Structural and Functional Abnormalities on Magnetic Resonance Imaging in
Idiopathic Generalized Epilepsy

By

Megan McGill

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Thomas Thesen, PhD
Dedication

This thesis is dedicated to my son Jasper, who taught me that work should be play and play is often work.
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Abstract

Idiopathic generalized epilepsy (IGE) is a neurologic disorder in which periods of normal neuronal activity are suspended by brief intervals of spontaneous, synchronous spike-and-wave discharges and clinical manifestations of seizures. Though the brains of patients appear completely normal on diagnostic imaging, including magnetic resonance imaging (MRI), the 3 Hz synchronous oscillatory features on electroencephalography (EEG) cast little doubt that pathological neuronal function causes the manifestation of seizures. Given our limited ability to study human brains in vivo, the main aim of the research presented here is to show the extent to which emerging MRI techniques can be used to quantify brain abnormalities, even when standard MRI scans reveal no obvious pathology.

This thesis reviews the most predominant manifestations of generalized epilepsy including myoclonic, absence and general tonic-clonic seizures. A close examination of evidence supporting corticocentric, centrencephalic and cortico-reticular theories of the primary cause of epileptogenesis is presented in the context of evolving animal models of IGE and electrophysiologic recordings. Finally, I highlight MRI techniques and analyses that can be employed to quantitatively measure morphometric differences, the integrity of anatomic networks and functional abnormalities focally and in distributed networks. $T_1$-
weighted MRI scans, diffusion tensor imaging (DTI) and resting state functional
MRI (fMRI) provide an integrated means to study focal and network pathology in
IGE.

We collected MRI scans from 27 patients with IGE and 27 age- and sex-
matched controls. To study cortical abnormalities, we used morphometric
measures including cortical thickness and gray-white contrast comparing IGEs to
normal controls. We also studied a cortical functional network, the default mode
network (DMN) to determine if widely distributed functional architecture is
preserved in generalized epilepsy. Because abnormal activity in the thalamus is
thought to be the primary node of pathologic activity in IGE, we performed
volumetric studies of the thalamus, correlated volumes with cortical measures and
studied the fractional amplitude of low frequency fluctuations (fALFF) in specific
thalamic subregions. Finally, DTI was employed to examine the integrity of
tracts throughout the brain and tracts connecting the thalamus and prefrontal
cortex, which is thought to be preferentially involved in IGE.

Our results fail to show morphometric network abnormalities in IGE.
Differences in resting state functional connectivity of the DMN point to abnormal
integration of the anterior medial prefrontal cortex (amPFC) and fALFF analysis
in the thalamus shows decreased amplitude of the BOLD signal in subregions that
are connected to the prefrontal cortex and premotor cortex, as well as the entire
thalamus. The lack of structural changes that can be quantified by cortical
thickness and gray-white blurring measures, in addition to normal thalamic volumes compared to controls in our study population, suggest that cell structures remain intact in IGE, though only histopathological studies would confirm this. Rather, abnormal function in the prefrontal cortex and correlated areas of the thalamus exist during interictal periods. Deviant or unregulated activity of voltage-gated ion channels are potential candidates of primary pathology contributing to spontaneous 3 Hz synchronous discharges throughout the brain. These findings are discussed in the context of the current literature on IGE. Study limitations and recommendations for future work are explored.
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1. INTRODUCTION

1.1 EPILEPSY

Epilepsy is a neurologic disease characterized by a predisposition to have recurrent seizures, which are the hallmark of the disease. An epileptic seizure is “a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain” (Fisher et al., 2005). Seizures are clinical events that can affect sensory and motor activity; consciousness; memory; behavior; emotions; autonomic functioning; and post-ictal states often contribute to changes in behavior or sensation after the epileptic activity itself. Though at least one seizure is required for an epilepsy diagnosis, not all people who experience a seizure are diagnosed with epilepsy. It is estimated that 10% of the population has experienced, at some point, seizure activity, primarily as a febrile seizure in youth but only 1% of the population is diagnosed with epilepsy by the age of 20 (Hauser, Annegers, & Rocca, 1996).

