FWHM Gaussian kernel and was bandpass filtered with a lower and upper cut-off frequency of 0.01 Hz and 0.2 Hz respectively. BOLD signal contributions from white matter and cerebrospinal fluid and motion were regressed from the BOLD signal.

It has been postulated that task-negative networks (such as the DMN) are anti-correlated with task-positive networks [123, 126]. Such anti-correlations have a physiological basis as neu-
rornal anti-correlations tend to correlate with BOLD anti-correlations [207]. A body of literature, however, also suggests that this may be an artifact as a result of global signal regression during pre-processing, as it introduces greater anti-correlations in the data [8, 282, 334]. Since regression of global variations in BOLD signal is advantageous in that it may reduce spurious correlations among brain regions [424], in the current study, all analyses were performed with and without regression of the global signal and found robust and reliable differences using both preprocessing pipelines. The figures presented in this article show data preprocessed using whole brain signal regression.

3.2.3 Graph Theoretical Analysis of Whole Brain Connectomes

To identify and subsequently compare resting-state hubs in children with localization-related epilepsy and typically developing controls, a whole brain connectome was defined based on synchronized low-frequency BOLD oscillations for each subject. Graph theoretical analysis was then performed to characterize and quantify each connectome's global organization (See Section 2.5 on page 40). This approach is increasingly considered advantageous over other analytical methods to provide insight into functional topographic reconfiguration of the brain in response to various physiological and pathological conditions (refer to Wang et al. for review [415]).

Whole brain connectomes were created for each individual subject by generating individual voxel-based graphs, where each voxel represents a node. Each functional connection, indexed by the square of the Pearson correlation coefficient between the time series of any voxel pair was defined as an edge (Figure 3.1 on page 53). All voxels within a functional brain mask for each child were included in the analyses. The voxel-based graph is a mathematical representation of the whole brain resting-state connectome, from which network topologies may be inferred [450]. A threshold was applied to the adjacency matrix for each subject to an Erdős-Rényi entropy (S) of 2.0 (Watts and Strogatz. 1998), which ensures that the small-world features of the network were consistent across all subjects [136]. Of note, for both children with epilepsy and propensity-matched controls, minimal thresholding was required to reach an S-value of 2.0. For both groups, over 90% of connections were included.
Figure 3.1: Graph theoretical analysis of whole brain connectome.

From the connectivity matrix, eigenvector centrality (EC) was calculated for each node using the Brain Connectivity Toolbox [332]. EC is an index of the extent to which a given region represents a hub within a network. This metric was chosen for several reasons. First, this topological measure directly evaluates a node’s importance within a graph by assigning relative scores to nodes based on the principle that connections to high scoring nodes contribute more to the score of a given node than equal connections to low scoring nodes. Functional connections to hub regions within a given network may therefore be prioritized while rejecting functional connections to non-hubs. Second, previous studies have demonstrated that EC is a robust measure to index changes in functional brain connectivity [136]. A final advantage of EC over other metrics that may identify brain hubs (such as betweenness or closeness centrality) is that it is computationally less expensive, allowing analysis of a large number of brain voxels. Once the EC maps were created, they were registered to the MNI152 template. Differences in hub-like topographies between children with medically-intractable epilepsy and normal controls as well as between different subgroups of children with epilepsy were evaluated using a non-parametric randomization algorithm (FSL randomise) [289]. Significance thresholds for cluster-based statistics were determined using 3dClusterSim from the AFNI toolbox. Coding was performed using custom scripts written in-house in MATLAB.
3.2.4 Seed-Based Functional Connectivity Analysis

To evaluate brain regions to which identified hub regions were connected, seed-based connectivity analysis was subsequently performed (See Section 2.4.3 on page 34). Seed regions were chosen from selected ICNs that possessed differential hub properties in the previous whole brain connectome analysis. For example, if significant group differences in EC were identified in the posterior cingulate cortex (PCC), central nodes of the DMN were seeded. In order to account for inter-subject variability in assigning seed points for connectivity analysis, a 15 mm spherical region of interest (ROI) was defined and centred upon published coordinates based on a systematic review of literature [46]. An iterative algorithm adapted from prior work [139] was then employed whereby the ROI was eroded within each subject’s functional space until an internal correlation of 0.7 was achieved. This method is advantageous as it may account for minor differences in the organization of intrinsic resting state networks [139]. A detailed explanation of this method and inter-subject variability in centroid locations of eroded ROIs is presented in Appendix 1 (Table 8.4 on page 144).

First-level analysis was performed by correlating the mean time series of the eroded ROIs with the time series of all voxels in the brain. This analysis was performed using FEAT (FMRI Expert Analysis Tool) Version 5.98, part of FSL (FMRIB’s Software Library, www.fmrib.ox.ac.uk/fsl). Time series statistical analysis was carried out using FILM with local autocorrelation correction [438]. Mixed-effects higher-level analysis was subsequently performed to contrast the first level statistical parameter connectivity maps between children with epilepsy and propensity-matched controls. Z (Gaussianised T/F) statistical images were thresholded using clusters determined by Z>2.3 and a (corrected) cluster significance threshold of P<0.05 [439].

3.2.5 Hierarchical Clustering

To further characterise inter- and intra-network patterns of connectivity, the mean BOLD time series of nodes of multiple ICNs were extracted. ICN selection was based on a literature review performed by Brier and colleagues [46]. Adjacency (correlation) matrices were constructed in a similar manner to the voxel-based analysis previously described, but with each network region as the node and the Pearson correlation coefficient between any two nodes as the edge. Two network-based graph theoretical measures were then calculated: The mean network clustering and characteristic network pathlength. The former is a measure of functional segregation, measuring the fraction of a node’s neighbours that are also neighbours of each other [332]. The latter is a measure of functional integration and describes the average path length between all pairs of nodes in the network [421].

Agglomerative hierarchical clustering was also performed on the adjacency matrices to visualize the segregation of nodes into individual networks based on the strength of the correlations between them. This algorithm creates a cluster tree (dendrogram) based on the shortest Euclidean distance between the correlation value of any two nodes. The agglomerative hierarchical clustering is based on the single linkage algorithm using Euclidean distances among the rows.
of the correlation matrix. Unweighted average distance (unweighted pair group method with arithmetic mean), also known as group average was used. The iterative algorithm continued until no further clusters could be created. Hierarchical clustering was performed in MATLAB (Natick, Massachusetts).

### 3.2.6 Neuropsychological Testing

To test whether the identified ICNs were associated with cognitive deficits, network measures were correlated with neuropsychological testing scores in children with epilepsy. Since the DMN connectivity has previously been associated with working memory [151, 441], forward and backward digit recall was measured using the Working Memory Test Battery for Children (WMTB-C) [308]. The full scale intelligence quotient (IQ) of the Wechsler Intelligence Scale for Children was also correlated with neuroimaging data as a measure of global cognitive function [422]. Age-adjusted z-scores were derived from raw scores [333, 422]. Multivariate linear regression modeling was performed with network connectivity as the dependent variable and subject age, neuropsychological score and group classification as independent variables. Backwards elimination, stepwise conditional regression was performed in MATLAB software using the sum squared error as the criterion to remove model terms [164].

### 3.3 Results

#### 3.3.1 Children with epilepsy demonstrate decreased centrality in important hubs of default mode and salience networks

Eigenvector centrality maps were calculated on connectivity matrices composed of approximately 30,000 voxels. When the whole brain connectome of children with epilepsy was contrasted with propensity-matched controls, significant group effects and age interactions were identified in the centrality of important nodes of the DMN and salience networks. Children with epilepsy demonstrated significantly lower eigenvector centrality in the insulae bilaterally (Figure 3.2 on page 56; right insula: group effect: $F(1,50)=28.16; p<0.01$; age effect: $F(1,50)=0.5, p=NS$; group x age interaction: $F(1,50)=0.30; p=NS$; left insula: group effect: $F(1,50)=31.48; p<0.01$; age effect: $F(1,50)=0.14, p=NS$; group x age interaction: $F(1,50)=0.73; p=NS$). A significant age interaction was also observed in the posterior cingulate cortex, where increases in centrality with age were significantly different between the two groups (group effect: $F(1,50)=11.47; p<0.01$; age effect: $F(1,50)=7.71, p=0.021$; group x age interaction: $F(1,50)=6.36; p=0.015$). Typically-developing children demonstrated a decrease in centrality with age within a large cluster of the frontal lobe, those with epilepsy demonstrated persistence in frontal lobe centrality with age (group effect: $F(1,50)=4.69; p<0.035$; age effect: $F(1,50)=6.94, p=0.011$; group x age interaction: $F(1,50)=13.46; p<0.01$).
Figure 3.2: Differences in whole brain connectome hubs in children with epilepsy compared to controls.

(A) Group effects show that insulae of children with epilepsy are weaker regional hubs compared to controls. (B) Age effects show that the PCC and frontal lobe demonstrate age-related interactions in children with epilepsy. The PCC becomes more central as a function of age in controls compared with children with epilepsy, while the centrality of the frontal lobe fails to weaken with age in children with epilepsy. Axes scaled to show variance in data; steepest increases with age identified in the PCC (slope_{control}=1.1 \times 10^{-3}; \text{slope}_{epilepsy}=7 \times 10^{-5})

3.3.2 Impaired functional network integration and segregation in children with epilepsy

To characterize the patterns of connectivity of hub regions that showed significant differences between children with epilepsy and controls, ROI-based functional connectivity analyses were performed. Brain regions that are known to be important nodes of the default mode (PCC and ventromedial prefrontal cortex) and salience networks (right insula and anterior cingulate cortex) were included as seeds for the ROI analysis. The mean connectivity maps for both children with intractable epilepsy and controls demonstrated expected anti-correlation of task-positive and task-negative ICNs (Figure 3.3 on page 57). Children with epilepsy, however, showed significantly greater connectivity between networks that are normally anti-correlated in
comparison to controls. For instance, when evaluating two core nodes of DMN, the ventromedial prefrontal cortex (vmPFC) and PCC, it was found that the vmPFC showed greater connectivity to the bilateral insulae and somatosensory cortices in children with epilepsy. The PCC also showed greater connectivity to the bilateral insulae, as well as the thalamus. In controls, the PCC demonstrated stronger connectivity to other regions within the DMN, namely the ventromedial prefrontal cortex and lateral parietal cortices. Similarly, when core nodes of the salience network, the right insulae and anterior cingulate cortex (ACC), were evaluated, it was found that controls demonstrate greater connectivity of the right insulae to the ACC, relative to children with epilepsy. Conversely, children with localization-related epilepsy showed greater connectivity of the right insula and ACC to nodes of the DMN, the vmPFC, PCC and lateral parietal cortices.

Figure 3.3: Seed-based analysis reveals stronger inter-network connectivity and weaker intra-network connectivity in children with epilepsy.

Using core nodes of the DMN and salience networks as regions-of-interest, it was determined that children with epilepsy demonstrate significantly stronger inter-network connectivity and weaker intra-network connectivity compared to controls, suggesting a failure of network integration and segregation during development. Colour bars represent z-scores.

To quantify the extent of intra-network connectivity, two graph theoretical properties were extracted: mean network clustering and average network pathlength (Figure 3.4). With increasing age, the network topology of the DMN was characterized by greater clustering and lower characteristic path lengths (network clustering: age: F(1,50)=13.98; p<0.01; group ef-


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fect: $F(1,50)=0.33, p=\text{NS}$; group x age interaction: $F(1,50)=0.08, p=\text{NS}$; characteristic path length: age: $F(1,50)=11.26, p<0.01$; group effect: $F(1,50)=0.46, p=\text{NS}$; group x age interaction: $F(1,50)=0.36, p=\text{NS}$). Children with epilepsy also possessed lower clustering ($F(1,50)=6.78, p=0.01$) and higher pathlength ($F(1,50)=9.5, p<0.01$) of the salience network. Although inter-network correlations between important nodes of the DMN and salience networks decreased with age (PCC-ventral ACC correlation; age effect: $F(1,50)=5.95, p=0.018$), children with epilepsy also demonstrated significantly higher inter-network correlations than controls (PCC-right insula correlation; group effect: $F(1,50)=3.99, p=0.05$).

Figure 3.4: Differing trajectories for functional integration and segregation of DMN and salience networks with age among children with epilepsy and controls.

Mean clustering and characteristic path length of the DMN increase and decrease with age, respectively. Although children with epilepsy demonstrated less steep slopes, the interaction was not significant. Mean clustering and characteristic path length of the salience network were significantly lower and greater in children with epilepsy, respectively. While inter-network correlations decreased with age, children with epilepsy demonstrated greater inter-network correlations than controls among various nodes.

3.3.3 Hierarchical clustering reveals greater network disorganization in children with epilepsy

Given the evidence for weaker segregation and integration of resting-state networks in children with epilepsy, the time series of various nodes of the DMN and task/attention control networks
were extracted in order to further characterize the preceding findings. Connectivity matrices were constructed using correlations between mean ROI time series (Figure 3.5). Using agglomerative hierarchical clustering, the ROIs within individual ICNs tended to cluster together in control subjects. In children with epilepsy, agglomerative clustering revealed greater disorganization of ICNs. When network edges that were significantly different between the two cohorts were identified and contrasted, it was determined that children with epilepsy possessed significantly more inter-network connections, whereas the controls possessed a greater number of intra-network connections (P=0.0005 uncorrected, P=0.01 corrected; Fisher’s Exact test).

Figure 3.5: Agglomerative hierarchical clustering reveals more disorganized network architecture in children with epilepsy compared to controls.

Less separation of individual networks was observed in children with epilepsy. Children with epilepsy also had a higher frequency of significant inter-network connection and fewer significant intra-network connections relative to controls (P<0.01, uncorrected; P=0.01, corrected).

### 3.3.4 Epilepsy duration, location and semiology associated with specific network disturbances

Whole brain connectome analyses were subsequently performed on subgroups of children with medically-intractable epilepsy to determine the contributions of specific clinical features on connectome topology: epileptic focus (temporal versus extra-temporal), duration of epilepsy and semiology (secondarily generalized versus non-generalized). For all subgroup analyses, age was modeled as a covariate.

Children with secondarily generalized seizure semiologies demonstrated decreased centrality
of left hippocampus (Figure 3.6; $F(1,22)=11.38; p=0.027$), anterior thalamus ($F(1,22)=8.31; p=0.0086$), caudate head ($F(1,22)=10.19; p=0.0042$) and left globus pallidus ($F(1,22)=6.4; p=0.019$), relative to other children with epilepsy. Age was not a significant covariate in these analyses, although an interaction with age approached significance in the head of the caudate ($F(1,22)=3.67; p=0.069$). Children with secondarily generalized seizure semiology also demonstrated greater centrality of regions of the DMN, namely the precuneus ($F(1,22)=9.39; p=0.0057$) and right parietal cortex ($F(1,22)=7.03; p=0.014$). Seed-based connectivity demonstrated that despite these regions being stronger hubs in children with secondarily generalized seizures, they were more strongly connected to brain regions outside of the DMN, such as the right insula and ventral ACC and less strongly connected to intra-network nodes, such as the vmPFC (Supplementary Figure 8.4 on page 146).

To investigate the effects of epilepsy duration on ICNs, duration was included as a continuous covariate in the multivariate regression (Figure 3.7). Increasing duration of epilepsy was associated with greater centrality of DMN regions, namely the precuneus ($t(19)=4.85; p<0.001$),
vmPFC ($t(19)=6.08; p<0.001$) and right parietal cortex ($t(19)=5.20; p<0.001$). Again, although these regions were associated with greater centrality, seed-based connectivity revealed greater inter-network connections involving these areas with increasing epilepsy duration (Supplementary Figure 8.5 on page 147). A significant interaction was identified between duration of epilepsy and child age with respect to inter-network correlation. Children with a longer epilepsy duration exhibited greater correlation between core nodes of different ICNs (the PCC and ventral ACC) with increasing age, while children with a shorter duration of epilepsy showed age-related anti-correlation between these two regions (age x duration interaction: $p=0.007$). Changes in ICNs as a function of the proportion of life with epilepsy, defined as the ratio of duration of epilepsy to child age were also evaluated. These data yielded similar results to regression against epilepsy duration.

![Figure 3.7: Duration of epilepsy is associated with weaker intra-network integration and inter-network separation. With longer duration of epilepsy, nodes of the DMN express reduced eigenvector centrality and show greater disorganization. Similar patterns were observed when evaluating proportion of life with epilepsy and age of epilepsy onset as independent variables. Colour bars represent distribution of subject ages.](image)

Next, it was evaluated whether the localization of epileptic foci was associated with unique functional connectome topologies. Children with epilepsy were dichotomized by the location of the seizure focus into those with temporal and those with extra-temporal foci (Figure 3.8).
Compared to children temporal epileptic foci, those with extra-temporal foci showed greater centrality of the temporal lobes and insula bilaterally (right: $F(1,22)=12.93; p=0.0016$; left: $F(1,22)=5.47; p=0.029$) as well as to the left posterior temporal cortex ($F(1,22)=9.82; p=0.0048$). The only region showing decreased centrality in children with temporal lobe seizure foci compared to extra-temporal foci was the left parietal cortex ($F(1,22)=8.14; p=0.0092$), where an interaction with age was also significant ($F(1,22)=4.74; p=0.04$). No significant differences in DMN and salience network connectivity were identified using a seed-based approach between children with temporal and extra-temporal seizure foci.

Figure 3.8: Extra-temporal epilepsy location associated with weaker centrality in the salience network.
Bilateral insular regions were weaker hubs in the connectomes of children with extra-temporal epilepsy relative to children with temporal lobe epilepsy. The latter demonstrated greater centrality of the lateral parietal cortex (a component of the DMN). No significant differences were observed with seed-based connectivity between the two groups.

3.3.5 DMN Network Integration Associated with Working Memory while Inter-network Correlations Associated with Global Deficits

On multivariate linear regression, after adjusting for child age, increased clustering of the DMN was associated with higher scores on neuropsychological testing for working memory (Supplementary Figure 8.6 on page 148; digit span recall score: $t(24)=2.10; p=0.046$; age effect: $t(24)=4.36; p<0.01$). There was no significant group effect ($p=0.70$) or two-way interactions, suggesting that maturation of DMN clustering is associated with improved working memory scores in both subject cohorts. Similarly, decreased pathlength of the DMN was associated with
higher working memory scores (digit span score: $t(24)=-2.28; p=0.032$; age effect: $t(24)=-4.16; p<0.01$). Again no significant group effect ($p=0.64$) or two-way interactions were identified. The findings were specific to the DMN, as neither salience network clustering nor path length were associated with working memory function. Interestingly, although graph theoretical measures of DMN integration were associated with working memory, increased centrality of the PCC in itself was more strongly associated with digit span recall in controls than children with epilepsy, as indicated by the significant interaction term (Supplementary Figure 8.7 on page 148; digit span: $t(23)=2.19; p=0.039$; digit span x group interaction: $t(23)=-2.08; p=0.048$; backwards digit span: $t(21)=3.44; p<0.01$; age: $t(21)=2.98; p<0.01$; backwards digit span x group: $t(21)=-2.15; p=0.043$; age x group: $t(21)=-2.46; p=0.022$).

Greater inter-network anti-correlation, indexed by the correlation coefficient between the PCC (DMN) and ventral anterior ACC (salience network), was also associated with higher full-scale IQ scores in children with epilepsy (IQ score: $t(16)=-3.20; p<0.01$; age effect: $t(16)=-2.67, p=0.016$; IQ score x age interaction: $t(16)=-1.04; p=0.31$). A near-significant interaction was also identified between IQ score and group classification (IQ x group: $t(41)=-1.82; p=0.075$), suggesting that the association between IQ and network segregation may be stronger in children with epilepsy compared to controls (Supplementary Figure 8.8 on page 149). Weaker centrality of the frontal lobe was also associated with higher IQ scores in all children (IQ score: $t(41)=-2.37; p=0.022$; age effect: $t(46)=-4.60, p<0.01$; group effect: $t(41)=-4.29; p<0.01$). There was no significant interaction between group and IQ with respect to centrality in the frontal lobe ($p=0.47$), although, as previously noted, a significant interaction was observed between group and age ($p<0.001$).

### 3.4 Discussion

The intersection of childhood epilepsy, brain network development and cognitive outcome is poorly understood. The current chapter provides the first evidence of impaired development of long-range connectivity in children with medically-intractable epilepsy, and uniquely demonstrates associations between abnormal ICN development and cognitive deficits exhibited by these patients, as previously suspected [158, 357, 358]. The study is also the first to examine the effects of intractable epilepsy on the normative development of ICNs, showing that important regions of the default mode and salience networks demonstrate less hub-like connectivity properties compared to propensity-matched controls. Normative patterns of anti-correlation between task-positive and task-negative networks were disrupted with greater inter-network connectivity and weaker intra-network integration in children with epilepsy and these alterations in intrinsic brain connectivity were associated with poor cognitive outcome. Furthermore, it was shown that the duration of epilepsy affects the developmental trajectory of these networks, supporting the model of earlier surgical treatment of children with localization-related epileptic foci. By understanding impaired emergence of ICNs in children with epilepsy, markers of disease-
related impairment may be elucidated in order to benchmark therapeutic medical and surgical interventions.

Changes observed in DMN and attention control networks during neurodevelopment are consistent with other reports in the literature of increasing functional segregation of adjacent regions and integration of distributed hubs into common networks [9, 109, 208]. Others have also shown that typical development is associated with increased cingulo-insular connectivity and decreased fronto-parietal connectivity [95]. It was demonstrated herein for the first time that localization-related epilepsy interacts with normal developmental trajectories, resulting in deviations from expected patterns of intra-network integration and inter-network segregation.

In the analysis of whole brain connectomes using graph theoretical metrics, a significant interaction between group and age in was found in the PCC, which became a stronger hub as a function of age in controls compared to children with epilepsy. Intra-network DMN connectivity involving the PCC has been shown to exhibit more rapid maturation during adolescence than any other ICN [208]; therefore, the interaction of typical development with epilepsy in this region may explain dysfunction of DMN activity that has been previously documented in young adults with epilepsy [205, 263, 444] (Refer to Section 2.4.5 on page 37 for additional literature). The finding that the PCC demonstrated significantly greater inter-network connectivity in children with epilepsy may explain why PCC centrality was not associated with digit span recall in this cohort, although increases in DMN clustering and decreases in pathlength were associated with working memory.

These data also show that hub properties of a large portion of the frontal lobe fails to decrease with age in children with epilepsy, relative to the control children. Other studies have shown that the process of development over a similar age range involves a transition from "local" to "globally distributed" networks [110]. A hallmark of this transition is the loss of extensive cross-correlations that exist within the frontal lobe and the fractionation of frontal lobe subregions that become integrated into various ICNs [110]. The interaction between age and group classification strongly suggests a disruption of typical fractionation of frontal regions into the task-control and task-negative regions, which was confirmed in the current study through seed-based connectivity analyses. Interestingly, both decreasing frontal lobe centrality and greater inter-network segregation were associated with higher IQ, further supporting the claim that they may be inter-related processes.

The association identified between epilepsy duration (as well as proportion of life with the disease) and failure of network integration and segregation demonstrates that the burden of epilepsy adversely affects the development of brain networks. Previous studies have identified cognitive difficulties at the onset of epilepsy, suggesting that brain network dysfunction cannot be exclusively interpreted as the long-term effects of the disease or seizures [159, 189, 300]. The majority of participants in the current study were children with chronic epilepsy. It was shown that the cumulative burden of epilepsy affects developmental trajectory, independent of the child’s age. Importantly, the entire current population also consisted of children with
localization-related medically-intractable epilepsy, which is potentially amenable to surgical intervention. Given these findings, it is suspected that early surgical treatment of affected children may mitigate the detrimental effects of chronic, repetitive seizure activity on the development of these networks. Future studies should also evaluate whether network disturbances return to the normative developmental trajectories following intervention, which would allow these findings to serve as markers of therapeutic effect. Indeed, a single case report recently suggested that following corpus callosotomy, ICNs showed greater inter-network separation [311].

Of particular importance was the finding that children with secondarily generalized seizure semiologies possessed less centrality in the anterior thalamus, left hippocampus and left globus pallidus compared to those without secondarily generalized seizure activity. Generalized seizures are recognized as the end result of a common pathway in seizure expression. While the corpus callosum is the primary pathway for interhemispheric propagation of seizure activity, other routes have been described involving basal ganglia, thalami and brainstem reticular formation [35, 389]. Thalamocortical circuits are thought to play an important role in generalized seizure activity [315]. Morphological studies in adults with idiopathic generalized epilepsy (IGE) demonstrated gray matter deficits involving the thalamus, caudate, putamen and cortical structures [70] and thalamic deactivation has been observed in response to generalized interictal discharges [140, 274]. Other resting-state studies have also proposed that functional alterations in subcortical structures are evident in adults with IGE [418, 446]. This is the first study to show disengagement of the thalamus and other subcortical structures from whole brain connectomes in secondarily generalized seizures in children with localization-related epilepsy. These findings support the hypothesis that active inhibition of subcortical arousal systems may be involved in seizure semiologies characterized by decreased levels of consciousness. The translational utility of these findings is buttressed by the fact that stimulation of the anterior thalamus [115] and hippocampi [265] are putative strategies for the treatment of medication-resistant epilepsy. These findings lead us to speculate that these interventions may exert a therapeutic effect by engaging these disconnected hubs within the network architecture of the brain.

The current study is unique in that it applied graph theoretical analysis and hierarchical clustering methods to examine the integration and segregation of ICNs during a critical developmental phase. Importantly, stringent quality-assurance reduced the impact of head motion on connectivity results, as half of the original subject cohort was excluded due to excessive motion. Furthermore, whereas the majority of previous studies evaluated patients with idiopathic generalized epilepsy, all subjects herein were affected by localization-related epilepsy, which is amenable to surgical treatment. Finally, this study is the first to examine the intersection of epilepsy and development of ICNs. Increased awareness of deviations from normative trajectories may yield markers to assess efficacy of future interventions.
3.4.1 Limitations

The manifestation of focal epilepsy in childhood is much more heterogeneous than adults. In contrast to adults, nearly half of children may present with extensive extra-temporal or multi-lobar epileptic foci [157, 212]. Furthermore, surgically remediable syndromes are much more heterogeneous in children. It is hypothesized that ICNs would vary depending on the expression of epilepsy syndromes in children and indeed identified differences based on epilepsy duration, seizure focus location and clinical semiology. A larger database of functional imaging of children with intractable epilepsy is required to disentangle the effects of other variables.

Another consideration is the effect of antiepileptic drugs (presented in Supplementary Table 8.1 on page 135) on the findings observed. In this patient cohort, Levetiracetam was the most commonly utilized across multiple drug regimens [185]. It is unknown whether Levetiracetam affects resting-state connectivity. The drug combinations administered were, however, distributed in a non-specific pattern across patient subgroups and therefore unlikely to affect group differences.

Although it was demonstrated that epilepsy affects the development of ICNs, the pathological processes that drive these deviations from typical developmental trajectories have yet to be elucidated. One putative mechanism involves interictal discharges, which have been shown to suspend DMN activity in previous studies evaluating adults with epilepsy [140]. Ictal dynamics have also been previously shown to disrupt synchronization of neural oscillations in eloquent brain regions that may be distant from site of seizure origin [179]. Such disruptions have been associated with specific clinical deficits that persist beyond the ictal period. Evaluation of ICNs in children with epilepsy using multiple modalities with varying temporospatial resolutions is indicated to better characterize the mechanisms of network impairment.

3.5 Future Directions and Ongoing Analyses

3.5.1 Magnetoencephalography ICNs and their Impairment by Epileptic Dynamics

The study of the development and impairment of ICNs in children with epilepsy offers avenues to better understand the effects of the disease in childhood and guide informed decisions regarding treatment. Unfortunately, all BOLD-fMRI related methods to identify and study ICNs are limited in that they are indirect measurements of brain activity, related to blood flow rather than direct neural processes. Another modality that has been studied to characterize ICNs is magnetoencephalography (MEG), which may record direct neural oscillations, which are intimately involved in network activity (See Section 2.4.6 on page 39 for a summary of current understanding of how BOLD coherence and direct neural coherence are related and the study of ICNs using MEG). There are several advantages to measuring ICNs using MEG. Firstly, oscillatory activity may be analyzed into physiologically-relevant frequency bands, which may
provide novel insights into the underlying physiological processes involved in the development and disruption of ICNs. Second, given the millisecond temporal resolution of MEG, one is able to precisely determine how pathological activity may affect these oscillatory networks. Finally, as described in Section 2.3.1 on page 25, magnetic fields are not distorted by inhomogeneous conductivity in the head, thereby providing a more accurate projection of neural activity in source space.

Studies that have performed simultaneous scalp EEG-fMRI have suggested that interictal epileptiform discharges (IEDs) may be responsible for ICN impairments in patient populations with epilepsy (see Section 2.4.5 on page 37 for detailed review). In these preliminary analyses, ICN will be reconstructed from MEG recordings using resting-state fMRI-informed coordinates. The effects of IEDs on network topologies will be studied on a millisecond timescale. The extent of resilience and vulnerability to IEDs will be compared against neurocognitive outcomes. These preliminary analyses will provide a first demonstration of the ability of MEG to reconstruct and investigate ICN dynamics in epilepsy at high temporal resolution.

**fMRI-informed MEG Beamforming** As outlined in Section 2.3.1 on page 25, the disadvantage of MEG is that calculating the spatiotemporal distributions of neural current generators does not result in a unique solution, since the generators lie within the head, which is a conducting medium. This is known as the inverse problem. Inverse models may be parametric (global solutions accounting for entire measured fields in terms of a small number of sources) or imaging models, such as local linear estimators, that estimate the source activity at points of interest. The latter models each point of interest as independent from all other locations, thereby minimizing the contributions of all other sources to estimates of source activity at this point (referred to as a spatial filter or beamformer). Spatial filtering approaches produce volumetric images of brain activity by dividing the brain into voxels and choosing a voxel-of-interest to reconstruct. These approaches may be adaptive (relying on forward solution and field measurement) or non-adaptive (only relying on forward solution). Using a combined fMRI-MEG approach, ICNs may be identified and localized using fMRI with the corresponding MEG time series reconstructed using beamformer (adaptive spatial filtering) approaches. While beamforming is a robust method to solving the inverse problem, it is important to remember that it may suffer from two primary limitations, reconstruction of activity in the presence of highly temporally correlated activity and the influence ("leakage") of strong sources from outside a region of interest. ICNs exhibit weak temporally correlated activity and have been constructed using beamforming methods previously. Furthermore, analysis algorithms, such as the PLI or wPLI (described in detail in Section 2.3.3 on page 26) may be useful at removing the common influence of leakage from strong sources.

To perform spatial filtering on MEG data and extract fMRI-informed networks of interest, source activity at time $t$ from a brain location of interest, $r$, may be calculated as follows:
In Equation 3.1, \( W(r) \) is the weight vector used to create a weighted sum representing an estimate of source activity from a desired location and \( b(t) \) is the measurement vector (in sensor space with \( M \) number of sensors). The challenge is often to calculate the weighting vector, \( W(r) \) which may be achieved using the forward problem, whereby a head model is created (also called the lead-field matrix, \( l(r) \)), which models the head as a conducting sphere (or multi-sphere) such that a relationship is established between an intracranial source and the output in the sensor array is known. By imposing a constraint on the system, to attenuate sources outside the spatial location of interest, a spatial filter is created, as follows:

\[
\min_{W} W^T(r) RW(r)
\]

In Equation 3.2, \( R \) represents the covariance matrix calculated from the sensor array (\( R = \langle b(t)b^T(t) \rangle \)). The choice of the constraint determines the beamformer properties in terms of location bias, resolution and artefacts. In these preliminary analyses, the weights are calculated by imposing the unit-noise-gain (UNG) constraint, which imposes a constraint on the gain such that the weights satisfy: \( W^T_{UNG}(r_p)W_{UNG}(r_p) = 1 \). The weights are therefore calculated as shown in Equation 3.3.

\[
W_{UNG}(r) = \frac{R^{-1}l(r)}{[l^T(r)R^{-1}l(r)]^{1/2}}
\]

Once the weights, \( W(r) \) are calculated, source time courses, known as “virtual sensors” at the locations of interest based on fMRI data can be easily calculated, as shown in Equation 3.1. A vector beamformer is used in the current dissertation, measuring the three components of the weight vector to track the three components of the source activity vector. Since radial components of activity within a conducting sphere are not detectable outside the sphere, the magnitude of the tangential components of the virtual sensor is used to reconstruct source activity.

**MEG Acquisition and IED Marking**

For these preliminary analyses, MEG data were acquired from 26 children with medically-intractable LRE (age range 7-17 years) who were sleep deprived the night prior to the acquisitions in order to accentuate focal interictal discharges. A whole-head gradiometer-based Omega CTF system (151 channels, MISL, Coquitlam, BC, Canada) in a magnetically shielded room was utilized. Fifteen two-minute periods of spontaneous data were recorded with simultaneous MEG and scalp EEG (International 10-20 system placement), as previously published. Data from patients who had greater than 5 mm head displacement between the beginning and end of the each two-minute recording were discarded.
Interictal epileptiform discharges (spikes, polyspikes and sharp waves) were reviewed by clinical electrophysiologists from the 151-channel raw MEG wave forms with band pass filter of 1-70 Hz, which were cross-referenced to simultaneous EEG recordings. As per institutional protocol, when polyspikes or repetitive spikes occur, the earliest spike peak with a reasonable magnetic field topography for dipole source analysis is selected. For localization purposes, this is valuable as the zone of the earliest spike shows a high correlation with the seizure onset zone, defined by intracranial recordings. Some interictal spikes can be better visualized by either MEG or EEG, therefore, selection of interictal spikes should use both methods simultaneously. The earliest peak of IEDs were marked as events for subsequent analysis.

**Constructing ICNs using Neuromagnetic Oscillations**

In order to identify the coordinates of ICN nodes for MEG beamforming, an independent component analysis (ICA; as described in Section 2.4.3 on page 34) was first performed on temporally concatenated fMRI data (Figure 3.9). Four typical ICNs were extracted using ICA, the default mode, salience, dorsal attention and motor networks. The coordinates of the spatial maxima of the important nodes of these networks were then identified in standard MNI space (Table 3.1).
Figure 3.9: Independent component analysis of ICNs in children with epilepsy (age range 7-17). DMN, Default Mode Network, SAL, Salient Network, DAN, Dorsal Attention Network, MN, Motor Network
<table>
<thead>
<tr>
<th>Network</th>
<th>Node</th>
<th>Group-ICA Coordinates (mm, MNI)</th>
<th>Literature Coordinates (mm, MNI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>x  y  z</td>
<td>x  y  z</td>
</tr>
<tr>
<td>DMN</td>
<td>PCC</td>
<td>2  -60  22</td>
<td>0  -51  29</td>
</tr>
<tr>
<td></td>
<td>vmPFC</td>
<td>-2  52  -14</td>
<td>0  61  22</td>
</tr>
<tr>
<td></td>
<td>Left Lateral Parietal</td>
<td>-50  -72  28</td>
<td>-48  -66  34</td>
</tr>
<tr>
<td></td>
<td>Right Lateral Parietal</td>
<td>52  -68  22</td>
<td>53  -61  35</td>
</tr>
<tr>
<td></td>
<td>Medial Thalamus</td>
<td>-8  -8  14</td>
<td>0  -9  7</td>
</tr>
<tr>
<td>SAL</td>
<td>Right Insula</td>
<td>48  14  -8</td>
<td>43  7  2</td>
</tr>
<tr>
<td></td>
<td>Left Insula</td>
<td>-48  14  -6</td>
<td>-42  6  4</td>
</tr>
<tr>
<td></td>
<td>ACC</td>
<td>2  20  34</td>
<td>12  32  30</td>
</tr>
<tr>
<td>DAN</td>
<td>Left FEF</td>
<td>-24  2  56</td>
<td>-29  -5  55</td>
</tr>
<tr>
<td></td>
<td>Right FEF</td>
<td>24  6  50</td>
<td>31  -5  54</td>
</tr>
<tr>
<td></td>
<td>Left IPS</td>
<td>-64  -30  36</td>
<td>-45  -37  48</td>
</tr>
<tr>
<td></td>
<td>Right IPS</td>
<td>66  -30  34</td>
<td>43  -36  46</td>
</tr>
<tr>
<td>MN</td>
<td>Right Motor</td>
<td>42  -36  54</td>
<td>41  -22  48</td>
</tr>
<tr>
<td></td>
<td>Left Motor</td>
<td>-40  -38  52</td>
<td>-40  -23  53</td>
</tr>
<tr>
<td></td>
<td>SMA</td>
<td>-6  -16  50</td>
<td>1  -18  49</td>
</tr>
</tbody>
</table>

Table 3.1: Spatial maxima of nodes of ICNs calculated using Group-ICA in children with epilepsy and comparison with literature

Literature coordinates from Brier and colleagues, 2012 [46]; ACC, anterior cingulate cortex; DAN, dorsal attention network; DMN, default mode network; FEF, frontal eye fields; IPS, intraparietal sulcus; MN, motor network; PCC, posterior cingulate cortex; SAL, salience network; vmPFC, ventromedial prefrontal cortex

Time series were then reconstructed to represent the activity of each source (node) using beamformer analysis, described above. Multisphere head models (lead-field matrices, $l(r)$) were reconstructed for each subject using their individual structural MRI data. In this study, the sources were defined by resting-state fMRI-guided MNI coordinates (Table 3.1), which were fitted to the standard MNI brain and unwarped into individual head space. Reconstructed data from each source were filtered into narrowband frequency ranges from 1-70 Hz with a bandwidth of 3 Hz using custom-designed FIR filters. The instantaneous phase time series were then obtained for each source and frequency range using the Hilbert transform, as described in Section 2.3.2 on page 25. The PLI was then calculated across trials as described in Section 2.3.3 on page 26. Network properties, namely clustering coefficient and characteristic path length were then derived for individual subjects. The effects of IEDs on network properties were then evaluated. A schematic of this analysis pipeline is shown in Figure 3.10.
Chapter 3. Intrinsic Connectivity Networks in Children with LRE

Figure 3.10: Extraction of spontaneous neuromagnetic oscillations using fMRI-guided coordinates.

Group-ICA coordinates are used to reconstruct the ICNs using beamformer analysis in MEG. The raw time series for each node is then derived. Using the Hilbert transform, the instantaneous phase time series is calculated. The PLI is then calculated across trials (IEDs) and network topologies are then derived based on the PLI connectivity matrices. Changes in network topologies occurring around the time of the IED are evaluated for each subject. The resilience and vulnerability of network topology to IEDs was then correlated with fMRI data as well as neurocognitive outcomes.

ICN Resilience to Interictal Epileptiform Discharges is Associated with Cognitive Outcome

When event-related changes in network topology were calculated, it was found that both before and after IEDs, there was an increase in network clustering and a decrease in characteristic path length amongst the network formed by ICN nodes. The mean event-related network properties are shown in Figure 3.11. These changes were most apparent in the 200 ms window before and after the IED.
Chapter 3. Intrinsic Connectivity Networks in Children with LRE

Figure 3.11: Event-related changes in functional network topology

The network changes associated with spikes both in the pre-IED and post-IED window varied between subjects. Subjects that had larger increases in mean network clustering in response to IEDs were considered more vulnerable to IEDs, whereas those who did not have large changes in network topology were considered resilient. The magnitude of changes in network topology both preceding and following IEDs were entered as regressors in the fMRI analysis. Dual regression was employed to determine how ICNs are related to resilience or vulnerability of network topologies to IEDs [114]. The group-average ICA maps were first regressed into each subject’s 4D dataset (as a spatial regressor in a multiple regression) to give a set of time courses, resulting in a set of subject-specific time series, one per group-level spatial map (stage 1). The time courses were then regressed into the same 4D datasets (as temporal regressors in a multiple regression) to derive a subject-specific set of spatial maps that estimate a version of the group-level spatial maps (stage 2). Permutation testing was then used to evaluate differences in spatial maps associated with increased resilience or vulnerability to IEDs using the randomise permutation-testing tool in FSL.

As shown in Figure 3.12 and summarized in Tables 3.2 and 3.3, network topologies that were resilient to IEDs in MEG were associated with stronger ICN connectivity in resting-state fMRI. Conversely, network topologies that were vulnerable to IEDs were associated with weaker ICNs and greater inter-network functional connectivity.
Table 3.2: Differences in networks associated pre-IED network clustering.

<table>
<thead>
<tr>
<th>Network</th>
<th>Contrast</th>
<th>Region</th>
<th>Cluster Size</th>
<th>Coordinates (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMN</td>
<td>Less change in pre-IED network topology</td>
<td>PCC/Precuneus</td>
<td>797</td>
<td>8 -50 32</td>
</tr>
<tr>
<td></td>
<td>Greater change in pre-IED network topology</td>
<td>Ventral ACC</td>
<td>1297</td>
<td>-8 16 34</td>
</tr>
<tr>
<td>SAL</td>
<td>Less change in pre-IED network topology</td>
<td>Left STG</td>
<td>1469</td>
<td>-66 -32 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left Insula</td>
<td>903</td>
<td>-36 16 4</td>
</tr>
<tr>
<td>MN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3.12: Regression of event-related change in functional network topology against functional network components.
Table 3.3: Differences in network associated post-IED network clustering.

<table>
<thead>
<tr>
<th>Network</th>
<th>Contrast</th>
<th>Region</th>
<th>Cluster Size</th>
<th>Coordinates (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMN</td>
<td>Less change in post-IED network topology</td>
<td>Left IFG</td>
<td>2103</td>
<td>-34  22  -20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vmPFC</td>
<td>1089</td>
<td>0  52  32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ACC</td>
<td>810</td>
<td>4  24  16</td>
</tr>
<tr>
<td></td>
<td>Greater change in post-IED network topology</td>
<td>Right IPS</td>
<td>1178</td>
<td>-32 -52  34</td>
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<tr>
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<td>Less change in post-IED network topology</td>
<td>Left Insula</td>
<td>2082</td>
<td>-30  18  -12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ventral ACC</td>
<td>1830</td>
<td>10  20  24</td>
</tr>
<tr>
<td></td>
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<td>Left STG</td>
<td>1437</td>
<td>-48 -36  14</td>
</tr>
<tr>
<td></td>
<td>Greater change in post-IED network topology</td>
<td>Occipital Cortex</td>
<td>2552</td>
<td>-30 -32 -24</td>
</tr>
<tr>
<td>DAN</td>
<td>Greater change in post-IED network topology</td>
<td>Right Hippocampus</td>
<td>902</td>
<td>28 -20 -22</td>
</tr>
<tr>
<td>MN</td>
<td>Less change in post-IED network topology</td>
<td>Left Rolandic</td>
<td>1652</td>
<td>-60 -16  38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right Rolandic</td>
<td>1510</td>
<td>-44 -26  42</td>
</tr>
</tbody>
</table>

Chapter 3. Intrinsic Connectivity Networks in Children with LRE
Interestingly, network topologies that were more resilient to IEDs (with lesser magnitude of change in network clustering coefficient following an IED) were associated with higher full-scale IQ scores (\(R=-0.49; p=0.017\)). When subjects with fewer than 50 recorded IEDs (6 subjects) were excluded from the analysis, presumably because those individuals may introduce noise in the measurements of phase-locking, the inverse relationship between network vulnerability to IEDs and full-scale IQ became stronger (\(R=-0.64; p<0.01\)).

The electrophysiological basis for DMN deactivations coincident with epileptic activity is not well understood. While previous studies have evaluated the effects of IEDs on ICNs using fMRI (see Section 2.4.5 on page 37 for review of salient literature), these preliminary results are the first to suggest, that network changes are not only associated with IEDs, but are present at their onset, indicating that altered ICN topology may be involved in the expression of IEDs. These findings link numerous previous findings, including the detection of increased delta frequency power in the DMN, which is associated with impaired consciousness in patients with complex partial seizures [34, 106]. Importantly, it is only by combining fMRI with MEG that it is possible to disentangle the temporal relations between ICN disruption and IEDs. Furthermore, the resilience of ICNs to IEDs is associated with stronger network functional connectivity as well as better neurocognitive outcomes in children with epilepsy. These findings therefore provide valuable evidence of the relationship between IEDs disruption of ICNs and neurocognitive outcomes in this patient population.

### 3.6 Conclusions

The current study provides the first evidence of impaired development of ICNs in children with medically-intractable localization-related epilepsy. Regions of the brain that are central to the DMN and salience networks were weaker hubs in children with epilepsy compared to matched controls. Furthermore, affected children demonstrated weaker integration and segregation of ICNs. Atypical ICN development was associated with poor cognitive outcomes, suggesting that epilepsy may interfere with maturation of communication of functional brain networks, thereby contributing to cognitive deficits that are prevalent in this population. Patterns of network dysfunction also differed in various clinical epilepsy phenotypes. These findings support the model of early treatment of epilepsy and may provide a benchmark to assess efficacy of therapeutic interventions, once validated by longitudinal studies.
Chapter 4

Ictal Desynchronization of Functional Networks

The findings presented in this chapter have been published as follows:


The findings have also been presented at the following meeting:

(a) American Association of Neurological Surgeons, Pediatrics Section (St. Louis, Missouri, USA, November 25-28, 2012).

Contributions: GMI (conception/design of study, analysis/interpretation of data, drafting/revising article, final approval); TA, AO, HO, MLS, MJT, EJD, JTR, OCS, SMD (conception/design of study, analysis/interpretation of data, final approval)

4.1 Introduction

As discussed in Chapter 2, children with epilepsy are known to exhibit varying levels of neuropsycho logical impairments ranging from motor weakness to deficits in cognition, perception and memory [167, 266]. Prolonged refractory seizures are associated with increased functional impairment, and early control of epilepsy is imperative to counteract developmental deficits [156, 202]. Furthermore, it is common for patients with focal epileptogenic lesions to present with diffuse alterations of cognitive function, which cannot be exclusively attributed to the location of the lesion. Although recent research has suggested that performance difficulties associated with epilepsy may arise due to disruption of functional connectivity within distributed brain networks [447, 448], the mechanism of seizure-induced network impairment remains unclear. In the previous chapter, it was shown that impaired development of oscillatory BOLD ICNs is related to neurocognitive outcomes in children with epilepsy and that IEDs may me-
diate some of these deficits. There remain important gaps in knowledge, however, regarding how epilepsy interferes with the oscillatory neural networks that are supported by synchronous oscillations of neural cell assemblies.

As described in Section 2.2.4 on page 18, and Section 2.2.5 on page 19 oscillatory neural synchronization in the gamma frequency range (\(>30 \text{ Hz}\)) is thought to dynamically modulate functional connectivity among neural populations \([105, 141]\). Synchronization of gamma oscillations is also understood to underlie coordination of activity within distributed task-dependent neuronal assemblies \([63, 406]\) supporting numerous processes including sensory integration, attention, action selection as well as learning and response inhibition \([403]\). Oscillatory synchronization among brain regions has been implicated in motor control, and its disturbance has been studied in clinical populations \([344]\). Maturation of gamma oscillations is related to the development of cognition and perception during childhood and adolescence \([23, 394]\), and atypical patterns of oscillatory coherence are associated with conditions affecting childhood cognitive development \([261, 281]\).

Aberrant brain synchronization has long been thought to play a critical role in epileptic seizures \([341, 426]\) and experimental observation has confirmed abnormal synchrony within epileptogenic brain regions \([301, 419]\). Recent application of graph theoretical analysis has also revealed that network properties of functional connectivity are abnormal in epileptic cortex and that these areas are functionally disconnected from other brain regions \([172, 419, 434]\). Despite convergent evidence implicating network connectivity in cognitive, perceptual and motor function, their impairments in clinical populations, and recent findings linking oscillatory power to functional difficulties in epilepsy \([219]\), the relationship between oscillatory synchrony and functional deficits in epilepsy remains poorly understood.