Epilepsy is not a singular disease entity but a variety of disorders reflecting underlying brain dysfunction. Electroencephalography (EEG) recordings aid in the diagnosis of seizures, though are not required as some patients demonstrate normal brain activity between ictal events. The ictal
enhanced synchrony of neurons imposed on an otherwise normal background is characteristic on EEG from epilepsy patients.

The International League Against Epilepsy (ILAE) classifies seizures as partial or generalized (Panayiotopoulos, 2011). Partial seizures arise from a localized epileptogenic zone, which may spread throughout the brain. Generalized seizures involve both hemispheres simultaneously and show no focus of seizure initiation (Engel, 2001). Loss of consciousness can be present in both partial and generalized seizures, though the terms “complex partial” and “simple partial,” previously used to denote unconscious and conscious seizures, respectively, are now obsolete (Berg et al., 2010).

Idiopathic generalized epilepsy (IGE) encompasses three classes of seizures: myoclonic, absence and/or generalized tonic-clonic seizures (GTCS). These seizure types associated with IGE can occur alone or in varying combinations and with variable severity (Mattson, 2003). Though the clinical manifestations of these seizures vary greatly, all show generalized epileptiform discharges seen as 3 Hz spike and wave discharges synchronously across the entire brain during ictal events. On visual inspection, all brain MRIs from patients with IGE look normal and no focus of abnormal anatomy can be detected (Koutroumanidis, Aggelakis, & Panayiotopoulos, 2008; Mattson, 2003). Intellectual functioning and neurological examination are typically normal as
well. The main classes of generalized epilepsy are described in more detail below.

1.1.1 Juvenile Myoclonic Epilepsy

Juvenile Myoclonic Epilepsy (JME) is one of the manifestations of IGE. Janz, when first describing it, called JME “Impulsiv Petit Mal” but it has also been referred to as myoclonic epilepsy of adolescence and benign myoclonic juvenile epilepsy (Janz, 1985). JME has a prevalence of 10-20 per 100,000 persons (Jallon & Latour, 2005) and most cases present in teenage years. The myoclonic jerks of JME are co-morbid with general tonic-clonic seizures in 80-97% of cases of IGE and occur in patients with a history of absence seizures in up to 54% of cases (Mehdiratta & Aggarwal, 2002). JME is relatively benign with respect to mortality, with premature death reported in 9 per 10,000 patient-years (Genton & Gelisse, 2001).

The diagnosis of JME rests on identification of myoclonic jerks, 3-4 Hz spike-wave discharges on EEG, a normal neurologic exam and normal appearing MRI on visual inspection (Panayiotopoulos, Tahan, & Obeid, 1991). Because of the nature of its presentation, namely limb jerks that can be asymmetric, JME is often misdiagnosed as partial epilepsy. These jerks can also go unappreciated, leading to an under-diagnosis of JME (Renganathan & Delanty, 2003).
Ictal myoclonic jerks themselves are usually bilateral, abrupt, involuntary movements, predominantly in the shoulders or arms (Faught, 2003; Renganathan & Delanty, 2003). Consciousness is preserved throughout these seizures. The jerks often occur on waking from sleep or a nap (Pedersen & Petersen, 1998). Precipitating factors for myoclonic jerks are sleep deprivation (89.5% of cases), fatigue (73.7%), photosensitivity (36.8%), menses (24.1%), intense concentration (22.8%), stress (12.3%), and alcohol ingestion (51.2%) (Panayiotopoulos, Obeid, & Tahan, 1994; Pedersen & Petersen, 1998).