The current chapter investigates relations among network synchrony and functional impairment in epilepsy. Specifically, the experiment evaluates the consequences of uncontrolled epileptic seizures on motor networks by evaluating their impact on functional connectivity involving the Rolandic cortex. As stated in Hypothesis H1.2 in Section 2.6 on page 45, it is expected that ictal disruption of functional connectivity in a specific cortical region (i.e. the motor cortex) would be associated with specific neurological deficits (i.e. motor deficits). The identified network properties are, therefore, compared with neuropsychological assessments to identify associations between network connectivity and clinical motor deficits. Furthermore, to evaluate whether these effects are due to relations among neural synchrony or rather a reflection of the location of the epileptogenic cortex, patients with Rolandic and extra-Rolandic epilepsy were included. The study adjusted for the location of the intracranial electrocorticography (ECoG) seizure-onset zone (SOZ) and/or the epileptogenic lesion on magnetic resonance imaging (MRI) relative to the Rolandic cortex.
4.2 Methods

4.2.1 Patient Population

ECoG recordings from fifteen children undergoing invasive monitoring for surgical treatment of medically-intractable focal epilepsy at the Hospital for Sick Children were obtained. The underlying pathology in all cases was focal cortical dysplasia (FCD), as classified by the International League Against Epilepsy [33]. The protocol for the analyses described herein was reviewed and approved by The Hospital for Sick Children research ethics board. Retrospective electrophysiological data were reviewed and no prospective patient consent was required.

The mean age was 11.3 ± 4.2 years with a mean duration of epilepsy of 5.3 ± 3.1 years and a mean daily seizure frequency of 4.8 ± 4.4 seizures per day. The majority of children (10 patients; 67%) had type II FCD with balloon cells visible on microscopic examination. Subdural grids were used (median 111 electrodes; range 98-122). A sample case is presented in Figure 4.1. There were no significant differences between children with normal and abnormal motor function with respect to duration of epilepsy (p=0.35), seizure frequency (p=0.15), number of distinct seizures (p=0.56) or size of the SOZ (p=0.17). The distance between the SOZ and the Rolandic cortex was, however, significantly less in children with abnormal motor function (p=0.02). None of these children exhibited post-ictal (Todd’s) paresis.

Figure 4.1: Rolandic Region of Interest.
(A) Intraoperative photograph showing placement of grid over right hemisphere. Asterisk indicates motor hand area as determined by cortical stimulation. (B) Three-dimensional reconstruction showing grid (light pink dots), MRI lesion (dark pink area), magnetoencephalographic (MEG) cluster (green dots). Square shows 3x3 montage used for PLV analysis.

The technique of subdural grid implantation and intra- and extra-operative functional mapping of the epileptogenic and eloquent cortices have been previously described [24]. Subdural grids of 4-mm diameter electrodes embedded in a silicone elastomer sheet with interelectrode
distances ranging from 8 to 10-mm were used. Patients underwent digitally recorded intracranial video-EEG using a Harmonie system (Stellate, Montreal, QC, Canada) with a sampling rate of 1 kHz and anti-aliasing filter at 300 Hz (Butterworth, -20 dB/oct) applied prior to sampling. An averaged reference was selected by clinical electrophysiologists from two channels in a relatively inactive area of the grid during seizures, which was also distant from Rolandic cortex. Children were included in the study if the subdural grid covered the motor cortex and the hand motor area was reliably identified by cortical stimulation.

Ictal and interictal epochs were selected based on ECoG tracings. Ictal periods were of variable length and were comprised of rhythmic ECoG activity demonstrating evolution over time and associated with clinical seizures. Interictal epochs were each two minutes in length and selected at least an hour apart from ictal events. The interictal periods were chosen by experienced electrophysiologists as representative background activity, which in most cases, included interictal epileptic discharges. The ECoG sections were exported as European Data Format Plus (EDF+) files [209] and imported into MATLAB software for subsequent analyses (The MathWorks, Natick, MA, USA). At least three epochs of each type were analyzed for each patient and the mean phase-locking and clustering values were calculated.

### 4.2.2 Phase Synchronization Analysis

To determine synchronization within the motor cortex, a Rolandic region of interest (ROI) was extracted. This was defined as the electrode determined by extra-operative stimulation to be recording the hand motor area and its adjacent electrodes (e.g. a 3 by 3 electrode montage centred over the motor hand area). An internal control was chosen by defining another 3 by 3 montage of electrodes at least three electrodes away from the motor hand area and as equidistant as possible to the SOZ. To investigate long-range phase synchronization involving motor cortex, a secondary analysis was performed including all pairs of electrodes within the grid. The data were band-pass filtered digitally into physiological frequency bands with a notch filter applied to all resonance frequencies of 60 Hz to exclude line noise. The analytical signal of the filtered waveform, $\varsigma(t)$, for each ictal and interictal epoch, $f(t)$, was calculated to obtain the instantaneous phase $\theta(t)$, as described in Section 2.3.2 on page 25.

Inter-electrode phase synchronization was quantified using phase-locking values (PLVs). PLVs were calculated by comparing the instantaneous phases of signals recorded by pairs of electrodes across time as shown in Section 2.3.3 on page 26. As it was hypothesized that seizures alter the functional connectivity of eloquent cortex, an average PLV value for each pair-wise electrode relation was derived for the entire course of each ictal and interictal epoch.

PLVs range between 0 (random phase difference) and 1 (maximum phase-locking). To determine inter-electrode synchrony within the motor cortex, the PLV values associated with all pair-wise comparisons of the electrodes within the motor ROI were averaged across each frequency band. The derived PLV was subsequently averaged across defined frequency bands: delta (1 - 4 Hz), theta (5 - 8 Hz), alpha (8 - 13 Hz), beta (14 - 30 Hz), gamma1 (36 - 44 Hz),...
Chapter 4. Ictal Desynchronization of Functional Networks

gamma2 (45 – 80 Hz), gamma3 (81 – 150 Hz), HFO1 (151 – 200 Hz), HFO2 (201 – 250 Hz) and HFO3 (251 – 300 Hz). In order to quantify the disruption of functional connectivity associated with seizures, the mean interictal phase-locking from ictal values within each analyzed frequency range was subtracted, creating a composite variable describing the ictal phase desynchronization (hereafter referred to as ‘ictal desynchronization’).

4.2.3 Graph Theoretical Analysis

To assess network connectivity involving motor cortex, graph theoretical analysis was performed using the Brain Connectivity Toolbox [332]. Each electrode on the grid was defined as a node and the calculated PLV values represented the edge weights, creating a weighted undirected network, as described in Section 2.5 on page 40. A key measure of networks is the clustering coefficient [287]. This is defined by the ratio of the number of connections between the neighbours of a node and the number of all the possible connections between its neighbours [443]. By providing an indication of the embeddedness of a single node, clustering coefficients quantify the degree of connectivity within the synchronization network. Graphs were constructed using weighted undirected edges defined by PLVs and therefore derived a weighted clustering coefficient, representing the average ‘intensity’ of triangles around a node [298]. A frequency of 80 Hz was selected to investigate clustering in the gamma-band, as this was consistent with the frequency range which showed the strongest relationship between ictal desynchronization and motor function in the ROI analysis. Once the connectivity matrix of the entire grid was analyzed, all possible connections between the electrode recording from the motor cortex (the hand motor node) and the remainder of the electrodes (i.e. all functional neighbours) were considered in the analysis and the clustering coefficient was extracted [332]. As with the analysis of phase synchronization within Rolandic cortex, the interictal clustering values were subtracted from ictal values in order to index the seizure-induced disturbance of connectivity within functional systems involving motor cortex. This composite variable is termed ‘ictal declustering.’

4.2.4 Neuropsychological Testing

The majority of children underwent a battery of neuropsychological testing including two motor-related tasks: the grooved pegboard (N=10) [260] and finger tapping tasks (N=9) [323]. Non-motor aspects of the children’s function were assessed with the matrix reasoning (N=13) and vocabulary subsets (N=12) of the Wechsler Intelligence Scale for Children [422]. Age- and sex-adjusted z-scores were derived [333, 422]. Children were dichotomized as having normal (N=9) or abnormal (N=6) motor function according to their neuropsychological evaluations with those performing under one standard deviation from the mean on the grooved pegboard task and/or with gross motor weakness of the contralateral hand on neurological examination. The grooved pegboard task was used to define abnormal hand function as it is the more complex motor task, requiring both speed and dexterity. All testing was administered and interpreted by neuropsychologists and neurologists during clinical care.
4.2.5 Statistical Analysis

Data are presented as means with error bars representing the standard deviation unless stated otherwise. Binary and dichotomized categorical variables were analyzed using the two-tailed Fisher’s exact test. The means of continuous variables were compared using the nonparametric randomization test, which employs resampling techniques to yield exact significance levels [116]. To adjust for confounders, a one-way analysis of variance (ANOVA) or analysis of covariance (ANCOVA) was performed for categorical and continuous variables respectively. A multivariate logistic binary regression was also performed with variables selected for inclusion based on a priori hypotheses. Outcomes were considered statistically significant at a \( p<0.05 \). Statistical analysis was performed using SAS Statistical Software 9.3 (Cary, North Carolina).

4.3 Results

4.3.1 Ictal Gamma-Band Desynchronization and Motor Impairment

There was a trend towards a significant difference between ictal PLVs in the Rolandic ROI (3 by 3 electrode montage) in children with normal and abnormal motor function, most notably in the gamma2 \( (p=0.053) \) and gamma3 \( (p=0.06) \) frequencies. When the composite variable was tested, “ictal desynchronization” – which has the notable advantage of accounting for baseline (interictal) differences in Rolandic connectivity between subjects – those with motor deficits were significantly more likely to have ictal desynchronization within the contralateral Rolandic ROI than those without deficit. This was observed across numerous frequency bands and was most significantly expressed in the gamma3 band \( (81-150 \text{ Hz};\ p<0.01; \text{ Figure 4.2}) \). Because this frequency band yielded the greatest difference, it was selected for comparison with neuropsychological testing.
Figure 4.2: Differences in Rolandic phase-locking between ictal and interictal epochs between children with normal and abnormal motor function across defined frequency bands. Children with motor deficits had ictal desynchronization (relative to interictal period), most significantly in the gamma3 (81-150 Hz) band.

Performance on the grooved pegboard task of fine motor dexterity of the hand contralateral to recording showed a strong linear correlation with extent of ictal desynchronization at the gamma3 band (Pearson coefficient: 0.62; p=0.05; Figure 4.3 on page 84). The correlation of ictal desynchronization in the gamma3 frequency band with fine motor dexterity outcomes for the hand ipsilateral to recording showed a weaker and non-significant trend (Pearson coefficient: 0.44; p=0.18) and correlation with non-motor neuropsychological function, the verbal and matrix reasoning subtests of WISC-IV showed no association (Pearson coefficients -0.18 and 0.19 respectively; p=0.57 and 0.52 respectively). There was no significant correlation between ictal desynchronization and the tapping task in the contralateral or ipsilateral hands (Pearson correlations: 0.41 and 0.02; p=0.27 and 0.96 respectively).
Figure 4.3: Linear regression of (A) motor tasks and (B) non-motor tasks with differences in phase-locking between ictal and interictal epochs.

Extent of ictal desynchronization (relative to interictal epochs) was significantly correlated with degree of neuropsychological impairment for the grooved pegboard task in the hand contralateral to recording, but not the ipsilateral hand or non-motor tasks.

When adjusting for the presence of the ECoG defined seizure-onset zone (SOZ) and/or MRI lesion within the motor cortex using a two-way ANOVA, significant differences were identified in ictal desynchronization in the gamma3 frequency band (81-150 Hz) which exhibited the most significant association with motor function remained between children with normal and abnormal contralateral hand function (F-value=6.48; \( p=0.03 \)). Furthermore, there was no interaction between ictal desynchronization in this frequency band and the presence of ECoG defined SOZ and/or MRI lesion (F-value=0.73; \( p=0.41 \)). The frequency yielding the strongest difference between normal and abnormal motor function (gamma3, 81-150 Hz) was included in a multivariate logistic regression model and found that gamma-range ictal desynchronization in that frequency band was a stronger independent predictor of motor deficit (OR 29.97; 95% CI 1.41-637.67) than the presence of the ECoG-defined SOZ or MRI lesion within the motor cortex (OR 3.45; 95% CI 0.11-112.61).

Following adjustment for epilepsy duration using ANCOVA, the association between ictal desynchronization at the 81-150 Hz band and abnormal contralateral motor function remained significant (F-value=13.79; \( p<0.01 \)). Longer duration of epilepsy (greater than 6 years) was also independently associated with worse neuropsychological outcomes on the grooved pegboard task (F-value 8.70; \( p=0.03 \)), but not the finger-tapping task (F-value=0.86; \( p=0.40 \)). There was no significant interaction between ictal desynchronization and epilepsy duration (F-value=3.12 and 0.29; \( p=0.18 \) and 0.61 on grooved pegboard and finger-tapping tasks respectively).

In the internal control montage, located at least three electrodes away from the hand motor area and equidistant from the SOZ, there was no difference in ictal desynchronization between children with normal and abnormal motor function at any frequency band (delta \( p=0.38 \); theta \( p=0.10 \); alpha \( p=0.09 \); beta \( p=0.29 \); gamma1 \( p=0.25 \); gamma2 \( p=0.29 \); gamma3 \( p=0.64 \); HFO1
p=0.80; HFO2 p=0.78; and HFO3 p=0.96). Furthermore, there was no significant correlation between ictal desynchronization at the gamma3 frequency band of the control montage and any neuropsychological test.

4.3.2 Graph Theoretical Analysis

Analysis of graph theoretical properties of Rolandic cortex connectivity involving the entire electrode grid revealed that patients with hand motor deficits had significantly higher ictal declustering at a frequency bin centered around 80 Hz involving the hand motor area of the contralateral Rolandic cortex compared relative to children without motor impairment, despite no significant differences in ictal and interictal clustering between these two groups (Figure 4.4). Of note, however, the trend towards significance during the ictal period (p=0.06) suggests that seizures are driving the differences observed. Neuropsychological outcomes for the hand tapping task for the hand contralateral to recording showed a strong linear correlation with extent of ictal declustering at 80 Hz (Pearson coefficient: 0.74; p=0.04; Figure 4.5). There was no significant association between the finger tapping of the ipsilateral hand, or non-motor tasks, and ictal declustering of gamma connectivity. For the grooved-pegboard test, there was a significant association between ictal declustering and outcome for the ipsilateral hand (Pearson coefficient: 0.66; p=0.04), but not the contralateral hand (Pearson coefficient: 0.44; p=0.23) or non-motor tasks.

Figure 4.4: Graph theoretical analysis-based topographic mapping of grid showed significant local declustering in the Rolandic cortex during the ictal period relative to interictal epoch in the gamma band in children with abnormal motor function. There was no significant difference between the two groups of children interictally and there was a trend towards more declustering in the ictal period (p=0.06). There was a significant difference between the extent of ictal declustering (relative to interictal epochs) in children with normal and abnormal motor function.
Figure 4.5: Linear regression of (A) motor tasks and (B) non-motor tasks with differences in clustering between ictal and interictal epochs.

Extent of Rolandic ictal declustering (relative to interictal epochs) significantly correlated with the degree of motor impairment based on the Finger tap test in the contralateral, but not ipsilateral hand and did not show significant correlation with non-motor deficits.

After adjusting for the presence of the ECoG-defined SOZ and/or MRI lesion within the hand-motor area of the Rolandic cortex using a two-way ANOVA, the decrease in clustering coefficient at 80 Hz between the ictal and interictal period remained significantly different between children with normal and abnormal contralateral hand function (F-value=6.23; p=0.03). There was no significant interaction between ECoG-defined SOZ and/or MRI lesion location in Rolandic cortex and abnormal contralateral hand function (F-value=0.02; p=0.90). The observed differences in ictal declustering between children with normal and abnormal motor functions remained significant following adjusting for duration of epilepsy using ANCOVA (F-value=12.96; p<0.01). There was no significant interaction between duration of epilepsy and ictal declustering properties (F-value=0.15; p=0.30). In a multivariate logistic regression model, neither a binarized ictal declustering variable nor the presence of the SOZ/MRI lesion in Rolandic cortex was predictive of abnormal hand function.

4.4 Discussion

The current study uniquely demonstrates that seizures alter the functional connectivity of eloquent cortical areas and that these alterations are predictive of clinical neurological deficit. Direct evidence is also provided for seizure-induced alterations in connectivity of functional networks, which may be distant from ECoG-defined SOZ or presumptive epileptogenic MRI lesions, which are in turn associated with neurological impairments. Evidence is also provided supporting the notion that invasion of function-specific areas of eloquent cortex by ictal connectivity dynamics are selectively related to impairment of the relevant functional domain (e.g. ictal desynchronization of motor cortex is selectively relevant to motor impairment). Hand motor
function was selected to test this hypothesis as it represents a relatively simple, robust network, for which the implicated cortical regions are reliably identified through cortical stimulation [24]. Based on these findings, one may speculate that ictal phase desynchronization and disruption of functional connectivity within a variety of distributed brain networks may underlie the broad spectrum of impairments affecting children with epilepsy. This view is consistent with EEG evidence linking gamma-band synchronization to the formation of distributed neuronal coalitions supporting a variety of cognitive and perceptual processes [53, 113, 325, 328, 382] as well findings of transient ictal desynchrony during aberrant emotional behaviour [17]. The observed association between ictal reduction gamma-band synchrony within Rolandic cortex and motor deficit may therefore represent a mechanism through which epileptic seizures exert long-lasting effects on cortical network dynamics and consequently neuropsychological function.

It remains unclear why in the present study seizure-induced changes in functional connectivity were found to be independently associated with motor weakness, although this finding is supported by clinical associations between longer duration of epilepsy and increased baseline functional impairment. One possible explanation is that prolonged desynchronization and disconnection of functional networks may facilitate network plasticity within the Rolandic cortex, resulting in clinical motor deficit (see Section 2.2.5 on page 19 for a review of how oscillatory synchrony facilitates network development). As previously described, the capacity of individual neurons to exhibit adaptive changes or plasticity is influenced by gamma synchronization [417, 257]. Furthermore, coherence of oscillatory gamma-band EEG activity has been previously studied as a basis for cognitive processes necessitating neuronal plasticity, such as learning and memory [112, 257, 271]. In spike-timing dependent plasticity, pre- and postsynaptic spiking within several milliseconds of a critical window is of profound functional importance [255]. Even when networks engage in oscillations in the beta and gamma ranges, synaptic plasticity is exquisitely sensitive to the timing of discharges with long-term potentiation occurring at the peak of membrane potential oscillations and long-term depression at the trough [427]. A critical implication of these findings is that aberrant gamma synchrony may act to pathologically decorrelate neurons comprising a functional circuit, resulting in long-lasting disruptions in connectivity and thus motor impairments.

Another explanation for these findings involves the role of interictal discharges in disrupting networks beyond the ictal period, further contributing to network destabilization, as suggested by previous findings in Chapter 3. However, it has also been shown that patients with epilepsy exhibit network impairments during interictal periods without interictal epileptic discharges and that functional connectivity is negatively correlated with disease duration [252]. Based on the findings of the current study, it is hypothesized that dysfunctional network integration may be related to repeated ictal desynchronization and functional disconnection within brain networks supporting motor function. This viewpoint is further buttressed by recent demonstration that phase correlations among gamma oscillations in distributed neuronal coalitions contribute to the formation of task specific network interactions involving the motor system [63].
A wealth of literature has also recently emerged suggesting that pathological high frequency oscillations (pHFOs; above 80 Hz) are a signature of cortical epileptogenicity (see Section 2.2.6 on page 20 for review of pertinent literature). One study showed that the presence of ictal motor symptoms was related more to pHFO amplitude in Rolandic cortex than in the SOZ, and that augmentation of ripple-band pHFOs (80-200 Hz) occurred approximately 400 ms prior to EMG onset of ictal motor phenomenon [285]. In contrast to studying oscillation amplitude, the current study examined phase-locking synchrony and graph theoretical properties of gamma-band networks and showed that ictal disturbances in connectivity are associated with baseline motor deficit. Furthermore, it was demonstrated that the magnitude of motor impairment is correlated with the extent of desynchronization and functional disconnection within Rolandic cortex. Using multivariate analysis, it was also shown that these findings are more predictive of deficit than epileptogenicity (SOZ presence) in the Rolandic cortex. It is also interesting that in the current study, the most significant frequency was the high-gamma band (81-150 Hz) (p<0.01), suggesting that ictal desynchronization was strongest within the gamma-band, which has been reliably implicated in the formation of networks supporting cognition, perception and motor control. Finally, while previous studies have shown that the SOZ is itself functionally disconnected [419], the current study showed that ictal disconnection of eloquent cortical areas independent of the SOZ location is associated with neurological deficits. That this may be a leading mechanism for neurological and cognitive impairment in children with epilepsy.

The present study possesses numerous advantages over previous works. Importantly, large subdural grids were used for electrocorticography, allowing us to capture a greater number of nodes and to more accurately apply graph theoretical analyses to map local and inter-regional connectivity involving Rolandic cortex. Secondly, electrophysiological synchrony and graph theoretical properties were associated with clinical and neuropsychological impairments. Finally, the epileptogenic pathology was the same (focal cortical dysplasia) for all children. The main limitation is the absence of a control group, which has been previously reported for intracranial EEG [419].

4.5 Future Directions and Ongoing Analyses

An interesting finding of the current analysis is that changes in functional connectivity during the ictal period involving a specific cortical region are associated neurological deficits attributable to that brain area. An important next step in characterizing these findings is to apply non-invasive neuroimaging methods to attempt to uncover how ictal desynchronization is related to resting- and task-evoked responses within specific networks. In this regard, a recent investigation of functional MEG responses following median nerve stimulation in this patient cohort revealed the expression of excessive gamma responses from Rolandic cortex, which were negatively associated with motor function [92]. The magnitude of the abnormal responses was also associated with the ictal desynchronization, presented herein, providing further evidence that ictal disruption
of motor networks is associated with altered functional responses from motor cortex and motor impairment. How these findings generalize to other brain regions and to what extent ictal changes in functional connectivity are associated with other deficits exhibited by children with epilepsy remains to be investigated.

4.6 Conclusions

The current chapter bridges a gap in knowledge between disturbances in functional connectivity caused by epilepsy and clinical impairments observed in affected children. It was shown that seizure-induced desynchronization of Rolandic cortex is associated with contralateral motor hand deficits independently of the location of the SOZ. Furthermore, ictal disruptions in functional connectivity, shown by local declustering of the Rolandic cortex, are also associated with motor deficit following adjustment for SOZ location. Finally, the study has the advantage of correlating observed seizure-induced changes with clinical and neuropsychological outcomes, rather than statistical differences in BOLD signal on fMRI. These findings were highly significant in the gamma frequency range, and disturbance of network dynamics involving motor cortex were selectively related to motor function. Evidence was presented for a plausible mechanism for network impairment due to epileptic seizures.
Part II

The Network Organization of Epileptogenic Cortex
Chapter 5

Cross-Frequency Phase-Amplitude Coupling in Epileptic Cortex

The data presented in this chapter have published as follows:


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Contributions: GMI (conception/design of study, analysis/interpretation of data, drafting/revising article, final approval); RA, GSC, TA, TV (analysis/interpretation of data, final approval); AO, HO, TO, EJD, JTR, OCS, SMD (conception/design of study, analysis/interpretation of data, final approval)

5.1 Introduction and Background

As previously discussed, the human brain is intrinsically organized into dynamic cell assemblies supported by synchronous neuronal oscillations at various frequencies [59]. This oscillatory activity is thought to result in rhythmic fluctuations in neuronal excitability, creating temporal windows for inter-regional communication [130]. Frequency-specific oscillations may subserve perceptual binding [130], synaptic plasticity [61], and the coordination of distinct brain regions [224]. Low-frequency rhythms modulate activity over large spatial regions and across long temporal windows, whereas high frequencies are restricted to small regions and short temporal windows [64, 413]. Interactions among neural oscillations at different frequency bands (cross-frequency coupling; CFC) have accordingly been proposed to regulate neural processing occurring across multiple spatiotemporal scales [64, 226] (see Chapter 2 for literature review). Previous chapters have examined how impaired functional connectivity of oscillatory neural
networks is related to clinical impairments in children with localization related epilepsy and the contributions of ictal and interictal events to dysfunctional oscillatory activity. The current segment examines the functional connectivity of epileptogenic cortex with a view towards understanding functional relations involved in seizure genesis and mapping epileptogenic brain regions.

Epilepsy is a disorder of neuronal synchrony within specific networks, resulting in recurrent, unprovoked seizures. As described in greater detail in Section 2.2.6 on page 20, one increasingly recognized feature of epileptic cortex is its tendency to express excessive pathological high frequency oscillations (pHFOs; pathological ripple frequencies: 80-150 Hz; pathological fast-ripple frequencies >200 Hz) [5, 296, 194]. Epileptic pHFOs are thought to occur through different mechanisms than their physiological counterparts, which are discrete oscillations at less than 100 ms generated by perisomatic inhibitory interneuron activity [254]. PHFOs may represent out-of-phase firing of neural assemblies in the absence of physiologically-relevant inhibitory and/or regulatory mechanisms (see Jeerys and colleagues for review [197]). Resection of cortical areas expressing pHFOs has been associated with improved seizure outcomes [5, 296, 194]. Recent studies have also suggested that epileptic cortex may also demonstrate atypical cross-frequency interactions [405, 6, 76] and that these cross-frequency interactions may themselves predict seizure onset [6] and occur in regions demonstrating high inter-regional epileptic network connectivity [76]. The phase of low frequency signals reflects fluctuations in neuronal excitability [63, 64], and has also been shown to modulate the occurrence of interictal epileptic activity during sleep [405].

It remains unclear, however, whether CFC during seizures is concentrated in the epileptogenic zone, or whether ictal CFC dynamics are related to progression of seizures or pHFOs. Furthermore, the clinical utility of CFC in the localization of epileptogenic cortex for pre-surgical planning has not been determined. Using electrocorticographic (ECoG) collected from 17 children with focal medically-refractory epilepsy secondary to focal cortical dysplasia (FCD), the hypothesis that excessive CFC involving pHFOs is concentrated within epileptogenic cortex was tested (Hypothesis H2.1, as described in Section 2.6 on page 45). Second, it was evaluated whether these topographically specific increases in CFC were strongest during seizures and investigated the frequencies at which ictal CFC within epileptogenic brain areas was most reliable. Data simulations were performed to evaluate whether observed CFC increases were attributable to true phase-amplitude coupling or other signal characteristics. Finally, the hypothesis that reliable cross-frequency phase-amplitude relationships would vary at seizure initiation and termination was tested to study the role of CFC in ictal dynamics.
5.2 Methodology

5.2.1 Study Population

Data were obtained from seventeen consecutive children with medically-intractable localization-related epilepsy secondary to FCD undergoing pre-surgical invasive monitoring. These included 9 males and 8 females with a mean age of 9.9 years (range 5-16 years) and a mean epilepsy duration of 4.8 years (range 1-11 years). A comprehensive description of the subjects’ clinical demographics and epilepsy syndromes may be found in Table 9.1 on page 150. Individual seizure morphology, including spectral characteristics are presented in Appendix 2 (Chapter 9; Supplementary Figure 9.2 on page 154 and Supplementary Figure 9.3 on page 155). This research was approved by the Hospital for Sick Children Research Ethics Board.

5.2.2 Electrocorticographic Recording and Processing

The technique of subdural grid implantation has been previously described [24]. Data were recorded from subdural grids of 4-mm diameter electrodes embedded in a silicone elastomer sheet with inter electrode distances between 8-10 mm. In addition, strip and depth electrodes were implanted, as clinically indicated. Patients underwent digitally recorded intracranial video-EEG using a Harmonie system (Stellate, Montreal, QC, Canada) with a sampling rate of 1 kHz and anti-aliasing filter at 300 Hz (Butterworth, -20 dB/oct) applied prior to sampling. An averaged reference electrode was selected from two channels in an inactive area of the grid. These represent the same sampling criteria employed to identify pHFOs clinically using multiple band frequency analysis, as previously published [296]. An ictal and interictal epoch was selected for each participant. The former was selected by clinical electrophysiologists based on ECoG tracings, correlated with seizure activity on video-EEG, whereas the latter were chosen at least 2 hours apart from ictal activity. Ictal epochs varied in length according to seizure duration, whereas interictal epochs were uniformly two minutes in duration. The seizure-onset zone was defined as the electrodes with earliest ictal activity (namely, focal fast waves). The "early spread zone" was classified as secondary areas of seizure propagation, which were subsequently resected. Non-epileptic cortex was defined by electrodes that were not included in the resective surgical plan. The ECoG sections were exported as European Data Format Plus (EDF+) files [209] and imported into MATLAB software for subsequent analysis (The MathWorks, Natick, MA, USA).

5.2.3 Derivation of Signal Amplitude And Phase

The raw data for each individual electrode were band-pass filtered into frequency ranges reflecting canonical delta, theta, alpha, beta, gamma, ripple and fast ripple bands using custom filters created through the Filter Design and Analysis (FDA) toolbox of MATLAB. Although the slower frequencies are represented by relatively narrow-band filters, wide increments between the stop and pass frequencies were chosen to minimize ringing effects associated with
digital filtering. Furthermore, although fast-ripples have been noted in excess of 300 Hz, the sampling frequency of 1 kHz precluded reliable sampling above this threshold. The properties of the finite impulse response (FIR) band-pass filters were as shown in Supplementary Table 9.2 on page 155. The analytic signal, $\zeta(t)$, was then calculated from the filtered signal, $f(t)$, and its Hilbert transform, $\tilde{f}(t)$, for each frequency band as described in equation 2.2 on page 26.

From this result, $A(t)$ and $\theta(t)$ were derived, representing the instantaneous envelope amplitude, and instantaneous phase of the analytic signal, respectively (Figure 5.1 on page 95). To account for distortions at each end of the analyzed epochs resulting from the high filter order (ranging from 127 at the fast-ripple band to 1265 at the delta band), the analytic signal was truncated at the beginning and end of the time series by a number of samples equivalent to two-thirds of the highest filter order.
Figure 5.1: Derivation of instantaneous amplitude and phase for a representative one second interval.

Raw signal (black) is band-pass filtered into theta (blue) and ripple (red) frequency bands. The analytic signal is derived using the Hilbert transformation resulting in the envelope amplitude and instantaneous phase for theta and ripple band frequencies, respectively.

5.2.4 Modulation Index Calculation And Electrode Dichotomization

In order to assess the strength of cross-frequency coupling between the high frequency amplitudes and low frequency phases, the modulation index described by Canolty and colleagues [62] was calculated. This index computes the modulus (or length) of the average value of a complex-valued time series in which each sample point has a modulus of the high frequency amplitude and a phase of the low frequency phase (i.e. the analytic signal represents a hybrid of high frequency amplitude and low-frequency phase). If the distribution of low frequency phase is uniform and statistically independent from the high frequency amplitude time series, the distribution of modulus values of the complex-valued time series would be radially symmetric and the modulus of its average value would be zero. If, however, the time series are dependent (such that cross-
frequency coupling exists), the distribution would be eccentric towards the preferred phase and the modulus of the average value of the complex-valued time series would represent the raw modulation index (MI).

To normalize the raw MI, and determine statistical significance of the cross-frequency coupling, 200 surrogate modulation index values were calculated (see Supplementary materials in Canolty et al., 2006 [62] for details). The amplitude and phase time series were shifted by large time lags to dissociate any cross-frequency relations while maintaining the structure of the individual time series. According to the central limit theorem, the histogram of modulation indices derived from the surrogation procedure follows a near-normal distribution, from which one may test the hypothesis that the observed raw modulation index is more extreme than the surrogated data (i.e. the alternative hypothesis being that a non-random relationship exists between amplitude and phase). A z-score was calculated to quantify the deviation of the observed raw modulation index from the random surrogate distribution. These steps were repeated using narrow-band filters from 1 Hz to 300 Hz in 2 Hz bins. Filtering was done with a two-way least squares finite impulse response (FIR) filter (eegfilt.m from EEGLAB toolbox) [87].

In order to determine the efficacy of CFC in delineating epileptogenic brain regions within individual patients, a data-driven, automated algorithm was also developed to dichotomize the electrodes by cross-frequency modulation signatures. In this routine, the sums of the MI of frequency pairs exceeding the statistical threshold determined by Bonferroni correction were projected onto a 1-dimensional space. This reflects the expectation that in epileptic cortex, pathological modulation of high frequency activity by low-frequency rhythms would be evident. Electrodes demonstrating excessive CFC were defined as those exceeding the overall centroid location of the scatter distribution (see Section 9.4.1 on page 161 for detailed description). Fisher’s exact test was used to determine significant associations between electrodes within the SOZ and those demonstrating elevated CFC.

5.2.5 Data Simulations

Data simulations were performed to investigate whether increased CFC could be attributable to factors other than true increases in coupling between low frequency phase and high frequency amplitude, such as the presence of disproportionately higher frequency activity in epileptogenic regions or spikes in the data, which may represent broad-band power increases or introduce "false ripples" in the signal following digital filtering [22]. Eight simulated scenarios were examined with signals comprised of (1) randomly-placed pHFO with randomly-placed spikes; (2) randomly-placed pHFO with spikes synchronized to theta oscillatory phase; (3) pHFOs synchronized to theta phase with randomly-placed spikes; (4) pHFO and spikes synchronized to theta phase; (5) randomly-placed pHFO only; (6) pHFO synchronized to theta phase only; (7) randomly-placed spikes only; and (8) spikes synchronized to theta phase only. For each of these scenarios, signal characteristics were generated, and overlaid on normal background (interictal) ECoG data from one electrode recorded from a child with medically-intractable epilepsy who
was not included in the current analysis. These data were then analysed using the MI, as described above, to assess the impact of each scenario on the measure of CFC. The generation of the simulated data is described in detail in Section 9.2 on page 151.

5.2.6 Phase-Cycle Relationships

We characterized phase cycle relations at various times during the seizures to test the hypothesis that the nature of CFC changed throughout seizure progression. To determine the point along the preferred low frequency phase where maximal high frequency amplitude occurred, the instantaneous amplitude (envelope) of each of the high frequency bands was sorted by the phase of low frequency bands (delta, theta, alpha, beta) into 60 bins of 0.105 radians width. The mean amplitude within each bin was obtained. Statistical significance was evaluated using another surrogation method (1000 re-samplings), whereby low-frequency phase time series were shuffled and the mean high-frequency amplitude was derived for each sample. Data from subjects were normalized (by subtracting the mean and dividing by the standard deviation) and pooled across subjects. A Bonferroni correction factor was applied to adjust for multiple comparisons.

We then projected each normalized phase-sorted amplitude histogram onto a single pair of sine and cosine waves at the fundamental (lowest) frequency. This is equivalent to the dot product of the high frequency amplitude histogram and the cosine and sine waves respectively (see Section 9.4 on page 155). A single complex-valued coefficient was calculated with a real component consisting of the projected cosine wave and an imaginary component consisting of the projected sine wave. These steps are essentially performing a one-point Fourier transform for the single coefficient at the fundamental frequency only. The phase of this coefficient represents the offset of the peak of the best-fitting sinusoid to the histogram data, thus quantifying the low-frequency phase at which the maximal amplitude of high frequency oscillations occurs.

The seizures were divided into 10 equal bins and the phase relations derived for each were plotted on a polar histogram. A threshold was set to disregard all the bins whose high frequency amplitude was within the lower quartile, as the phases of low-amplitude data likely add uninformative noise. Validation testing was performed to ensure that this did not bias the findings (Figure 9.6 on page 160). The Rayleigh test for non-uniformity of circular data was used to identify significant amplitude to phase-cycle associations. Under the null hypothesis, the phases at which maximal pHFOs occur are uniformly distributed around a circle. The alternative hypothesis is that pHFO amplitude maxima tend to occur at specific points in the slower frequency cycle.
5.3 Results

5.3.1 Ictal Modulation of pHFOs by Phase of Slow Oscillations During Seizures is Concentrated in Epileptogenic Cortex

Within the seizure onset zone, it was found that theta and alpha frequency (~6-14 Hz) phase modulated broad band activity encompassing the pathological ripple and fast-ripple frequencies (Figure 5.2 on page 99; p<0.05, Bonferroni corrected). Electrodes that were resected, but were not the site of seizure origin (i.e. the early propagation zone) also demonstrated considerable CFC between the same frequency bands; however these values did not reach the threshold for statistical significance following Bonferroni correction (p<0.05, uncorrected). Electrodes that were beyond the resected cortex (non-epileptic regions) did not exhibit any significant ictal cross-frequency modulation (p>0.05). During the interictal period, CFC did not exceed the threshold for Bonferroni correction in any region. Furthermore, the pattern of the modulation of pHFOs by alpha and theta rhythms observed in the ictal period was not apparent.
Figure 5.2: Modulation of high frequency amplitude by low-frequency phase.
In the seizure-onset zone, significant modulation of high-frequency amplitude (40-300 Hz) is observed, mainly by the phase of theta and alpha oscillations during the ictal period. In the interictal period, no specific CFC with slower oscillations is observed. There is also less cross-frequency coupling in the early propagation zone during seizures and no significant coupling is noted in non-epileptogenic cortex. The z-axis demonstrates the modulation of amplitudes of different narrow-band frequencies (x-axis) by the phases of other narrow-band frequencies (y-axis). Lower and upper planes represent uncorrected and corrected statistical thresholds at p<0.05, respectively.

When all electrodes were dichotomized based on their CFC signatures in the ictal period, those within the SOZ were significantly more likely to have elevated CFC (Figure 9.8 on page 163; p<0.0001; Fisher’s Exact test). Figure 5.3 on page 100 demonstrates topographic plotting of the CFC occurring at individual electrodes on a subdural grid during a single seizure, showing that elevated cross-frequency interactions occur near the site of seizure-onset. For all subjects, the mean CFC of each electrode was plotted across all frequencies as a function of the electrodes’ Cartesian distance from the seizure-onset zone and resected cortex (Figure 5.4 on page 101). With increasing distance from epileptic cortex, CFC demonstrated a logarithmic decrease. Of note, only a subset of electrodes involved within the visual seizure-onset zone and the cortices that were subsequently resected demonstrated elevated CFC. Indeed, when the specificity and
sensitivity of CFC in identifying epileptogenic cortex for all subjects was evaluated using the electrode dichotomization algorithm described above (Table 9.3 on page 164), it was found that CFC possessed high specificity (79%-100%), but low sensitivity. These analyses suggest that CFC is localized to epileptogenic cortices, but that not all brain regions identified as epileptogenic using current clinical methods necessarily express elevated ictal CFC.

Figure 5.3: Topographic mapping of cross-frequency interactions in a representative subject.
(A) Intraoperative image of grid demonstrating seizure onset and early propagation zones. (B) Fast-ripple amplitudes sorted by alpha phase for all grid electrodes. Cosine wave represents alpha phase from \(-\pi\) to \(\pi\). Increased pHFO-to-low-frequency coupling occurs in resected cortex (black borders). Values normalized by 95% confidence interval such values above 0 are significant at \(p<0.05\). (C) Cross frequency modulation index for all electrodes, where the X-axis represents low frequency phase (1 to 40 Hz; left to right), and the Y-axis denotes envelope amplitude (1 to 300 Hz; top to bottom). Values exceeding Bonferroni correction threshold shown. Significant modulation of pHFO amplitudes by low-frequency phase is observed in epileptogenic cortex.

Figure 5.4: Mean ictal electrode pHFO amplitude and cross-frequency coupling as a function of Cartesian distance from epileptogenic cortex. Pooled data across all subjects demonstrates that cross-frequency coupling (red trend line) declines sharply relative to pHFO amplitude (blue trend line), indicating that pHFOs and CFC are dissociable measures, which differ in their topographic relation to epileptogenic cortex. Shaded regions represent standard deviation.
5.3.2 pHFOs, Spikes and CFCs: Simulated Data

Since epileptogenic cortex is known to exhibit excessive high frequency activity relative to non-epileptic regions, the study sought to determine to what extent pHFOs that are not coupled to slower oscillations (i.e. random pHFOs) would bias the metric of CFC. This is particularly important, since it was demonstrated that the within this dataset, the power spectra of electrodes within the seizure-onset zone express greater activity at all frequencies (Supplementary Figure 9.3 on page 155), including pHFOs, which are already known to be associated with epileptogenic brain areas [5, 296]. Furthermore, since spikes and polyspikes are commonly encountered in epileptic cortex, the study also sought to determine whether CFC between different frequencies may be an artifact of these morphologies. This is also valuable since other authors have previously noted “false ripples” that may be seen when applying convolution-based filtering methods to intracranial data [22].

The modulation index describing CFC was measured across multiple parameters for each scenario, as shown in Table 5.1 on the following page. In simulated datasets, only the presence of pHFOs synchronized to a slower cortical rhythm resulted in significant CFC. The presence of random pHFOs and spikes in the data did not result in significant phase-amplitude coupling. Furthermore, the presence of synchronized spiking activity in the absence of pHFOs also did not result in significant CFC.
Table 5.1: Simulated data showing effects of synchronized and random pHFOs and epileptic spikes on phase-amplitude coupling measurement.

<table>
<thead>
<tr>
<th>Case</th>
<th>Event Frequency (Fraction of Theta Cycle)</th>
<th>pHFO Amplitude (RMS)</th>
<th>Spike Amplitude</th>
<th>Spike Width (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Mod. Index</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[1]</td>
<td>0.02</td>
<td>0.08</td>
<td>0.11</td>
<td>0.10</td>
</tr>
<tr>
<td>[2]</td>
<td>0.02</td>
<td>0.08</td>
<td>0.12</td>
<td>0.11</td>
</tr>
<tr>
<td>[3]</td>
<td>0.08</td>
<td>0.33</td>
<td>0.82</td>
<td>1.63</td>
</tr>
<tr>
<td>[4]</td>
<td>0.08</td>
<td>0.33</td>
<td>0.82</td>
<td>1.64</td>
</tr>
<tr>
<td>[5]</td>
<td>0.02</td>
<td>0.08</td>
<td>0.12</td>
<td>0.11</td>
</tr>
<tr>
<td>[6]</td>
<td>0.08</td>
<td>0.33</td>
<td>0.81</td>
<td>1.62</td>
</tr>
<tr>
<td>[7]</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>[8]</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

| Norm. Mod. Index (z) | | | | |
| [1] | 0.64 | 0.08 | 0.52 | 0.52 | 1.12 | 0.87 | 0.15 | 1.02 | 0.00 | 1.00 | 0.62 | 0.02 |
| [2] | 0.84 | 0.09 | 0.80 | 0.42 | 1.40 | 0.78 | 0.14 | 0.90 | 0.10 | 1.02 | 0.64 | 0.34 |
| [3] | 2.00 | 7.58 | 12.23 | 16.49 | 3.06 | 5.37 | 6.4 | 6.88 | 4.64 | 5.78 | 5.61 | 3.8 |
| [5] | 0.75 | 0.05 | 0.66 | 0.37 | 1.19 | 0.89 | 0.16 | 0.99 | 0.00 | 1.01 | 0.63 | 0.03 |
| [6] | 2.23 | 7.54 | 11.67 | 15.56 | 3.05 | 5.15 | 6.37 | 6.82 | 4.51 | 5.69 | 5.26 | 3.88 |
| [7] | 1.43 | 1.56 | 0.74 | 1.27 | 1.4 | 1.09 | 1.23 | 1.11 | 1.14 | 1.11 | 1.08 | 1.72 |
| [8] | 1.27 | 1.22 | 0.17 | 0.06 | 1.0 | 1.21 | 0.82 | 1.14 | 0.90 | 0.27 | 0.64 | 0.98 |

| Pref. Phase (Rad) | | | | |
| [1] | 2.1 | -2.3 | 3.0 | 2.5 | 1.5 | 2.1 | 1.0 | -0.7 | 2.1 | -2.7 | -2.6 | -0.8 |
| [2] | 2.1 | -2.3 | 3.0 | 2.1 | 1.6 | 2.1 | 0.9 | -0.7 | 2.0 | -2.8 | -2.5 | -0.8 |
| [3] | -1.1 | -1.1 | -1.1 | -1.1 | -1.1 | -1.1 | -1.0 | -1.0 | -1.0 | -1.1 | -1.1 | -1.1 |
| [4] | -1.0 | -1.0 | -0.9 | -0.9 | -1.0 | -1.0 | -0.8 | -0.9 | -1.0 | -1.0 | -0.9 | -1.1 |
| [5] | 2.2 | -2.3 | 2.9 | 2.3 | 1.6 | 2.1 | 0.9 | -0.8 | 2.1 | -2.8 | -2.7 | -0.9 |
| [6] | -1.1 | -1.1 | -1.1 | -1.1 | -1.1 | -1.1 | -1.0 | -1.0 | -1.0 | -1.1 | -1.1 | -1.1 |
| [7] | -2.9 | -2.5 | -2.2 | -1.7 | -2.6 | -2.3 | -2.3 | -2.4 | -2.5 | -2.5 | -2.2 | -2.4 |
| [8] | -2.4 | -2.2 | -1.8 | -1.3 | -2.3 | -2.3 | -2.4 | -2.5 | -2.6 | -2.1 | -1.7 | -1.5 |
Where cases are as follows: [1] Random pHFOs with Random spikes; [2] Random pHFOs with Synchronized spikes; [3] Synchronized pHFOs with Random spikes; [4] Synchronized pHFOs with Synchronized spikes; [5] Random pHFOs only; [6] Synchronized pHFOs only; [7] Random spikes only; and [8] Synchronized spikes only. Only cases [3],[4], and [6] reached statistical significance at \( p < 0.05 \). No \( p \)-values of CFC reached statistical significant under any circumstance unless pHFOs were synchronized to the phase of slower oscillations (theta phase).

### 5.3.3 Consistent Cross-Frequency Coupling Between pHFOs and Slower Rhythms at Seizure Termination

To further characterize the relations between pHFO amplitude and low frequency phase, the preferred slow oscillatory phase at which high amplitude pHFOs occurred at various times throughout the seizure was measured. When the preferred phases from all bins from all subjects were plotted cumulatively on polar histograms, it was observed that pathological fast-ripple amplitudes preferentially occurred during the trough of alpha oscillations, whereas pathological ripple amplitudes preferentially occurred between 0 radians and \( \pi/2 \) radians of alpha and theta oscillatory cycles (Figure 5.5 on page 105; \( p < 0.01 \) and \( p < 0.01 \), respectively; Rayleigh test). At seizure initiation (i.e. the first bin), there was no preferred phase for coupling between pathological fast-ripple or ripple amplitudes and the phase of alpha or theta oscillations (\( p = 0.14 \) and \( p = 0.68 \), respectively). At seizure termination (i.e. the last bin), pHFO amplitudes occurred at the trough of the alpha oscillatory cycle (pathological ripple amplitude: \( p < 0.01 \); pathological fast-ripple amplitude: \( p = 0.03 \)). Ripple amplitude maxima were also found at the peak of delta phase irrespective of the seizure progression (Supplementary Figure 9.9 on page 165). To ensure that differences in bin length did explain measures of CFC, a reanalysis of the data with fixed length segments comprised of the first and last 2000 ms of seizures, revealed the same pattern.
Chapter 5. Cross-Frequency Phase-Amplitude Coupling in Epileptic Cortex

Figure 5.5: High-frequency amplitude maxima occur at distinct locations in the low frequency oscillatory cycle as seizures progress.

Top panels show alpha phase-sorted fast-ripple (A) and ripple (B) amplitude histograms for the seizure-onset zone (red) and non-epileptic cortex (blue) for the entire ictal period. Dashed and dotted lines show uncorrected and Bonferroni corrected 95% confidence intervals, respectively, while the black line denotes a single alpha-band phase cycle. Lower panels show polar histograms of this relationship cumulatively, at the beginning of seizures and at seizure termination. Over the entire seizure, phase is biased towards 0 and $\frac{\pi}{2}$ for fast-ripple and ripple frequencies respectively. In the first tenth of the seizure, phases are biased to $-\frac{\pi}{2}$ and $\frac{\pi}{2}$ respectively, while at seizure termination, both fast-ripple and ripple amplitude peaks shift towards trough of alpha phase. P-values indicate Rayleigh test of non-uniformity.

5.4 Discussion

The study of the functional connectivity of oscillatory neural activity may be critical to understanding fundamental aspects of epileptogenic networks with a view towards seizure mapping and treatment. The current chapter provides evidence that ictal CFC involving pHFOs is significantly elevated in SOZ relative to non-resected cortex. Data simulations indicate that these observations are not attributable to other signal characteristics such as increased pHFO amplitude or sharp transients. The study also demonstrated that the relationship between high
frequency amplitude and low frequency phase becomes markedly consistent at seizure termination, implicating cross-frequency interactions in ictal dynamics. These findings may lay the foundation for novel methods for presurgical delineation of epileptogenic cortex based on the topography of CFC.

The pathophysiology of pHFOs in epilepsy has been the subject of considerable recent interest. One prevailing hypothesis is that pHFOs represent out-of-phase (or jittered) firing of independent neural assemblies [29, 82, 355, 201] (See Section 2.2.6 on page 20 for further details). Factors that may be implicated in such out-of-phase firing include unreliable firing, synaptic noise, recruitment delays, non-random connectivity and neuronal loss specific to the epileptogenic regions (see Jeerys and colleagues for review [197]). Interestingly, other have shown that seizure initiation and termination are characterized by loss [286] and subsequent recovery [341, 180] of phase synchrony, respectively. The findings that seizure termination is associated with consistent phase-amplitude coupling suggests that establishing stable CFC is implicated in seizure cessation and thus, conversely that atypical cross-frequency interactions may be relevant for generation of pHFOs and seizures. This view is supported by findings indicating that low-frequency phase reflects rhythmic fluctuations in local neuronal excitability, while high frequency power (envelope amplitude) reflects either a generalized increase in population synaptic activity (broad-band power increase) or the selective activation of a connected neuronal subnetwork (narrow-band power increase) [64]. Cross-frequency interactions may therefore regulate neural processing occurring across multiple spatiotemporal scales [64, 226]. In the context of previous studies, the observed changes in CFC near seizure termination suggest not only recovery normal oscillatory activity within epileptogenic cortex, but also functional connectivity in large-scale brain networks, which are thought to be integrated through coherence in the theta and alpha bands [413].

Recent microelectrode recordings in humans suggest that only during an ictus and within the ictal zone, is there strong spike-field coherence to low frequency components (2-50Hz) of the local field potential [340]. Thus coherent spiking accompanies recruitment into the ictus. The study showed increased CFC between theta/alpha and high-gamma within the epileptogenic zone. High-gamma has been suggested to be a surrogate for increased spiking activity [269], which would be expected during ictal activity. However, spike coherence is more effective than asynchronous spike rate increases at increasing high-gamma power recorded with macroelectrodes [321]. At the cellular level then the CFC observed may reflect increased spike coherence, the hallmark of epilepsy, and suggests, as the current study shows that increases in theta-high gamma CFC may be a surrogate measure for recruitment into ictal activity. How such theta-high gamma CFC differs from that associated with cognitive activity [62] remains to be elucidated. A number of putative metrics may include the absolute magnitude of theta-high gamma CFC, the phase at which high gamma is most strongly modulated, and a broader range of low frequency oscillations that modulate high-gamma amplitude during ictal activity as compared to cognitive activity. Another interesting possibility may be related to the observation that physiological
CFC is usually accompanied by a decrease in low frequency amplitude [237]. Conversely the pathological CFC observed here is associated with increases in low frequency power. Further work elucidating the cellular and microcircuit generators of theta-high gamma CFC in human cortex will be required to specifically address the nature of physiological versus pathological theta/alpha high gamma CFC observed here [118].

The preferred phase and modulating frequency of cross-frequency coupling may also provide new insights into the functional network structure of ictal events in light of the current literature on the neurophysiology and function of cross-frequency interactions. Physiological high frequency oscillations have been previously shown to occur at the trough of low frequency phases in neocortex [62, 190]. Similarly, the low-frequency phase at which maximal pHFO amplitudes were generated at seizure termination was consistently at trough of the oscillations. Additionally, this relation was most significant for modulation of pHFO amplitude by alpha phase. Pulsed alpha inhibition is understood to play an important role in functional networks by exerting a strong inhibitory influence on spike time and firing rates, and therefore, the processing capabilities of a given cortical region [198, 262]. Alpha oscillations reflect fundamental mechanisms of cortical inhibition and idling that may direct information flow within brain networks across different contexts [213].

Furthermore, these findings may be interpreted in the context of existing treatments for epilepsy, such as deep brain stimulation of the anterior thalamic nucleus, which is thought to render the cortex less susceptible to seizures [375, 115]. Thalamocortical cells are robust generators of low-frequency oscillations [96, 295, 377]. Excessive cortical gamma activity and cross-frequency interactions have been proposed to arise from alterations in inhibitory drive on the thalamus (or thalamic deafferentation) leading to increased thalamocortical coherence and dysregulation of cortical inhibition [336, 337]. Stimulation of the anterior nucleus of the thalamus has been shown to improve seizure control and very recently, closed-loop transcranial electrical stimulation has been shown to reduce spike and wave episodes in a rodent model of generalized epilepsy and alter neocortical phase relationships, suggesting that normalization of thalamocortical cross-frequency interactions may be relevant for seizure termination [115, 25]. These speculations are all the more interesting given findings described in Chapter 3, pertaining to loss of centrality of the thalamus within the whole-brain connectome with more severe epilepsy syndromes.

In the present study evidence is provided for ictal increases in CFC that are concentrated within epileptogenic cortex. These findings indicate that CFC is a highly specific but not a particular sensitive marker for epileptogenic cortex. These results suggest that regions expressing elevated ictal CFC are a subset of the epileptogenic zone. This raises questions for future research regarding whether epileptogenic brain areas expressing increased ictal CFC play a different role in ictogenesis and seizure termination than epileptogenic brain regions not associated with elevated CFC. Accordingly, delineation of epileptogenic cortex using topographic mapping of elevated ictal CFC alone is very unlikely to be effective, at least using present methods. This
metric may provide some complimentary mapping utility, however, in that expression of elevated ictal CFC could provide confirmatory evidence that a brain region is epileptogenic. It is attractive to speculate that the resection of cortical regions expressing excessive cross-frequency interactions may provide additional benefit over standard localization modalities. This is foreseeable as pHFO mapping is increasingly used to define epileptic cortex \[5, 296, 194\]. In light of the growing literature indicating a critical role for CFC in regulating interactions among brain regions, future studies should examine whether CFC may be included in network-based approaches that attempt to identify epileptic regions for surgical treatments.

5.5 Conclusions

The current study provides evidence for cross-frequency phase-amplitude coupling in the regulation of epileptic pHFOs. The data also uniquely demonstrate that the SOZ is a site of significant ictal cross-frequency coupling, a finding which may be useful in presurgical planning. The first characterization of dynamic changes in coupling between high and low frequency bands are presented as seizures progress and terminate, which is associated with the occurrence of maximal pHFO amplitude at the trough of the alpha-band oscillatory phase cycle, indicating a potential return to physiological neocortical oscillatory dynamics. It is likely that cross-frequency interactions may play a central role in regulatory circuitry facilitating seizure termination. The study of the functional connectivity of epileptogenic cortex may, therefore, provide critical information for the treatment of epilepsy.
Chapter 6

Frequency-Specific Phase Synchrony in Epileptic Cortex

The findings presented in this chapter have been published as follows:


The findings have also been presented at the following meeting:

(a) World Congress of the Society for Brain Mapping & Therapeutics (Toronto ON, June 2-4, 2012)

Contributions: GMI (conception/design of study, analysis/interpretation of data, drafting/revising article, final approval); RA, TA, GSC (analysis/interpretation of data, final approval); AO, HO, EJD, JTR, OCS, SMD (conception/design of study, analysis/interpretation of data, final approval)

6.1 Introduction

Oscillatory neuronal synchronization has been proposed to mediate functional integration of discrete brain regions into functional cell assemblies, supporting a wide variety of brain functions [141, 105]. Oscillatory activity occurs across multiple spatiotemporal scales. The slower the rhythm, the wider the window of opportunity for synchronization, and the greater the spatial extent of synchrony because synaptic and axonal conductance delays are less limiting [58]. Accordingly, high frequency oscillations have been associated with local neuronal interactions, whereas rhythms at slower frequencies are more relevant for long-distance integration [413, 94].

The previous chapter described abnormal coupling of pHFO amplitudes, a marker of epileptogenicity, to slower cortical rhythms during seizure initiation and propagation. At seizure termination, normative patterns of CFC were restored, suggesting that the hierarchical regu-
lation of high frequency amplitudes by slower cortical rhythms is important for understanding seizure dynamics. As described in Section 2.2.6 on page 20, while clinical applications of pHFO biomarkers are rapidly advancing, the mechanisms of pHFO generation remain poorly understood and their relationship to the reorganization of epileptic network synchrony remains unclear. The associations identified in Chapter 5 indicate that removal of the SOZ from normative regulatory (inhibitory) processes and its isolation from large-scale networks formed by slow-wave modulation over a widespread brain region are associated with ictogenesis.

The present chapter tests the hypotheses that inter-regional functional connectivity of epileptogenic cortex during seizures is altered in a frequency-dependent manner and that the expression of pHFOs by regions involved in epileptic networks is associated with specific connectivity patterns (Hypothesis H2.2, as described in Section 2.6 on page 45). Furthermore, given the dysregulation of functional connectivity measures in the SOZ shown in Chapter 5, the associations between inter-regional functional connectivity and abnormal CFC is evaluated. Phase synchrony among intracranial electroencephalographic (ECoG) electrodes implanted in children with medically-intractable epilepsy were calculated and graph theoretical analyses were used to characterize the involvement of individual electrodes in cortical networks. Correlations were evaluated between the mean pHFO envelope amplitude of each electrode and its associated cortical network topologies, as indexed by graph measures within each frequency range. Subsequently, the association between specific connectivity patterns and pHFO expression in epileptic networks was evaluated. A time-resolved analysis was performed to evaluate changes in the association between pHFO expression and topographic functional connectivity as seizures progress and terminate.

6.2 Methods

6.2.1 Patient Population

Intracranial EEG recordings were obtained from 17 children undergoing invasive monitoring at the Hospital for Sick Children for surgical treatment of lesional medically-refractory localization-related epilepsy in whom the lesion was suggestive of or confirmed to be focal cortical dysplasia on histopathology (Section 9.1 on page 150). The demographics are described in detail in Appendix 2. The median number of electrodes was 111 per patient (range 68-122). The technique of subdural grid implantation and extra-operative mapping of epileptogenic cortical regions has been previously described [24]. Subdural grids were used of 4-mm diameter electrodes embedded in a silicone elastomer sheet with interelectrode distances ranging from 8 to 10 mm as well as strip and depth electrodes. Patients underwent digitally recorded intracranial video-EEG using a Harmonie system (Stellate, Montreal, QC, Canada) with a sampling rate of 1 kHz and anti-aliasing filter at 300 Hz (Butterworth, -20 dB/oct) applied prior to sampling. An averaged reference was selected by clinical electrophysiologists from two channels in a relatively inactive area of the grid. The common reference was chosen because it is routinely applied to clinical
acquisitions for pre-surgical evaluations designed to capture frequencies ranging from delta to pHFOs (up to 300 Hz) [296, 5, 194]. To ensure that choice of reference did not bias synchrony measures, the multi-channel ECoG data were digitally re-referenced to a common average and a post hoc reanalysis of the data was performed (not shown), which was consistent with findings using the common electrode reference.

Ictal events of variable length were selected by a clinical electrophysiologist based on ECoG tracings. The seizure onset zone (SOZ) was defined by the electrodes with the earliest ictal discharges. The ECoG sections were exported as European Data Format Plus (EDF+) files [209] and imported into MATLAB software for subsequent analyses (The MathWorks, Natick, MA, USA). The seizure segments (beginning, middle and termination) were derived by extracting non-overlapping 10 second recordings at the beginning, middle and end of the seizure. If seizure duration was less than 30 seconds, the first, middle and last 10% of the seizure was used, respectively.

### 6.2.2 Inter-Electrode Phase Synchronization

To construct synchronization networks, inter-electrode phase synchronization between all pairs of electrodes within each implanted grid for the each segment of the ictal period for each seizure at each analyzed frequency (delta [0-4 Hz], theta [4-8 Hz], alpha [7-14 Hz], beta [13-32 Hz], gamma [36-80 Hz], ripple [80-150 Hz] and fast ripple bands [180-300 Hz]) was measured. Phase synchronization was chosen as a measure of inter-electrode coupling since it has been described as a putative mechanism for long-range neuronal integration, supporting network formation and inter-regional communication [130]. Bandpass filtering was performed in the filter design and analysis (FDA) toolbox of MATLAB (a sample one second interval is shown in Figure 6.1B). Instantaneous phases and amplitudes, \( \theta(t) \) and \( A(t) \), respectively, were then obtained by calculating the analytic signal of the filtered waveform, \( f(t) \) and its Hilbert transform as described in equation 2.2 on page 26.

Phase synchronization between each pair of electrodes within each frequency band was calculated from the instantaneous phase time series (Figure 6.1C) and quantified using phase-locking values (PLVs) [225]. PLVs range from 0 (random phase difference) to 1 (maximum phase-locking) and represent the consistency of inter-electrode phase relations across time.

We also averaged the instantaneous amplitude of the signal of each electrode above 80 Hz to index ripple and fast ripple magnitude, hereafter referred to as pHFO amplitude. Although pHFOs are often described clinically as brief bursts of oscillatory activity in the high gamma frequencies, the averaged pHFO envelope amplitude describes the tendency of electrodes to express pHFOs across the entire duration of the seizure epoch.

### 6.2.3 Graph Theoretical Analysis

Connectivity (adjacency) matrices were subsequently constructed, whereby each electrode was a node and each inter-electrode PLV was an edge, thereby creating a weighted undirected graph
Edges within- and between- SOZ and non-SOZ nodes were included in the same graph, such that network properties were calculated for all electrodes. Network properties were measured by applying graph theoretical analysis using the Brain Connectivity Toolbox [332]. Two network properties were selected, the clustering coefficient and eigenvector centrality. The former is defined by the weight of connections among neighbours of a node and the total weight of possible connections between its neighbours [298, 443]. Since all electrodes were considered potential functional neighbours, the clustering coefficient is an index of the extent to which a given electrode is embedded within a network. Eigenvector centrality identifies hub regions within a network, that is, it is a measure of the importance of a node within the network [37, 38]. Scores are assigned to individual nodes based on the principle that connections to high scoring nodes contribute more to the score of the node in question than equal connections to low-scoring nodes. These two network parameters describe unique graph topologies. The former is a measure of embeddedness, whereas the latter is a tendency of nodes to form hubs. The clustering coefficient and eigenvector centrality were normalized for each patient by shuffling the edges of each graph 200 times and recalculating the graph properties at each surrogation, which resulted in a mean and standard deviation that was used to normalize the observed parameters (Figure 6.1E). The normalized network parameters were then averaged for each electrode across the different seizures recorded from each patient.
Figure 6.1: Analysis of a representative one second interval.
Raw data (A) is bandpass filtered (B) and the instantaneous phase (C) is derived from the filtered signal and its Hilbert transform (signals from two electrodes shown). Phase-locking values (PLVs) are calculated to index phase synchrony between electrodes. (D) The PLV is used as an edge to construct weighted, undirected graphs and network parameters of interest are calculated. (E) The observed metrics are normalized relative to a normal distribution of network parameters derived by shuffling the edges within the graph.

6.2.4 Statistical Analysis
To ensure that parametric testing was applicable, Q-Q Plots and density curves were modeled for clustering, eigenvector centrality and pHFO amplitude. pHFO amplitude appeared to follow a logarithmic curve, and therefore the log of the pHFO envelope amplitude, which was indeed Gaussian, was used for all calculations. The data were considered as a hierarchically structured set, whereby electrodes are nested within individual subjects. To evaluate the heterogeneity within the population, the pHFO amplitudes were plotted within each subject (Figure 6.2). Within-subject regressions were also performed (data not shown), both of which demonstrated considerable heterogeneity. Traditional regression analyses are limited by a single source of variability (about the regression line). A multilevel model, however, allows a separate regression
model to fit multiple subgroups within the data; therefore, multiple sources of variation may be included (around a given regression line and between different regression lines of groups within the dataset). This has the unique advantage of not only adjusting for heterogeneity within the dataset, but modeling it theoretically.

Figure 6.2: High frequency oscillatory (pHFO) envelope Amplitude for each electrode nested within 17 patients.

The heterogeneity in patient-level electrode pHFO envelope amplitude distribution indicates that a mixed-effects model would be well-suited for the study of the data.

A multilevel mixed-effects linear regression was therefore used to test the association between the seizure onset zone or logarithm of the pHFO envelope amplitude (the independent variable) and the network properties of grid electrodes (the dependent variable). To determine whether a random intercept or random coefficient model best fit the data, the log-likelihood test was performed, which demonstrated that a random coefficient model was superior for the regression with pHFO amplitude (p<0.0001). To preserve the degrees-of-freedom for the higher-level analysis, the number of patient-specific variables included in the model were restricted a priori to patient age, duration of the epilepsy and size of the SOZ.

Given the large number of first-level observations (i.e. electrodes) included in the study, relative to the few second-level observations (i.e. subjects), the primary risk with lower-level analysis is a Type I error, therefore, a stringent standard of statistical significance was imposed when analyzing electrode-level variables, adopting a threshold of p<0.0001. The foremost concern with the higher-level analyses is a Type II error, therefore, a threshold for statistical
significance for patient-level variables of $p<0.05$ was accepted. All analyses were performed in R statistical software.

6.3 Results

6.3.1 Increased pHFO Envelope Amplitude Within Epileptogenic Cortex

A random-effects regression with the logarithm of pHFO envelope amplitude as the dependent variable and SOZ-status of electrodes as the independent variable revealed that electrodes within the SOZ expressed significantly higher mean pHFO amplitudes than electrodes outside the SOZ (Fixed effects $\beta_1$: 0.26; Standard Error: 0.063; $p<0.0001$). Similarly, electrodes recording from cortical areas that were subsequently resected possessed higher pHFO mean amplitudes compared to electrodes recording non-resected regions (Fixed effects $\beta_1$: 0.14; Standard Error: 0.017; $p<0.0001$). Both effects remained significant following inclusion of the second-level effects of patient age, epilepsy duration and SOZ size as independent variables ($p<0.0001$).

6.3.2 SOZ Functionally Disconnected at High Frequencies but Relatively Hyperconnected at Slow Frequencies

To test differences in inter-regional functional connectivity (indexed through the network properties of clustering and centrality) of the SOZ relative to electrodes outside of the SOZ at the different frequencies, a pooled analysis was first performed (Figure 6.3 on page 116). The normalized network properties of electrodes outside the SOZ showed little variability at all frequencies throughout the seizure. The functional connectivity of the SOZ electrodes, however, appeared to vary considerably across the different frequency bands and across the different points in the seizure. At slow frequencies, SOZ electrodes were more strongly embedded in the network (as measured by the clustering coefficient) and showed an increased tendency to be network hubs (as measured by eigenvector centrality) than non-SOZ electrodes. At fast frequencies (gamma, ripple and fast ripple), the SOZ was strongly disconnected from the network. There appeared to be a dramatic transition at seizure termination characterized by loss of high frequency cortical disconnection of the SOZ.
Figure 6.3: Frequency-dependent seizure onset zone disconnection.
Compared to electrodes in the non-seizure onset zone (SOZ), those within the SOZ demonstrate greater embeddedness within the network as demonstrated by (A) the clustering coefficient; and greater centrality as demonstrated by (B) eigenvector centrality at slow frequencies, but not at fast frequencies. This is evident at the beginning and middle of seizures, but not at seizure termination. Error bars represent standard error.

Due to considerable between-subject heterogeneity which must be included in the inferential statistical model, a multivariate mixed-effects linear regression was again employed to quantify the differences in functional connectivity between the two classes of electrodes (SOZ vs. non-SOZ) (Table 6.1 on the next page). For the regression, which requires contrast levels within categorical variables, the delta band was used as a contrast to the other frequencies since it is at the extreme of the frequency spectrum and it was observed in the pooled analysis that functional connectivity varies approximately linearly by frequency. Non-SOZ electrodes were
used as contrasts for the SOZ. Significant interactions were identified at between SOZ-status of electrodes and frequency. Compared to the non-SOZ, the clustering coefficient and eigenvector centrality of the SOZ decreased significantly (compared to delta) at all frequencies above alpha. This is reflected in the interaction terms between SOZ-status and frequency (p<0.0001). At seizure termination, there was no difference between the SOZ and non-SOZ with respect to functional connectivity, and the interaction effect of high frequency disconnection and low frequency hyperconnection was absent. There were no significant two-way interactions between level-1 and level-2 covariates.

Table 6.1: Multilevel regression of functional connectivity of the SOZ.

<table>
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<tr>
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<td>Centrality</td>
<td>Clustering</td>
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<td>(0.043)</td>
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<td>(0.16)**</td>
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<td>(0.0017)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fast Ripple</td>
<td>0.050</td>
<td>(0.034)</td>
<td>0.052</td>
<td>(0.034)</td>
<td>0.066</td>
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### Chapter 6. Frequency-Specific Phase Synchrony in Epileptic Cortex

<table>
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<th>SOZ x Frequency***</th>
<th>SOZ x Theta</th>
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<th></th>
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<td></td>
<td></td>
<td>-0.11 (0.11)</td>
<td>-0.086 (0.11)</td>
<td>-0.29 (0.11)</td>
<td>-0.32 (0.11)*</td>
<td>-0.15 (0.11)</td>
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<td>-0.12 (0.11)</td>
<td>-0.23 (0.11)</td>
<td>-0.20 (0.11)</td>
<td>0.053 (0.11)</td>
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<td>-0.36 (0.11)*</td>
<td>-0.75 (0.11)**</td>
<td>-0.78 (0.11)**</td>
<td>-0.0033 (0.11)</td>
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<td>SOZ x Gamma</td>
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<td>-0.82 (0.11)**</td>
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<td>0.23 (0.11)</td>
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<td>SOZ x Fast Ripple</td>
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<td>-0.56 (0.11)**</td>
<td>-0.72 (0.11)**</td>
<td>-0.71 (0.11)**</td>
<td>-0.044 (0.11)</td>
</tr>
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| Random Effects | Intercept | 3.07e-05 | 3.08e-05 | 3.07e-05 | 3.072e-05 | 3.09e-05 | 3.09e-05 |
|               | σ2         | 0.98     | 0.98     | 0.98     | 0.98      | 0.98      | 0.98      |

*Denotes significance at p<0.01 **Denotes significance at p<0.0001, which was defined a priori as the threshold for statistical significance for level-1 covariates. ***Contrast is delta band Values in parentheses denote standard errors; SOZ denotes seizure onset zone.

#### 6.3.3 pHFO Envelope Amplitude Associated with Functional Disconnection in Fast Frequencies

The data have shown that the mean pHFO envelope amplitude is elevated in the SOZ and that the SOZ is strongly disconnected at fast frequencies at the beginning and middle of seizures. Importantly, it was subsequently tested whether the pHFO envelope amplitude of electrodes itself was correlated with similar frequency-dependent functional connectivity (Table 6.2 on the following page). Using the mixed-effect linear regression, the relationship between the mean pHFO envelope amplitude of electrodes and their network properties was evaluated. Increasing pHFO envelope amplitude was associated with greater disconnection at fast frequencies relative to delta. The interaction term also indicates that increasing pHFO amplitude is associated with a relative hyperconnectivity at the slower frequencies relative to the higher frequencies. The interaction between pHFO amplitude and frequency remained significant in the ripple band (p<0.0001), even at seizure termination, although the coefficient (i.e. slope) of the interaction was less than at the beginning and middle of the seizure (Table 6.2; -0.49 vs. -0.30).
### Table 6.2: Multilevel regression of functional connectivity of pHFO amplitude

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<td>-0.37</td>
<td>(0.22)</td>
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<td>***</td>
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</tr>
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<td>-0.10</td>
<td>(0.057)</td>
<td>-0.094</td>
<td>(0.057)</td>
</tr>
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<td>(0.058)</td>
<td>-0.094</td>
<td>(0.057)</td>
<td>-0.055</td>
<td>(0.064)</td>
</tr>
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<td>(0.064)</td>
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<td>(0.064)</td>
</tr>
<tr>
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<td>-0.17</td>
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<td>(0.057)</td>
</tr>
<tr>
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<td>(0.057)</td>
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<td>-0.44</td>
<td>(0.064)</td>
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<td>-0.44</td>
<td>(0.064)</td>
<td>-0.20</td>
<td>(0.064)</td>
</tr>
<tr>
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<td></td>
<td>-0.20</td>
<td>(0.064)</td>
<td>-0.20</td>
<td>(0.064)</td>
</tr>
</tbody>
</table>
6.4 Discussion

Although the roles of pHFOs and network phase synchrony in epilepsy have each been independently subjects of tremendous research interest, the results of the present chapter show that they are interrelated phenomena. The analyses also uniquely demonstrate that at seizure initiation and progression, the site of seizure onset is relatively functionally disconnected from surrounding cortex at beta, gamma, ripple and fast ripple frequencies. Furthermore, it was shown that there is a logarithmic decrease in functional connectivity in the beta, gamma, ripple and fast ripple (32-300 Hz) bands with increasing mean pHFO envelope amplitude (i.e. increasing tendency towards epileptogenicity). These data are consistent with a growing body of work that suggests that pHFO generation is related to out-of-phase or jittered firing of neuronal assemblies [29, 82, 355, 201]. The results of the present study are also congruent with previous demonstrations of disconnection of epileptogenic cortex [419], which was variably expressed across different frequencies. Interestingly, epochs of inter-regional functional disconnection of epileptogenic cortex correspond to epochs of abnormal CFC (as demonstrated in Chapter 5). This suggests that pathological regulation of functional cross-frequency interactions is associated with widespread abnormalities in network connectivity involving epileptogenic cortex, which are in turn related to ictogenesis. A return to normative patterns of CFC and loss of functional isolation of epileptogenic cortex occurs at seizure termination, highlighting the importance of the functional integration of these regions into large-scale, inhibitory networks. As described in
Section 2.2.6 on page 20, desynchronization of inter-regional interactions within epileptogenic cortex may represent depressed synaptic inhibition, isolating the region from the controlling influence of the embedded network.

These data also indicate that epileptogenic cortex may be able to recruit widespread cortical regions during seizures through interactions mediated by long-range slow rhythms which are relatively hyperconnected to the SOZ. This is particularly relevant as synchronization of slow rhythms are known to facilitate functional interactions among widely distributed neural populations [413, 241] and oscillatory synchronization is thought to promote functional interactions among neurons, as bursts of action potentials can be consistently exchanged during the depolarized phase of the receiving neurons’ ongoing membrane potential fluctuations [130]. Presumably these slow oscillations are pathological given the seizure state and the previous findings in Chapter 5 of impaired CFC involving these rhythms. Furthermore, it has been recently suggested that the SOZ and cortical regions expressing pHFOs are functionally disconnected at theta frequencies in the interictal period [401]. The shift from disconnection to relative hyperconnection merits further characterization in future studies as an indicator of seizure onset.

These results also support the emerging notion that epilepsy is not a condition that is universally characterized by hypersynchrony and hyperconnectivity, as previously described (refer to Section 2.2.6 on page 20 for a detailed review). In agreement with the current findings, recent studies have demonstrated desynchronization of oscillatory activity amongst brain regions during seizures [341, 342]. Using a rat model of pharmacologically-induced epilepsy, Cymerblit-Sabba and Schiller found that the transition to seizures is marked by a decrease in interneuronal synchrony followed by a late resynchronization phase in hippocampal recordings [79]. In their decapitated rat model, Neto and Schill used dual-cell patch-clamp techniques while simultaneously measuring network activity within the hippocampus to show that seizures were associated with desynchronization [286]. In four patients with focal epilepsy, implanted with 13-31 electrode channels each, Warren and colleagues showed that local field potential synchrony was decreased between pairs of electrodes located in the SOZ and non-SOZ in the interictal period, compared to non-epileptic controls [419]. Finally, studies that used similar methodologies to the current work have also found dynamic patterns of phase synchrony during seizures. Kramer and colleagues also show that overall synchronization changed only weakly, although topographical connectivity changed dramatically [218]. Furthermore, Schindler and colleagues performed a time-resolved analysis of intracranial EEG sampled at 200 Hz and found that initial ictal desynchronization was followed by synchronization prior to the end of the seizures and hypothesize that delayed synchronization may be a mechanism of seizure termination [343, 341].

The functional disconnection of epileptogenic cortex above the alpha band with increasing pHFO envelope amplitude may provide a physiological rationale for the use of pHFO power signatures as biomarkers for surgical mapping of seizure during resective procedures. Furthermore, the frequency-dependent functional connectivity of epileptogenic cortex may itself be further characterized as an additional marker for seizure mapping; within individual patients, topo-
graphic concentrations of beta, gamma, ripple and fast ripple disconnection and delta and theta hyperconnectivity often corresponded to brain regions that were identified visually as epileptogenic (Figure 6.4 on page 122). Future studies may also characterize the ability of these methods to complement standard mapping methods for delineation of epileptic cortex.

**Figure 6.4:** Mapping of epileptogenic cortex using frequency-dependent network properties. Topographic mapping of normalized clustering coefficients in the middle of a seizure in a representative subject (Subject M) in the delta and ripple bands. Colors (blue to red) denote increasing clustering coefficients; lines demarcate epileptogenic cortex. Each square represents an individual electrode. Note that epileptogenic cortical regions tend to express high delta-band and low ripple-band clustering. Asterisk denotes location of depth electrodes.

An important finding pertains to the functional disconnection of the SOZ in the early stages of seizure activity, followed by a loss of the disconnection in the fast frequencies at seizure termination. The paradoxical relative hyperconnectivity of the SOZ at slow frequencies, coincident with high frequency disconnection during seizure initiation and propagation is noteworthy. In agreement with the current findings, Cortic and colleagues have previously shown that epileptic regions that demonstrate local cross-frequency interactions are also areas of increased inter-electrode coherence [76] and Nariai and colleagues have demonstrated a tight coupling between the phase of slow waves (defined as less than or equal to 1 Hz in their study) during epileptic spasms and ictal pHFOs (greater than 80 Hz) [284]. The current finding of elevated inter-regional slow frequency connectivity within the SOZ during seizure initiation and propagation adds additional emphasis to the role of slow oscillations during seizures. The importance of slow oscillatory activity in epilepsy is an emerging area of research. It has been found that seizure semiologies characterized by impaired consciousness demonstrate abnormal slow-wave activity [442]. Theta-band connectivity has also been proposed as an early diagnostic test for epilepsy [98] and pathologically elevated theta connectivity has related to greater seizure frequency [97]. Finally, a treatment of epilepsy, vagus nerve stimulation, has been shown to increase and de-
crease gamma- and theta-band connectivity, respectively, underscoring the potential relevance of these findings for seizure control [256]. It may be extrapolated, therefore, from these data in the context of existing literature that the functional integration of epileptogenic cortex into large-scale networks mediated by slow-wave synchrony and CFC may be critical for aborting seizure activity.

Mounting evidence also indicates that interactions among cortical oscillations in various frequency ranges support cognitive processing [12, 62, 236, 88, 53]. One may speculate that relative hyperconnectivity of the SOZ in the slow frequency ranges may play a causal role in ictal states characterized by impaired consciousness, especially given the role of low-frequency oscillations in the synchronization of distant networks [413, 61], and the role of theta-gamma interactions in conscious processing [53, 91].

A significant advantage of the current study over previous work is the utility of a mixed-effects model to characterize the relations among epileptogenic cortex, pHFO amplitude and functional connectivity. By their nature, epileptic networks are highly heterogeneous. Mixed-effects models robustly model heterogeneity, but have thus far been underutilized in the epilepsy and neural network literature. Furthermore, a large number of electrodes were analyzed, thereby allowing the construction of robust synchronization networks, whereas previous studies typically analyzed considerably fewer electrodes per subject [419, 343]. A limitation of the current study is the lack of control subjects without epilepsy, as reported by other groups [419]. Furthermore, eigenvector centrality and clustering coefficient may have been influenced by a common parameter (such as degree distribution). Further investigation is required to determine whether these findings are lesion-specific. Given that cortical dysplasia represent a migration abnormality with secondary development of altered connectivity of neuronal circuits involved, the data may not be generalizable to other etiologies of medically refractory localization-related epilepsy.

### 6.5 Future Directions and Ongoing Analyses

The frequency interactions identified in these analysis and their associations with pHFOs may lay the foundations for future mapping methods of the epileptogenic zone. Based on these results, time-resolved frequency-specific analysis of ictal, preictal and interictal data is underway to identify epileptogenic networks based on phase relationships and correlate these networks to pHFO signatures. As shown in Figure 6.5, a graphical-user interface is being developed to topographically plot frequency-dependent functional connectivity increases (blue) and decreases (red) during the preictal and ictal periods relative to the interictal period.
Chapter 6. Frequency-Specific Phase Synchrony in Epileptic Cortex

Figure 6.5: Time-resolved connectivity analysis.
Dynamic phase relations in different frequency bands in the preictal/ictal period relative to the interictal period are shown, with pink lines indicating relative hyposynchrony, and blue relative hypersynchrony. Figure overlay shows eigenvector centrality for each node. The raw tracing of electrodes within the SOZ (red), the early propagation zone (green) and non-epileptogenic cortex (blue) are plotted.

First, these analyses may facilitate better characterization of dynamic changes in epileptogenic brain regions with a view towards the mapping of epileptogenic cortex. The functional disconnection of epileptogenic cortex at higher frequencies may be used to delineate epileptogenic regions. For instance, Figure 6.6 shows a functional connectivity map of derived from intracranial EEG data from a 9 year old boy with MRI-negative epilepsy and a 1-year history of partial seizures arising from the left frontocentral region. In the high gamma band, functional disconnection of the epileptogenic cortex from surrounding brain regions is apparent. The regions that demonstrate greatest functional disconnection correlate strongly with the proposed resection plan.
Figure 6.6: Functional disconnection of epileptogenic cortex.

In the preictal and early ictal period, the hypothesized epileptogenic zone (yellow rectangle) shows reduced inter-regional phase synchrony at higher frequency bands relative to the interictal period.

Second, the functional hyperconnection of epileptogenic regions may be useful in differentiating epileptogenic brain regions from cortical areas that are uninvolved in the epileptogenic zone. For instance, Figure 6.7 demonstrates the functional connectivity map of a 10 year old child with a history of secondarily generalized seizures arising from the left temporal lobe. At low frequencies, early in the ictal period, a strong epileptic network is apparent in the low-frequency delta band within the temporal lobe. Very shortly following seizure initiation, the epileptogenic networks involves the posterior temporal lobe, namely cortical regions that are strongly involved in language, as determined by extraoperative cortical stimulation mapping. On visual inspection of the EEG tracing, the language region is not dissociable from the epileptogenic zone.
6.6 Conclusions

The current chapter demonstrates a logarithmic decrease in functional connectivity in the beta, gamma, ripple and fast ripple band with increasing pHFO. Conversely, a relative increase in connectivity at delta and theta frequencies was found to be associated with high frequency activity and the seizure-onset zones. These findings indicate that cross-frequency interactions are implicated in the initiation and propagation of seizures. It may be extrapolated that functional isolation of the SOZ through dysfunctional CFC and inter-regional phase scattering is associated with ictogenesis. Conversely, a return to normative hierarchical regulatory control of pHFOs by slower oscillations, coincident with a loss of functional isolation of the epileptogenic zone indicates a return of functional integration of pathological cortex into large-scale, inhibitory brain networks, heralding seizure termination. The current findings establish that pHFOs and altered network synchrony in epilepsy are interrelated phenomena and encourage future studies for the evaluation of frequency-dependent functional connectivity for the mapping and treatment of epilepsy.
Chapter 7

Conclusions and Ethical Considerations

The issues presented in this chapter have been published as follows:


Contributions: GMI (conception/design of study, analysis/interpretation of data, drafting/revising article, final approval); AF, OCS, IE, JMD, MB, JTR (conception/design of study, analysis/interpretation of data, final approval)

7.1 Important Findings

Segment I: Epilepsy and the Brain's Developing Networks The first segment of this dissertation evaluated how the development and organization of the functional connectivity of physiological brain networks is affected by epilepsy and specifically, interictal and ictal events. Typical childhood development is characterized by the emergence of intrinsic connectivity networks (ICNs) by way of inter-network segregation and intra-network integration. The impact of childhood epilepsy on the maturation of ICNs is, however, poorly understood. The developmental trajectory of ICNs in 26 children (8-17 years) with localization-related epilepsy and 28 propensity-score matched controls was evaluated using graph theoretical analysis of whole brain connectomes from resting-state fMRI data. Children with epilepsy demonstrated impaired development of regional hubs in nodes of the salience and default-mode networks (DMN). Seed-based connectivity and hierarchical clustering analysis revealed significantly decreased intranetwork connections, and greater inter-network connectivity in children with epilepsy compared to controls. Significant interactions were identified between epilepsy duration and the expected developmental trajectory of ICNs, indicating that prolonged epilepsy may cause progressive
alternations in large-scale networks throughout childhood. DMN integration was also associated with better working memory, whereas inter-network segregation was associated with higher full-scale IQ scores. Furthermore, subgroup analyses revealed the thalamus, hippocampi and caudate were weaker hubs in children with generalized seizures, relative to other patient subgroups. These findings underscore that epilepsy interferes with the developmental trajectory of brain networks underlying cognition, providing evidence supporting the early treatment of affected children.

Using MEG to reconstruct the ICNs with high temporal resolution, it was found that interictal discharges (IEDs) are associated with changes in functional network topologies within these networks. Changes in network topologies of ICNs both preceded and followed IEDs, suggesting a role for disorganized large-scale inhibitory processes in the expression of IEDs. The resilience of ICNs to IEDs was also associated with improved neurocognitive outcomes in these children. Conversely, network topologies that were more vulnerable to change in response to IEDs were associated with worse neurocognitive outcome in children. ICN responses to IEDs, measured by MEG were also strongly associated with fMRI statistical parameter maps.

To study how seizures may impair neurological networks, electrocorticography was used to study synchronization of oscillatory neural activity, which is purported to be a mechanism mediating functional integration within neuronal networks supporting cognition, perception and action. The hypothesis that seizure-induced alterations in synchronization are associated with functional deficits was tested. By calculating synchrony among electrodes and performing graph theoretical analysis, functional connectivity and local network structure of the hand motor area of children with focal epilepsy from intracranial electroencephalographic recordings was calculated. A local decrease in inter-electrode phase synchrony in the gamma bands during ictal periods, relative to interictal periods, within the motor cortex was strongly associated with clinical motor weakness. Gamma-band ictal desynchronization was a stronger predictor of deficits than the presence of the seizure-onset zone or lesion within the motor cortex. There was a positive correlation between the magnitude of ictal desynchronization and impairment of motor dexterity in the contralateral, but not ipsilateral hand. There was no association between ictal desynchronization within the hand motor area and non-motor deficits, demonstrating that seizure-induced disturbances in cortical functional connectivity are associated with network-specific neurological deficits.

Taken together, this segment of the dissertation provides evidence for a critical association between functional interactions within the human brain and chronic morbidities in children with medically-intractable localization-related epilepsy. As described in Section 2.2.5 on page 19, oscillatory activity is thought to be inextricably involved in normative brain development and subserves important roles in neural communication and network formation. The disruption of oscillatory activity by ictal and interictal dynamics has been shown here to correspond to cognitive and neurological deficits in children with epilepsy, supplying opportunities for novel therapies and biomarkers.
Segment II: The Network Organization of Epileptogenic Cortex  The second segment of the dissertation evaluated functional connectivity of epileptogenic brain regions with a view towards mapping seizure foci and understanding functional relations preceding and during seizure activity. Pathological high frequency oscillations (pHFOs; > 80 Hz) have been proposed to be robust markers of epileptic cortex. Oscillatory activity below this frequency range has been shown to be modulated by phase of lower frequency oscillations, an interaction that reflects hierarchical regulation of cortical activity across different spatiotemporal scales. The hypothesis that dynamic cross-frequency interactions involving pHFOs are concentrated within epileptogenic cortex was tested. Intracranial electroencephalographic recordings from 17 children with medically-intractable epilepsy secondary to focal cortical dysplasia were obtained. A time-resolved analysis was performed to determine topographic concentrations and dynamic changes in cross-frequency amplitude-to-phase coupling (CFC). CFC between pHFOs and the phase of theta and alpha rhythms was found to be significantly elevated in the seizure-onset zone compared to non-epileptic regions (p<0.01). The phase of low frequency oscillations at which pHFO amplitudes were maximal was inconsistent at seizure initiation, yet consistently at the trough of the low frequency rhythm at seizure termination. Amplitudes of pHFOs were most significantly modulated by the phase of alpha-band oscillations (p<0.01). These results suggest that increased CFC between pHFO amplitude and alpha phase may constitute a marker of epileptogenic brain areas and may be relevant for understanding seizure dynamics.

The synchronization of neural oscillations is also thought to integrate distributed neural populations into functional cell assemblies. While epilepsy is widely regarded as disorder of neural synchrony, knowledge is limited regarding whether ictal changes in synchrony involving epileptogenic cortex are expressed similarly across various frequency ranges. It is, furthermore, uncertain how pHFO amplitudes are related to epileptic network connectivity on a meso-scale. By calculating phase-locking values among intracranial electrodes implanted in children with intractable epilepsy, ictal connectivity networks were constructed and graph theoretical analysis was performed to characterize their network properties at distinct frequency bands. Ictal data from 17 children were analyzed using a hierarchical mixed-effects model adjusting for patient-level covariates. Epileptogenic cortex was defined in two ways: (1) a hypothesis driven method using the visually-defined seizure-onset zone; and (2) a data-agnostic method using the high frequency amplitude of each electrode. Epileptogenic cortex exhibited a logarithmic decrease in inter-regional functional connectivity at high frequencies (>30 Hz) during seizure initiation and propagation, but not at termination. At slower frequencies, conversely, epileptogenic cortex expressed a relative increase in functional connectivity. These findings suggest that pHFOs reflect epileptogenic network interactions, yielding theoretical support for their utility in the pre-surgical evaluation of intractable epilepsy. The view that abnormal network synchronization plays a critical role in ictogenesis and seizure dynamics is supported by the observation that functional isolation of epileptogenic cortex at high frequencies is absent at seizure termination. By applying these frequency-dependent interactions as markers of epileptogenicity, several ex-
amples were shown of added benefits in the presurgical evaluation of children with intractable epilepsy.

This segment of the dissertation revealed that functional interactions within epileptogenic cortex may be critical to the understanding of epileptogenic networks and for the mapping of seizure foci. Functional isolation of the SOZ from large-scale brain networks subserved by CFC and inter-regional phase synchrony was associated with ictogenesis. Conversely, seizure termination was characterized by a return to presumably normative hierarchical regulatory control of pHFOs by slower oscillations, coincident with a loss of functional isolation of the epileptogenic zone, indicating a return of the controlling inhibitory influence of the embedded network. These data are buttressed by findings from Part I of the dissertation, where changes in brain network topology preceded IEDs, suggesting that reorganization of the controlling influence of large-scale networks is involved in the expression of epileptic dynamics. Future studies may capitalize on functional relations to identify epileptogenic networks and develop novel treatment strategies for epilepsy.

7.2 Clinical Relevance

In Section 1.2 on page 3, various challenges were discussed in the management of children with epilepsy. The first of these was that a minority of children with medically-intractable epilepsy are currently referred for presurgical evaluation. Within this patient cohort, there are also subgroups of children who are disproportionately affected by the lack of momentum to consider surgical treatments, namely younger children [235]. In the first section of this dissertation, it was shown that the developmental trajectory of the brain’s ICNs is more disrupted by a longer duration and greater proportion of life with epilepsy, adding further support for the earlier treatment of intractable epilepsy across the age span.

The findings that both ictal and interictal events are associated with changes in oscillatory brain network organization may also be relevant for the conduct of surgical procedures. The goal of surgery is typically to achieve seizure-freedom with minimal morbidity. While the benefits of achieving seizure-freedom are self-evident, the benefits of suppressing IEDs is much more controversial, with some authors advocating spike suppression on post-resection ECoG and others suggesting that it makes no difference to patient outcomes [221]. The finding that changes in network topology both precede and succeed IEDs suggests that further research is required to delineate the extent to which these events are causally related to network impairments in epilepsy and the extent to which medical and surgical treatments should attempt their suppression.

A second challenge in the management of childhood epilepsy, discussed in Section 1.2 on page 3, is the lack of robust biomarkers for disease severity. It was shown that network analysis of non-invasive neuroimaging may capture the neural correlates of impaired development in children with intractable epilepsy. Furthermore, specific network impairments were identified in subgroups of patients with distinct clinical features, including the loss of hub-like properties of
the anterior thalamus in children with secondarily generalized seizure activity. It is tempting to speculate that longitudinal measurement of network topologies may prove useful in monitoring the success of medical and surgical treatments. In this regard, a longitudinal study is underway to assess network changes following resective surgery in these children.

The identification of unique network impairments in distinct subgroups of children with epilepsy may also inform patient selection for specific treatment paradigms. For instance, network topologies characterized by loss of centrality of the thalamus may be more amenable to neuromodulation strategies involving this structure [115]. The study of network impairments, theoretically, therefore offers opportunities for personalized medical care to address the specific functional impairments in children. Whether this is indeed the case remains to be tested in appropriately designed trials using neural networks as a biomarker.

A final challenge discussed in the introductory chapter pertains to gaps in knowledge regarding the localization of the epileptogenic zone, precluding some children from undergoing potentially curative surgical treatment. An important advance in the surgical treatment of epilepsy has been the establishment of an association between cortical expression of pHFO expression and the epileptogenic zone. In physiological processes, high gamma activity is tightly linked to the phase of slower oscillatory activity. The finding that pHFO coupling to slower rhythms is highly specific (albeit not sensitive) to epileptogenic cortex suggests that it may be a useful marker of highly pathological brain regions. Indeed, recent studies using microelectrode recordings support this hypothesis [423]. The observation of a breakdown in coupling at seizure initiation suggests a role for the loss of regulatory cross-frequency interactions in ictogenesis, a theory supported by the functional isolation of epileptogenic cortex during seizure initiation also observed at high frequencies. Conversely, at seizure termination, a strict pattern of coupling of pHFOs to slower oscillations was identified and the functional isolation of epileptogenic brain regions was absent. These functional interactions may therefore serve as a basis for mapping epileptogenic networks and developing novel strategies to modulate seizure activity.

### 7.3 Ethical Issues in Knowledge Translation

The dissertation evaluated ways in which impaired functional connectivity is related to cognitive development and epileptogenic networks in children with epilepsy, with various clinical applications as outlined above. The enthusiasm for applying these novel functional connectivity-based methods to the clinical care of patient populations must, however, be tempered by pragmatism and ethical considerations. At present, there are no accepted guidelines or frameworks in place to guide the evaluation of surgical innovations and their ethical translation to direct patient care. Germaine ethical issues for clinicians regarding the use of novel biomarkers and mapping methods is the extent to which novel methods deviate from established procedures, the level of evidence required prior to widespread adoption and the effects of using these technique on healthcare distribution (See Ref [181] for further discussion).
In the Belmont report, outlining ethical criteria for the conduct of human research, innovation is defined as "practice that departs significantly from the standard or accepted" [120]. There is a further distinction between innovation and research. In contrast to the latter, the former is characterized by evolving techniques, outcome measures and patient selection [264]. The extent to which a novel biomarkers and mapping methods require careful oversight of their broad application to patient care is directly related to the degree to which they deviate from established practices. In this regard, Bernstein and Bampoe proposed a classification system to stratify neurosurgical innovations based on their need for such oversight [26]. Table 7.1 presents a modified version of this classification system with examples of surgical innovations in epilepsy. In keeping with this system, the application of network data from functional connectivity analyses as biomarkers in treatment, as well as mapping methods in resective procedures, is a modification of the established procedures.

<table>
<thead>
<tr>
<th>Level</th>
<th>Description</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1</td>
<td>New procedure (elective)</td>
<td>Human implantation with vagus nerve stimulators [306]</td>
</tr>
<tr>
<td>A-2</td>
<td>New procedure (urgent)</td>
<td>Multiple subpial transsections for refractory status epilepticus [90]</td>
</tr>
<tr>
<td>B</td>
<td>New procedure in RCT</td>
<td>Anterior thalamic deep brain stimulation for epilepsy [115]</td>
</tr>
<tr>
<td>C</td>
<td>Modification of a technique of an established procedure</td>
<td>Reconstructing 3D magnetic source imaging using neuronavigation to facilitate cortical resections [168]</td>
</tr>
<tr>
<td>D</td>
<td>Modification of other feature of an established procedure</td>
<td>Use of functional connectivity as biomarkers and to guide surgical resections</td>
</tr>
<tr>
<td>E</td>
<td>New procedure to an individual surgeon/institution</td>
<td>Use of monopolar trains of five for direct cortical stimulation in epilepsy surgery at the Hospital for Sick Children [288]</td>
</tr>
</tbody>
</table>

Table 7.1: Stratification of innovative surgical procedures based on their need for regulation (modified from Ref [26])

While it may be some time before these methods are subjected to a randomized controlled trial, their translation to patient care may be cautiously performed. In a recent position statement pertaining to the regulation of surgical innovations, the Society of University Surgeons suggested that if outcomes of an innovation have not been previously detailed, then a review by a local surgical innovations committee must be conducted, the innovation should be submitted to the national innovations registry and additional informed consent is required of the patient [28]. Appropriate progression to widespread adoption of such methods may proceed following
the conduct of observational studies with some degree of standardization and attempts to involve multiple clinicians at different centers [135].

7.4 Concluding Remarks

The study of the functional connectivity of oscillatory neural networks on a meso- and macroscale affords opportunities to study processes associated with physiological and pathological communication within the human brain. While epilepsy has historically been viewed as an intrinsic disorder of neural communication associated with a state of hypersynchrony, insights gleaned from more advanced analyses reveal a more complex and fascinating disturbance of the rhythms of the brain. A more rigorous investigation of these disturbances may supply novel avenues for the evaluation and treatment of affected children. With increasing emphasis in recent years on epilepsy surgery to treat intractable epilepsy in children, greater efforts to study oscillatory neural networks are needed to deliver appropriate, evidence-based and ethical care.
Chapter 8

Appendix 1

8.1 Subject Demographics and History

Forty-seven children were recruited into the study, of whom 26 were ultimately included in the analysis. For a flow chart of study design, please refer to Figure 8.1. Children were excluded from the analysis if they were unable to complete the scans (7 children), had previous surgical treatment or distorted anatomy that precluded reliable registration to a common MNI-space (3 children) or excessive motion during data collection (8 children). Excessive motion was defined as greater than 2 mm mean maximum displacement or one-third of volumes with a displacement greater than 2 mm. In addition, 3 subjects were excluded because they did not meet diagnostic criteria for localization-related epilepsy.
The children’s epilepsy syndromes were heterogeneous, which reflects the manifestation of childhood epilepsy. In contrast to adults, nearly half of children may present with extensive extra-temporal or multi-lobar epileptic foci [157, 212]. Furthermore, surgically remediable syndromes are much more heterogeneous in children, with cortical dysplasias (23-78%), and tumors (17-38%) comprising the most common pathologies [365]. Children with medically-intractable epilepsy may also be placed on multiple concurrent antiepileptic medications [185]. The demographic data for subjects enrolled in the study is presented in Table 8.1.

Table 8.1: Demographic data for 26 subjects included in the study.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (Years)</th>
<th>Sex</th>
<th>Epilepsy Duration (Years)</th>
<th>Seizure Semiology</th>
<th>Current (and Previous) Antiepileptic Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>F</td>
<td>3</td>
<td>CPS, drop attacks</td>
<td>Topiramate, Clobazam (Carbamazepine, Valproate)</td>
</tr>
<tr>
<td>#</td>
<td>Age</td>
<td>Gender</td>
<td>Dose</td>
<td>Type</td>
<td>Combinations</td>
</tr>
<tr>
<td>---</td>
<td>-----</td>
<td>--------</td>
<td>------</td>
<td>------</td>
<td>--------------</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>M</td>
<td>1</td>
<td>CPS</td>
<td>Phenytoin (Levetiracetam)</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>F</td>
<td>0.5</td>
<td>CPS</td>
<td>Levetiracetam</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>M</td>
<td>3</td>
<td>SPS</td>
<td>Lamotrigine (Levetiracetam, Oxcarbazepine)</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>M</td>
<td>Unclear</td>
<td>CPS, sGTC</td>
<td>Levetiracetam (Phenytoin)</td>
</tr>
<tr>
<td>6</td>
<td>17</td>
<td>F</td>
<td>9</td>
<td>CPS</td>
<td>Topiramate, Lamotrigine</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
<td>M</td>
<td>Unclear</td>
<td>CPS</td>
<td>Carbamazepine, Clobazam (Levetiracetam)</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>M</td>
<td>1</td>
<td>CPS</td>
<td>Valproate</td>
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<tr>
<td>9</td>
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<td>M</td>
<td>0.5</td>
<td>CPS</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>M</td>
<td>Unclear</td>
<td>SPS, drop attacks</td>
<td>Levetiracetam, Carbamazepine</td>
</tr>
<tr>
<td>11</td>
<td>15</td>
<td>M</td>
<td>2</td>
<td>CPS</td>
<td>Oxcarbazepine, Levetiracetam (Carbamazepine)</td>
</tr>
<tr>
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<td>14</td>
<td>M</td>
<td>4</td>
<td>CPS</td>
<td>Levetiracetam, Gabapentin, Clobazam</td>
</tr>
<tr>
<td>13</td>
<td>14</td>
<td>F</td>
<td>2</td>
<td>sGTC</td>
<td>Topiramate, Levetiracetam</td>
</tr>
<tr>
<td>14</td>
<td>9</td>
<td>M</td>
<td>5</td>
<td>sGTC</td>
<td>Valproate, Clobazam, Carnitor</td>
</tr>
<tr>
<td>15</td>
<td>16</td>
<td>M</td>
<td>3</td>
<td>CPS, sGTC</td>
<td>Carbamazepine, Levetiracetam, Valproate</td>
</tr>
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<td>16</td>
<td>M</td>
<td>7</td>
<td>CPS, sGTC</td>
<td>Levetiracetam (Carbamazepine, Valproate)</td>
</tr>
<tr>
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<td>M</td>
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<td>CPS, sGTC</td>
<td>Levetiracetam</td>
</tr>
<tr>
<td>18</td>
<td>16</td>
<td>F</td>
<td>12</td>
<td>CPS, sGTC</td>
<td>Oxcarbazepine (Levetiracetam)</td>
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<td>MRI Findings</td>
<td>Scalp EEG</td>
<td>Other Modalities</td>
<td>Surgical Resection</td>
<td>Surgical Pathology</td>
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<tr>
<td>19</td>
<td>16</td>
<td>F</td>
<td>4</td>
<td>SPS, sGTC</td>
<td>Topiramate (Levetiracetam, Oxcarbazepine, Gabapentin, Clobazam, Lamotrigine)</td>
</tr>
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<td>12</td>
<td>F</td>
<td>4</td>
<td>CPS, sGTC</td>
<td>Oxcarbazepine, Lamotrigine, Levetiracetam (Carbamazepine)</td>
</tr>
<tr>
<td>21</td>
<td>14</td>
<td>F</td>
<td>3</td>
<td>CPS, sGTC</td>
<td>Oxcarbazepine (Carbamazepine)</td>
</tr>
<tr>
<td>22</td>
<td>10</td>
<td>F</td>
<td>Unclear</td>
<td>sGTC</td>
<td>Oxcarbazepine (Levetiracetam)</td>
</tr>
<tr>
<td>23</td>
<td>13</td>
<td>F</td>
<td>13</td>
<td>SPS, CPS, sGTC</td>
<td>Levetiracetam, Valproate</td>
</tr>
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<td>Oxcarbazepine (Levetiracetam)</td>
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<td>10</td>
<td>M</td>
<td>5</td>
<td>CPS, sGTC</td>
<td>Valproate, Clobazam (Carbamazepine, Levetiracetam)</td>
</tr>
<tr>
<td>26</td>
<td>10</td>
<td>M</td>
<td>8</td>
<td>CPS, sGTC</td>
<td>Levetiracetam (Valproate)</td>
</tr>
</tbody>
</table>

CPS, complex partial seizures (focal seizures with dyscognitive features); F, female; M, male; sGTC, secondarily generalized seizures; SPS, simple partial seizures (focal seizures without dyscognitive features). Duration reported in years and all current and previous antiepileptic drugs listed, with previously administered medications in brackets.

To localize the seizures, several modalities were employed: structural MRI, scalp EEG, MEG dipoles, SPECT and PET. The results of these are presented in Table 8.2.

Table 8.2: Imaging and seizure localization for population included in analysis
<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Right fronto-temporal</th>
<th>MEG: Right orbitofrontal dipole cluster</th>
<th>Frontal and temporal lobectomy with amygdalohippocampectomy and inferior parietal topectomy</th>
<th>Gliosis</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>Normal</td>
<td>Central-Parietal</td>
<td>PET: Peri-Rolandic hypometabolism</td>
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<td>n/a</td>
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<td>3</td>
<td>Left temporal AVM</td>
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<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
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<tr>
<td>4</td>
<td>Left front-temporal malformation</td>
<td>Left fronto-temporal</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
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<tr>
<td>5</td>
<td>Right parietal tumour</td>
<td>Right central-parietal</td>
<td>n/a</td>
<td>Gross total resection of low grade glioma</td>
<td>Low grade astrocytoma</td>
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<tr>
<td>6</td>
<td>Band heterotopia</td>
<td>Posterior regions</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
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<td>PET: left anterior temporal hypometabolism</td>
<td>Left anterior temporal lobectomy with amygdalectomy</td>
<td>Subpial gliosis, glioneuronal heterotopia</td>
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<tr>
<td>8</td>
<td>Left temporal tumour</td>
<td>Left temporal</td>
<td>n/a</td>
<td>Left anterior temporal lobectomy with amygdalectomy</td>
<td>Ganglioglioma</td>
</tr>
<tr>
<td>9</td>
<td>Right frontal hypointensity</td>
<td>Right frontal</td>
<td>MEG: Peri-Rolandic spike cluster</td>
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<td>n/a</td>
</tr>
<tr>
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<td>Normal</td>
<td>Left frontal</td>
<td>PET: Left frontal hypometabolism</td>
<td>Left frontal topectomy</td>
<td>Focal cortical dysplasia Type 2A</td>
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<td>Left fronto-temporal</td>
<td>MEG: Left frontal dipoles</td>
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<td>n/a</td>
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<tr>
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<td>Left fronto-temporal</td>
<td>MEG: Left temporal dipole cluster</td>
<td>Left temporal lobectomy with hippocampectomy</td>
<td>Neocortical gliosis, mesial temporal sclerosis</td>
</tr>
<tr>
<td>Case</td>
<td>Diagnosis</td>
<td>Normalization</td>
<td>PET/MEG</td>
<td>PET/MEG</td>
<td>Surgery</td>
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<td>------</td>
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<td>---------</td>
<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td>13</td>
<td>Normal</td>
<td>Posterior</td>
<td>MEG: Right parietal dipole cluster PET: Right parietal hypometabolism</td>
<td>Right parieto-occipital topectomy</td>
<td>Focal cortical dysplasia Type 2B</td>
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<tr>
<td>14</td>
<td>Normal</td>
<td>Left hemisphere</td>
<td>PET: Left posterior frontoparietal MEG: Peri-Rolandic dipole cluster</td>
<td>Left anterior temporal lobectomy</td>
<td>n/a</td>
</tr>
<tr>
<td>15</td>
<td>Left mesial temporal and hippocampus changes</td>
<td>Left temporal</td>
<td>MEG: left temporal dipole cluster</td>
<td>Left temporal lobectomy with amygdalohippocampectomy</td>
<td>Neocortical gliosis and hippocampal sclerosis</td>
</tr>
<tr>
<td>16</td>
<td>Right hippocampal volume loss</td>
<td>Right temporal</td>
<td>n/a</td>
<td>Right anterior temporal lobectomy with amygadalohippocampectomy</td>
<td>Focal cortical dysplasia Type 3A</td>
</tr>
<tr>
<td>17</td>
<td>Left Parietal tumor</td>
<td>Left hemisphere</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>18</td>
<td>Normal</td>
<td>Left temporal</td>
<td>MEG: left inferior frontal dipole cluster</td>
<td>Left inferior Rolando topectomy</td>
<td>Gliosis</td>
</tr>
<tr>
<td>19</td>
<td>Bilateral hippocampal signal change</td>
<td>Right fronto-temporal</td>
<td>PET: Right temporal hypometabolism</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>20</td>
<td>Left frontal volume loss</td>
<td>Left hemisphere</td>
<td>MEG: Left peri-sylvian dipoles</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>21</td>
<td>Left parietal hyperintensity</td>
<td>Left hemisphere</td>
<td>PET: Left temporal hypometabolism</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>22</td>
<td>Left temporal tumor</td>
<td>Left temporal</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>
23 | Left developmental malformation | Left frontal | MEG: Left frontal dipole cluster | n/a | n/a
24 | Normal | Non-Localizable | MEG: Left hemisphere dipoles | n/a | n/a
25 | Normal | Left temporal | PET: Left temporal hypometabolism | Left anterior temporal lobectomy with amydalectomy | Gliosis
26 | Left temporal signal change | Left temporal | n/a | Left anterior temporal lobectomy | Gliosis

8.2 Resting-State fMRI Acquisitions

Resting-state fMRI data were acquired with an echo planar imaging (EPI) sequence on three separate scanners with slightly different protocols (Table 8.3). Ninety-six percent of subjects were scanned using either Protocol 1 or Protocol 3. Although an increasing body of literature suggests that networks are consistent across scanners and paradigms, several additional validation tests were performed to ensure that scanner differences did not explain significant group differences that were identified.

<table>
<thead>
<tr>
<th>Protocol 1 (Historical Data)</th>
<th>Protocol 2</th>
<th>Protocol 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Epilepsy Subjects</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Number of Control Subjects</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>Scanner</td>
<td>Siemens Trio</td>
<td>GE Signa</td>
</tr>
<tr>
<td>Head Coil Channels</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Field Strength</td>
<td>3T</td>
<td>3T</td>
</tr>
<tr>
<td>Resolution</td>
<td>64x64</td>
<td>64x64</td>
</tr>
<tr>
<td>Number of Slices</td>
<td>40</td>
<td>28</td>
</tr>
<tr>
<td>Voxel size (mm)</td>
<td>3.5x3.5x3.5</td>
<td>3.75x3.75x4</td>
</tr>
<tr>
<td>TR (ms)</td>
<td>2340</td>
<td>1500</td>
</tr>
<tr>
<td>TE (ms)</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Number of TRs Acquired</td>
<td>120</td>
<td>140</td>
</tr>
<tr>
<td>Flip Angle (Degrees)</td>
<td>70</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 8.3: Scanners and protocols used for data acquisition

MR imaging with long readout times, such as EPI sequences are particularly sensitive to non-uniformities in the applied magnetic field. These may result in blur and distortion artefacts. In general, the frontal (namely frontal pole, orbitofrontal) and medial temporal cortices are more
susceptible to field inhomogeneities. In order to evaluate brain regions that are susceptible
to such distortions using the different scanner protocols and ensure that they did not overlap
with regions in which group differences were identified, B1 inhomogeneity and field maps were
compared for phantoms and a subject that were imaged using the different protocols. The
phantom was comprised of a cylindrical container filled with 3.75g NISO4 x 6H20 x 5g NaCl
per 1000 g of distilled H2O.

Signal intensity changes dynamically according to changes in the flip angle (FA) when scan-
ning parameters (TR and TE) are fixed [186]. The ratio of signal intensities from images acquired
using two different FAs can therefore provide a measure of spatial irregularity of signal linearity
(i.e. a measure of uniformity of signal intensity). This can be calculated as shown in Equation
8.1.

\[ \text{Ratio} = \frac{S(FA_1, TE_0)}{S(FA_2, TE_0)} \]  (8.1)

Where \( S(FA_1, TE_0) \) and \( S(FA_2, TE_0) \) represent respective signal intensity of the FA1 and FA2
images. The TR/TE was fixed at 4000/47.65 ms and obtained spin echo (SE) EPI sequences at
two FAs of 60 and 120 degrees. The choice of these parameters was based on previously published
simulations that provided high precision with dual angle imaging [69]. Supplementary Figure
8.2 presents the ratio maps for phantoms and an adult control using each protocol. The ratio
maps calculated from signal sensitivity using variable FA showed a similar distribution of signal
intensity between the different protocols in regions found to be significant across groups.

![Figure 8.2: Ratio of signal intensities from spin echo EPI images acquired using variable flip
angles. Spatial distribution of ratio maps is even across both protocols in regions identified as different
between groups. Histogram of ratio maps shows that both protocols result in the majority of voxels
approaching a ratio of 1. Images are scaled identically.](image)
Another important consideration is whether local differences in neurovascular coupling (cerebrovascular reactivity) captured by the different scanners and/or paradigms may have contributed to regional group differences between children with epilepsy and controls. A test was therefore designed to measure BOLD sensitivity among the different scanners and ensure that regions with greatest differences in BOLD sensitivity between scanners did not overlap with group differences. To perform this validation, a healthy subject was placed into both scanners and measured regional BOLD responses related to vasodilation of carbon dioxide (CO2) by means of hypoventilation (breath holding) induced hypercapnia [47, 330]. Transient hypercapnia was used as a global physiological stimulus to alter BOLD contrast. The EPI sequences involved the same parameters described in Table 8.3 with similar pre-processing to that applied for the subjects included in the study. For this analysis, however, motion parameters were not regressed from the general linear model. This was because movement was closely associated with the onset and offset of breath-holding and mean displacement was less than 1 mm. A block design paradigm was utilized with alternating 30 seconds of normal respiration followed by 30 seconds of hypoventilation.

The analysis indeed demonstrated that robust cerebrovascular reactivity was present across the different scanners in the posterior cingulate and bilateral insulae (regions which were found to significantly differ between children with epilepsy and controls; Supplementary Figure 8.3). In fact, subtraction of both images demonstrated that the greatest differences in cerebrovascular reactivity were in regions of the cerebellum, occipital lobes, pineal gland and subcingulate frontal lobes, where no significant group differences were identified. These validation studies therefore indicate that the observed group differences cannot be attributed to regional differences in neurovascular coupling captured by the different scanners and/or paradigms utilized.
Figure 8.3: Cerebrovascular reactivity measured using the different scanner protocols. Robust reactivity was observed using different protocols. Regions that demonstrate greatest differences in cerebrovascular reactivity did not overlap with those showing significant group differences.

8.3 Graph Theoretical Analysis

To perform the graph theoretical analysis all voxels within brain masks in functional space were considered nodes and the Pearson correlation coefficients between all voxels were defined as the edges. Adjacency matrices were constructed describing the relationship of all brain voxels to all other voxels. This was squared to remove negative correlations, as the current analysis involved the identification of hub regions (irrespective of correlation of anti-correlation with other regions, as this was studied subsequently). This approach is advocated by other authors [136]. Following normalization, eigenvector centrality (EC) was calculated, as described in Section 3.2.3 on page 52.

A representative histogram is shown in Figure 3.1 on page 53 demonstrating the distribution of EC values in a typical brain ROI mask. Most often, this followed a power-law distribution, whereby few voxels had high EC and most voxels had very low EC. Following calculation of EC maps, the subjects’ brains were transformed into the space of the MNI152 2 mm atlas for group
comparisons.

8.4 Region-Of-Interest Analysis

In order to investigate functional connectivity of hub regions, whole brain connectivity analysis was performed by correlating the time series of the hubs regions (and other regions of interest [ROIs] in the same network) with the time series of all brain voxels in a generalized linear model (GLM). To account for individual variability in assigning seed points, a 15 mm sphere centred around each initial seed coordinate in MNI was space was used to find a local region of high internal correlation in the functional scans of each participant. An iterative algorithm was used whereby cross-correlation of the mean ROI signal and its surrounding voxels were calculated. With each iteration, the adjacent voxels with the lowest cross-correlation were removed from the ROI until either an internal correlation of 0.7 was achieved or the ROI had eroded to 30 voxels. The timeseries of the eroded ROI was used as a regressor against the timeseries of all other brain voxels. This method may robustly account for individual and age-related differences in the organization of ICNs. This approach was derived from previous studies demonstrating the benefits of an erosion-based method for finding highly self-correlated ROIs in fMRI [139].

The initial seed coordinates were based on a literature review performed by Brier and colleagues [46] who summarized central coordinates for various ICNs, including the default mode network (DMN) as well as task-positive networks, namely the dorsal attention network (DAN), ventral attention network (VAN), control network (CON) and somatosensory network (SMN). To ensure that the erosion procedure did not result in ROIs that were far removed from the original coordinates, the mean coordinates of eroded ROIs were evaluated in all subjects as well as their standard deviations (Table 8.4). As shown, the mean position of the centroid of the eroded ROIs was universally close to the original seed coordinate. Furthermore, the standard deviation in subjects was approximately 5 mm. This suggests that the erosion-based ROI method identifies inter-subject variability without changing the intended network of membership.

Table 8.4: Inter-subject variability in centroid location of eroded ROI based on erosion

<table>
<thead>
<tr>
<th>ROI</th>
<th>Original x (mm)</th>
<th>Mean x (mm)</th>
<th>σ of x (mm)</th>
<th>Original y (mm)</th>
<th>Mean y (mm)</th>
<th>σ of y (mm)</th>
<th>Original z (mm)</th>
<th>Mean z (mm)</th>
<th>σ of z (mm)</th>
</tr>
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<tbody>
<tr>
<td>A PCC</td>
<td>0</td>
<td>2.5</td>
<td>4.8</td>
<td>-51</td>
<td>-55.0</td>
<td>5.7</td>
<td>29</td>
<td>27.9</td>
<td>6.2</td>
</tr>
<tr>
<td>A vmPFC</td>
<td>0</td>
<td>1.2</td>
<td>5.8</td>
<td>61</td>
<td>61.0</td>
<td>5.4</td>
<td>22</td>
<td>19.9</td>
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</tr>
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<td>-46.0</td>
<td>4.7</td>
<td>-66</td>
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<td>5.1</td>
<td>34</td>
<td>35.0</td>
<td>5.7</td>
</tr>
<tr>
<td>A R Parietal</td>
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<td>52.8</td>
<td>5.0</td>
<td>-61</td>
<td>-62.7</td>
<td>4.1</td>
<td>35</td>
<td>33.2</td>
<td>5.1</td>
</tr>
<tr>
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<td>-61.3</td>
<td>5.7</td>
<td>-22</td>
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<td>-11.2</td>
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<td></td>
<td></td>
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<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
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<tr>
<td>C</td>
<td>lAPFC</td>
<td>-45</td>
<td>-42.2</td>
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<td>50.9</td>
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<td>raPFC</td>
<td>-46</td>
<td>-43.5</td>
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<td>49.0</td>
<td></td>
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<tr>
<td>C</td>
<td>rSP</td>
<td>-51</td>
<td>-48.3</td>
<td>5.4</td>
<td>-50</td>
<td>-52.5</td>
<td></td>
<td></td>
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</tr>
<tr>
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<td>lSP</td>
<td>53</td>
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<td>-49</td>
<td>-52.1</td>
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<tr>
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<td>rPG-ACC</td>
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<td>11.7</td>
<td>6.2</td>
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<td>36.7</td>
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<tr>
<td>D</td>
<td>lPG-ACC</td>
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<td>-14.3</td>
<td>6.4</td>
<td>34</td>
<td>39.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>rSG-ACC</td>
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<td>5.3</td>
<td>34</td>
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<td>4.0</td>
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<td>3.9</td>
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<tr>
<td>D</td>
<td>rPut</td>
<td>30</td>
<td>33.4</td>
<td>6.2</td>
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<td>-17.2</td>
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<td></td>
<td></td>
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<tr>
<td>D</td>
<td>lIns</td>
<td>-42</td>
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</tr>
<tr>
<td>D</td>
<td>rIns</td>
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<td>-42.0</td>
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<td>-22.8</td>
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<td></td>
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<td>41</td>
<td>44.8</td>
<td>5.8</td>
<td>-22</td>
<td>-21.0</td>
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<td></td>
<td></td>
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<td>SMA</td>
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<td>0.1</td>
<td>5.2</td>
<td>18</td>
<td>17.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>lV1</td>
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<td>-9.7</td>
<td>4.5</td>
<td>-83</td>
<td>-87.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>rV1</td>
<td>7</td>
<td>8.8</td>
<td>4.5</td>
<td>-83</td>
<td>-85.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>lA1</td>
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<td>-60.7</td>
<td>6.1</td>
<td>-28</td>
<td>-27.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Chapter 8. Appendix 1**
8.5 Supplementary Results

Figure 8.4: Seed-based analysis reveals stronger inter-network connections and weaker intra-network connections in children with secondarily generalized seizures.
Figure 8.5: Seed-based analysis reveals stronger inter-network connectivity and weaker intra-network connectivity with longer duration of epilepsy.
Figure 8.6: DMN network clustering and characteristic pathlength are associated with working memory capacity in children with epilepsy. Increasing DMN clustering and decrease pathlength are associated with higher scores on working memory in children. No group effects or two-way interactions were identified.

Figure 8.7: Increased centrality of the PCC is associated with greater memory capacity in children in controls than children with epilepsy. Greater centrality of the PCC is associated with higher scores on digit span recall and backwards digit span recall in controls compared with children with epilepsy (digit span x group interaction: p=0.048; backwards digit span x group interaction: p=0.043).
Figure 8.8: Greater inter-network anti-correlation and loss of frontal lobe centrality are associated with higher Full-Scale IQ scores.

Greater inter-network anti-correlation is associated with higher Full-Scale IQ scores in children with epilepsy ($p=0.016$), with a trend towards a significant group interaction ($p=0.075$). Loss of frontal lobe centrality is associated with higher IQ scores ($p=0.022$). Although no group interaction was identified ($p=0.47$), children with epilepsy had a slower loss of frontal lobe eigenvector centrality with age ($p<0.001$).
Chapter 9

Appendix 2

9.1 Subject Demographics

Table 9.1: Clinical and demographic information for children included in the study

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Epilepsy Duration (years)</th>
<th>Seizure semiology</th>
<th>Mean seizure duration (s)</th>
<th>Resection Location</th>
<th>Size of SOZ*</th>
<th>Histopathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M</td>
<td>16</td>
<td>6</td>
<td>SPS</td>
<td>7</td>
<td>Frontal</td>
<td>13</td>
<td>FCD IIB</td>
</tr>
<tr>
<td>B</td>
<td>F</td>
<td>12</td>
<td>6</td>
<td>(1) SPS; (2) CPS; (3) SGS</td>
<td>24</td>
<td>Frontal</td>
<td>29</td>
<td>FCD IIA</td>
</tr>
<tr>
<td>C</td>
<td>M</td>
<td>13</td>
<td>6</td>
<td>CPS</td>
<td>14</td>
<td>Frontal</td>
<td>10</td>
<td>FCD IIB</td>
</tr>
<tr>
<td>D</td>
<td>F</td>
<td>13</td>
<td>11</td>
<td>(1) GTC; (2) CPS</td>
<td>12</td>
<td>Frontal</td>
<td>11</td>
<td>FCD IIB</td>
</tr>
<tr>
<td>E</td>
<td>M</td>
<td>15</td>
<td>2</td>
<td>(1) GTC; (2) SPS</td>
<td>15</td>
<td>Parietal</td>
<td>6</td>
<td>FCD IIB</td>
</tr>
<tr>
<td>F</td>
<td>M</td>
<td>5</td>
<td>4</td>
<td>CPS</td>
<td>15</td>
<td>Parietal</td>
<td>19</td>
<td>FCD IIB</td>
</tr>
<tr>
<td>G</td>
<td>F</td>
<td>14</td>
<td>4</td>
<td>(1) SPS; (2) GTC</td>
<td>35</td>
<td>Parietal</td>
<td>13</td>
<td>FCD IIB</td>
</tr>
<tr>
<td>H</td>
<td>M</td>
<td>4</td>
<td>1</td>
<td>CPS</td>
<td>60</td>
<td>Parieto-occipital</td>
<td>7</td>
<td>FCD IIB</td>
</tr>
<tr>
<td>I</td>
<td>M</td>
<td>15</td>
<td>3</td>
<td>(1) CPS; (2) GTC</td>
<td>60</td>
<td>Frontotemporoparietal</td>
<td>2</td>
<td>Suspected FCD</td>
</tr>
<tr>
<td>J</td>
<td>M</td>
<td>7</td>
<td>6</td>
<td>GTC</td>
<td>60</td>
<td>Frontal</td>
<td>1</td>
<td>FCD IIB</td>
</tr>
</tbody>
</table>
### 9.2 Simulation Data

Since epileptic cortex is known to exhibit pHFOs and it was demonstrated within this dataset that the seizure-onset zone contains greater power across multiple frequencies, the current analysis sought to test whether the presence of random pHFOs that may not be associated with a slower cortical rhythm may bias the metric of CFC. Additionally, it has been previously proposed that digital filtering of spikes in ictal and interictal recordings may introduce "false ripples" into the analysis [22]. The effects of spikes in the data on CFC were also evaluated. To assess the effects of spike morphologies and pHFOs on CFC, simulated spikes and pHFOs were added to interictal data (baseline neurological noise) that was free of any spikes and pHFO activity. Spikes and pHFOs were then subsequently added to the data both randomly, and locked to a specific phase of the theta band in the signal.

#### 9.2.1 Baseline Data

Interictal electrocorticographic (ECoG) data absent of any spikes or pHFOs were obtained from an epilepsy patient not included in the current analysis from a channel distant from the seizure-onset zone. These data were used as background activity to ensure a physiologically plausible spectrum. The data were sampled at 2 KHz and were therefore down sampled to 1 KHz to more accurately simulate the sampling rate of the data used in the current study. In order to determine where to place the spikes and pHFOs (randomly or locked to a slower rhythm), the data were first bandpass filtered in the theta-band using finite-impulse response filters, as described in the main article. The instantaneous phase of the filtered signal was then calculated using the Hilbert transformation, as detailed in the methods of the present study.

Markers were then placed at all the points where the phase crossed an arbitrarily determined
point in the theta cycle (at -1.5 radians), resulting in a perfectly time-locked set of markers, relative to the theta-band cycle. In order to avoid having markers that were temporally too close to each other, the time difference between all adjacent markers was determined. Markers that were separated by a time difference shorter than the lower 5th percentile of all time differences were removed. The frequency of the markers (i.e. percentage of theta cycle at which events occurred) was varied in simulation. Two sets of randomly located markers (i.e. not time-locked to the theta cycle) were also generated, with the same length as the theta-locked markers (Supplementary Figure 9.1).

Figure 9.1: Simulated dataset generation.
Top panel: Markers that were time-locked to the theta oscillatory cycle (green) and randomly placed (red) were created in a time series absent of any pHFOs or spiking activity. Bottom panel: Different combinations of random and perfectly time-locked pHFOs and spikes were added to the data and CFC was calculated. Only pHFOs synchronized to the slower cortical rhythm resulted in significant CFC.

9.2.2 Time-Locked and Random Simulated pHFOs and Spikes

Simulated pHFOs were generated by bandpass filtering white noise between 120-400Hz. The theta-synchronized and random pHFO time-series were generated by enveloping the pHFOs with 25ms standard deviation Gaussian pulses centred at the locations specified by the synchronized and randomly-generated markers, respectively. The theta-synchronized and random spike time-series were generated by placing Gaussian pulses centred at the locations specified by the theta-locked and randomly-generated markers, respectively. The width of the Gaussian pulses as well as the amplitude of the simulated pHFOs and spikes were allowed to vary in the simulations.

The test data sets were generated by summing the appropriate simulated theta-synchronized
or random pHFOs and spikes with the baseline ECoG signal. The CFC of 8 test signals were evaluated using the methods discussed in the main text of the article. The test signals were comprised of the following combinations:

1. Random pHFOs with random spikes;
2. Random pHFOs with spikes synchronized to theta phase;
3. pHFOs synchronized to theta phase with random spikes;
4. pHFOs and spikes synchronized to theta phase;
5. Random pHFOs only;
6. pHFOs synchronized to theta phase only;
7. Random spikes only; and
8. Spikes synchronized to theta phase only.

The simulations demonstrate that CFC rejects signals with randomly scattered pHFOs and spikes, and shows that the mere presence of pHFOs, spikes or their combination cannot account for an observed increase in CFC (Table 5.1 on page 103). Furthermore, the theta-synchronized spikes do not cause a significant increase in CFC, suggesting that the observed CFC is neither an artifact of increased pHFOs within epileptic cortex nor the convolution-based signal processing.

### 9.3 Spectral Analysis

The individual spectra properties of the analyzed seizures are presented. The mean seizure length was 85.2 ± 99.3 seconds (range 13.8 - 312 seconds). Firstly the data were passed through a notch filter (Order =4) to exclude line noise (60 Hz and harmonics). Spectrograms of an electrode within the seizure-onset zone (Supplementary Figure 9.2) were created using the short-time Fourier transform with a Hamming window with a length of 200 samples and 75% sample overlap. As evident in the spectrograms, the seizure onset zone contained predominantly low-frequency power, which was fairly heterogeneous across the population. High frequency activity was also evident in all seizures as bursts of high power oscillatory activity. To compare spectral characteristics of electrodes in the seizure-onset zone and outside the seizure-onset zone, power spectral density (PSD) analysis was performed using Welch’s method with a Hamming window of 200 samples and 75% overlap. The results were averaged across subjects (Figure 9.3). The seizure onset zone contained greater broad-band power compared with the non-seizure onset zone.
Figure 9.2: Individual seizure short-time Fourier transform spectrograms.

The seizure onset zone contained predominantly low-frequency power, which was fairly heterogeneous across the population. High frequency activity was also evident in all seizures as bursts of high power oscillatory activity.
9.4 Phase-Cycle Relationships

The data was bandpass filtered into canonical frequency bands using custom-designed filters in the FDAtool graphical user interface of MATLAB. The filters were designed as showed in Table 9.2. It has been previously reported that amplitude-to-phase cycle relationships vary amongst distinct brain regions [62, 190, 12] and between different brain states [226]. It is therefore important when considering cross-frequency coupling (CFC) to relate the peak of high-frequency activity to the phase of low-frequency cycle, at which it occurs.

<table>
<thead>
<tr>
<th></th>
<th>Stop Frequency (1)</th>
<th>Pass Frequency (1)</th>
<th>Pass Frequency (2)</th>
<th>Stop Frequency (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta</td>
<td>0 Hz</td>
<td>2 Hz</td>
<td>4 Hz</td>
<td>6 Hz</td>
</tr>
<tr>
<td>Theta</td>
<td>4 Hz</td>
<td>6 Hz</td>
<td>7 Hz</td>
<td>9 Hz</td>
</tr>
<tr>
<td>Alpha</td>
<td>7 Hz</td>
<td>9 Hz</td>
<td>12 Hz</td>
<td>14 Hz</td>
</tr>
<tr>
<td>Beta</td>
<td>13 Hz</td>
<td>15 Hz</td>
<td>30 Hz</td>
<td>32 Hz</td>
</tr>
<tr>
<td>Gamma</td>
<td>36 Hz</td>
<td>40 Hz</td>
<td>75 Hz</td>
<td>80 Hz</td>
</tr>
<tr>
<td>Ripple</td>
<td>80 Hz</td>
<td>90 Hz</td>
<td>130 Hz</td>
<td>140 Hz</td>
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<tr>
<td>Fast Ripple</td>
<td>185 Hz</td>
<td>200 Hz</td>
<td>285 Hz</td>
<td>290 Hz</td>
</tr>
</tbody>
</table>

Table 9.2: List of FIR filter values used to isolate each physiologically-relevant frequency band.
In this section, the method used to reduce this relationship to a single scalar phase value \( \varphi \), which will represent the low-frequency phase location at which the maximum high-frequency amplitude occurs is demonstrated. The evolution of \( \varphi \) over the duration of the seizure may then be observed to determine whether this phase-amplitude relationship was consistent or dynamic as the seizure progressed. The procedure is demonstrated with the example plot in Figure 9.4, which is a typical set of mean high-frequency amplitudes, binned by low-frequency phase.

If \( a \) was an ideal sinusoid, determining the location of its maximum would be trivial. As it is instead noisy data which may have multiple maxima, one approach is to project it onto an ideal sinusoid (Supplementary Figure 9.4B) and sine (Supplementary Figure 9.4C) waves at the fundamental frequency with an equal number of coefficients (i.e. 60 coefficients). This is achieved by calculating the dot product; i.e. multiplying corresponding entries and summing those products, as shown in Equation 9.1.

\[
a \cdot b = \sum_{i=1}^{n} a_i b_i = a_1 b_1 + a_2 b_2 + \ldots + a_n b_n \tag{9.1}
\]

Where \( b \) is either the ideal sine \( b_{\text{sin}} \) or cosine wave, \( b_{\text{cos}} \). The resulting scalar value is a measure of affinity between \( a \) and each of \( b_{\text{sin}} \) and \( b_{\text{cos}} \); or in other words, it is a relative measure of how much \( b_{\text{sin}} \) and \( b_{\text{cos}} \) is in \( a \).

The period of the cosine and sine waves was chosen to be \( 2\pi \) so that it would exactly span across the bins of the low frequency phase/amplitude plot (Supplementary Figure 9.4A). Essentially, this projection found how far (in radians) the signal \( a \) was shifted from that of the sine and cosine waves. Since the maxima of the sine/cosine waves are constant, one can then quantify where (along the phase of the low frequency signal) the peak of the high frequency signal occurs. A phasor was subsequently created, according to Euler’s formula, from these two projection coefficients. The real component was the projection of amplitude-phase relationship onto the cosine wave, and the imaginary component was the projection of the amplitude-phase relationship onto the sine wave, as shown in Equation 9.2.

\[
A e^{i\varphi} = a \cdot b_{\text{cos}} + i a \cdot b_{\text{sin}} \tag{9.2}
\]

Where \( \varphi \) is the argument (angle) of the phasor (i.e. the arctangent of real and imaginary components consisting of the projection of \( a \) with the cosine and sine waves respectively) and the corresponding position along the low-frequency phase cycle. Note that the magnitude of the phasor, \( A \), represents the amplitude of the projected sinusoid. This phasor is illustrated in Supplementary Figure 9.4D. The angle \( \varphi \) is then added to a polar histogram, as shown in Supplementary Figure 9.4E. This process was repeated for each time interval for each of the
analyzed seizures.

Figure 9.4: Quantification of Amplitude-to-Phase Cycle Relationship.  
(A) Mean amplitudes of high frequency oscillations, sorted by concurrent low-frequency phase into 60 bins of 0.105 Radians, for an example time window during a seizure; (B) An ideal cosine; and (C) a sine is modeled. (D) Phasor demonstrating the amplitude-phase cycle relationship. (E) The argument (angle) of the example phasor is a single contribution to be incremented onto a cumulative polar histogram spanning multiple subjects for one of ten given time periods in the seizure.

The goal of defining the phasor is to quantify dynamic changes in the amplitude-to-phase
relationship as the seizure progressed. Seizures were divided into 10 non-overlapping bins and a single phasor was derived to describe the amplitude-to-phase cycle relationship for each bin. This was done since performing high resolution analysis with shorter time windows may not capture generalizable dynamics with seizure progression. Therefore, ten individual polar histograms may be plotted to separate the amplitude-to-phase cycle relationships by their timing in the seizure in order to show their dynamic behaviour as the seizure progressed. Supplementary Figure 9.5, shows the expected polar histogram for each of the four discrete possible scenarios where amplitude peak occurs at the \(-\pi\) (or \(\pi\)), \(-\frac{\pi}{2}\), \(0\), or \(\frac{\pi}{2}\) position of the low-frequency oscillatory cycle. As shown, when high frequency amplitude occurs at the peak and trough of the low frequency phase, the polar histograms will point towards \(\pi\) and 0, respectively.
Figure 9.5: Simulated data demonstrating expected polar histogram distribution. The high frequency amplitude is represented by the blue solid line, whereas the low-frequency phase is represented by the black dashed line. When the high frequency amplitude is maximal at the peak and trough of the low frequency phase, the polar histograms will indicate $\pi$ and $0$, respectively.

To reduce extraneous noise and increase the sensitivity of the angle measurements, a threshold was applied to the data to exclude bins whose phasors have low signal magnitude, which was accepted as the bins with the lowest 25% of the magnitude. In order to ensure that this threshold does not bias the data, three validation analyses were performed. The first was an evaluation of all amplitude-to-phase cycles relationships in all bins for all seizures without any threshold. The second employed the statistical threshold, as aforementioned, and the third employed a statistical threshold. The statistical threshold excluded bins where the sorted amplitude-phase relationship did not exceed the 95% confidence limits, as determined by the statistical surrogation method. Of note, if no consistent relationship between amplitude and phase were present,
the distribution of the polar plots would be expected to be circular symmetric. As shown in Supplementary Figure 9.6, there was no difference between distribution of phases for all bins using any of these methods; however, when evaluating the first and last bins of each seizure, the proportional method was associated with least noise and greatest precision in amplitude-to-phase cycle relationships.

Figure 9.6: Comparison of three methods to reduce extraneous noise in amplitude-to-phase relationships.

The first column shows all seizure bins, whereas the second and third columns show the first and last bin of all seizures, respectively for fast-ripple to alpha phase relationships.

The MATLAB code for phase-cycle analyses is as follows:

```matlab
%%
% Inputs:
% data  amplitude (of high frequency bands) sorted by the
% phase (of low frequency bands)
% % Create cosine and sine waves with the same number of
% coefficients as data (i.e. 60):
% cosinewave=cos(((1:60)/60)*2*pi);%  
% sinewave=sin(((1:60)/60)*2*pi);%  
% % Calculate dot product of amplitude-phase relationship and
% % cosine and sine waves:
% proj_cos=sum(data.*cosinewave);
```
proj_sin = sum(data.*sinwave);
% Create and plot phasor of dot product and calculate its
% argument (angle) and magnitude:
phasor = proj_cos + i*proj_sin; figure;
axis([imag(phasor) real(phasor)]);
angle = atan2(proj_sin,proj_cos);
magnitude = abs(phasor);
% Once angles and magnitudes of phasors for all time
% intervals for a given seizure have been calculated
% (vector ANGLE and MAGNITUDE respectively), we may
% threshold phase proportionally by amplitude to reduce
% noise:
[aa bb] = hist(MAGNITUDE,1000);
cc = cumsum(aa); cc = cc/(cc(end) + 1e-8);
mask = (MAGNITUDE > bb(find(cc > 0.25,1)-1));
ANGLE = ANGLE(mask);
% The result may be plotted on a polar histogram:
rose(ANGLE);

9.4.1 Electrode Dichotomization Based on CFC

It was observed that epileptogenic cortex (seizure-onset zone and early propagation zone) demonstrates greater CFC than non-epileptogenic zone. The current analysis subsequently sought to dichotomize electrodes based on CFC in order to evaluate and further quantify the significance of this relationship. To do so, an automated algorithm was developed to identify electrodes expressing high CFC. Initially, the modulation matrix of each electrode was considered individually. Each modulation matrix was composed of 150 bins of 2 Hz bandwidth for amplitude at 20 bins of 2 Hz bandwidth for phase, yielding a total of 3000 cross frequency pairs (Supplementary Figure 9.7A). The sum of elements (i.e. cross-frequency pairs) that exceeded the threshold for significance (corrected) was calculated (Supplementary Figure 9.7B). This is essentially equal to the area under the curve following Bonferroni correction. The centroid of the distribution was calculated, and electrodes exceeding this threshold were considered to possess high CFC, whereas the electrodes below this threshold were considered to have low CFC. Supplementary Figure 9.7C demonstrates electrodes that were identified as having high CFC in one representative subject. Singleton electrodes (i.e. those with high CFC, but all their nearest neighbours possessed low CFC) were excluded.
Figure 9.7: Electrode dichotomy based on cross-frequency coupling signature. (A) Topographic representation cross-frequency coupling signature for all electrodes analyzed. Boxed electrodes represent the seizure-onset zone. (B) Dichotomy strategy whereby the sum of all elements above the statistical threshold is plotted for each electrode and a dichotomy threshold is established based on the scatter distribution. Blue represents electrodes that were subsequently resected; red represents electrodes that were not subsequently resected. (C) Output of automated dichotomy algorithm, whereby electrodes expressing high CFC are identified.

Once the electrodes were dichotomized for all subjects based on their CFC signatures (i.e. elevated versus non-elevated), a 2x2 table was created as shown in Supplementary Figure 9.8. Electrodes within the SOZ were significantly more likely to exhibit elevated CFC than those outside the SOZ ($p<0.0001$; Fisher’s Exact Test).
As determined by Fisher’s exact test, electrodes within the SOZ were significantly more likely to exhibit elevated CFC than those outside the SOZ (p<0.0001).

<table>
<thead>
<tr>
<th>Seizure Onset Zone</th>
<th>Elevated CFC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>120</td>
<td>51</td>
</tr>
<tr>
<td>No</td>
<td>308</td>
<td>1223</td>
</tr>
<tr>
<td></td>
<td>428</td>
<td>1274</td>
</tr>
</tbody>
</table>

Figure 9.8: Two-by-two table contrasting elevated CFC in electrodes within the SOZ.

9.4.2 Sensitivity and Specificity of CFC in Identifying Epileptogenic Cortex

Although the previous analyses have shown that CFCs are topographically concentrated in epileptogenic regions, it appears that several electrodes within these areas also did not exhibit significant CFC (please refer to Figure 5.4 on page 101). To better characterize the relationship between CFC and epileptogenic regions, the sensitivity and specificity of excessive CFC in identifying electrodes that were subsequently resected were calculated (Table 9.3). The positive and negative predictive values for CFC were also computed as well as the agreement between resected regions and regions expressing elevated CFC, as indexed by Cohen’s kappa value [66].
### Table 9.3: Sensitivity and specificity of CFC in identifying resected cortex.

* Denotes significant agreement p<0.05; ** Denotes significant agreement p<0.01; Kappa score denotes agreement on a scale of 0 to 1; all other values denote percentages

Elevated CFC demonstrated high specificity in identifying epileptogenic cortex (79%-100%) across subjects, further emphasizing that CFC is highly localized to epileptogenic cortices. The metric, however, showed low sensitivity and moderate positive and negative predictive values, as well as heterogeneous agreement with resection. This suggests that not all regions included in the putative epileptogenic zone may necessarily express CFC.

#### 9.4.3 Changes in Amplitude-to-Phase Relationships for Different Low-Frequency Rhythms

We sorted ripple and fast-ripple amplitude into bins of delta, theta, alpha and beta phase. Dynamic cross-frequency amplitude-to-phase relationships for both fast-ripple and ripple amplitudes were evident only for coupling with alpha phase. In comparison to the beginning of seizures, seizure termination was characterized by the occurrence of high frequency amplitude maxima at the trough of alpha phase. Ripple frequency amplitudes, however, also demonstrate cross-frequency coupling with the phase of delta, theta and beta frequency signals, although
there were no evident dynamic changes with seizure progression (Supplementary Figure 9.9). The peak of ripple amplitudes occurred at the peak of delta phase irrespective of the timing during seizure. Furthermore, cumulatively, ripple amplitudes tended to peak at the $\pi/2$ position and trough of theta and beta cycles, respectively.

Figure 9.9: Changes in relationship between fast-ripple and ripple amplitudes and delta, theta, alpha and beta phase throughout seizure. Fast-ripple amplitude generally demonstrate significant phase-locking to the trough of alpha phase, however this was not significant at the beginning of seizure (which tended towards the $-\pi/2$ position), but was significant near the end of the seizure. Ripple amplitude generally demonstrate significant phase-locking to the oscillatory cycle of multiple low-frequency bands. The peak of ripple amplitude was locked to the peak of the delta phase cycle, $\pi/2$ position of theta and alpha. During the last tenth of the seizure, ripple amplitude remained locked to delta phase peak, but demonstrated a shift towards alpha trough. First column of each row denotes cumulative bins for all seizures; second column: first tenth of seizure; last column: last tenth of seizure.
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