Familial clustering of JME cases has opened the possibility of genetic inheritance. Although absent a clear inheritance pattern, both polygenic mechanisms and heterogeneous penetrance have been proposed. Genetic studies using genome-wide associations and candidate gene approaches have attempted to identify the genetic basis for JME. Genes encoding a voltage-dependent potassium channel (Zifkin, Andermann, & Andermann, 2005), and the voltage-gated Cl\(^{-}\) channel ClC-2 (Gardiner, 2005), have been implicated in JME. In a French-Canadian family showing autosomal dominant inheritance of JME, a missense mutation in a gene that codes for a subunit of the Gamma-Aminobutyric Acid (GABA)\(_{A}\) channel has been identified (Delgado-Escueta et al., 2003).

The first line of treatment for JME is valproic acid (VPA) (Glauser et al., 2006). VPA enhances the transmission of GABA and blocks sodium channels and T-type calcium currents (Rosenberg, 2007). If VPA monotherapy fails to
control seizures or jerks, the second line is usually lamotrigine, though clobazam, levetiracetam and topiramate are also frequently prescribed (Bergey, 2005).

1.1.2 Absence Epilepsy

Absence seizures are another manifestation of idiopathic generalized epilepsy. Like other forms of IGE, absence seizures show slow, 3 Hz discharges that are synchronous and bilateral throughout the brain on scalp EEG. Clinical features include arrest of activity, loss of awareness, staring spells and occasional eyelid movements (Sadleir, Farrell, Smith, Connolly, & Scheffer, 2006). Seizures typically last a few seconds and often occur multiple times a day. Reports suggest a higher incidence in females than males (Durá Travé & Yoldi Petri, 2006; Ureña-Hornos et al., 2004). Absence seizures are common, with 10-12% of children with seizures experiencing absences (M. Tanaka et al., 2008). Many cases may go undiagnosed, especially in children who are seen as being inattentive or with declining school performance (Durá Travé & Yoldi Petri, 2006).

Though the mean age of onset of absence seizures is 7.5 years (Ureña-Hornos, et al., 2004), the ILAE differentiates between childhood absence epilepsy (CAE) and juvenile absence epilepsy (JAE). In JAE, seizures usually start around age 12 years while in CAE, seizures appear between ages 6-8 years (Valentin et al., 2007). CAE carries a more favorable prognosis and likely spontaneous
seizure remission (Malik, Hamid, Ahmed, & Ali, 2008). Though less common, absence epilepsy in infants can be diagnosed, with mean onset age at 20 months, though absences with recorded 3 Hz slow wave (SW) discharges on EEG have been seen as early as 12 months (Shahar et al., 2007). Absence epilepsy in infants carries a favorable prognosis, is often well-controlled on anti-epileptic medication, and cognitive skills are in the normal range at school age (Shahar, et al., 2007).

Comorbid conditions with absence seizures can be distinguished. As in all forms of generalized epilepsy, general tonic-clonic seizures (GTCS) are also common, with nearly half of those with absence epilepsy experiencing a GTCS (Tovia, Goldberg-Stern, Shahar, & Kramer, 2006). The occurrence of a GTCS in absence epilepsy is predictive of a poorer outcome, as these patients are more likely to relapse after stopping medication. Also recognized is absence epilepsy with eyelid myoclonia (EM), which is described as a marked jerking of eyeballs, often with eye closure, which is seen in about 9% of cases (Burneo, Miller, Bebin, & Prasad, 2004). Compared to absence epilepsy patients without EM, those with EM are typically older at presentation and have a higher incidence of developmental delay (Joshi & Patrick, 2007). In EM patients, paroxysmal bursts in the occipital area precede more general spike-wave discharges on EEG (Ogura, Maegaki, & Koeda, 2005). The majority of absence epilepsy patients with EM also experience tonic-clonic seizures. Lastly, absence epilepsy with or without
photosensitivity has been described (Baykan, Matur, Gürses, Aykutlu, & Gökyiğit, 2005). This occurs more commonly in juvenile absence epilepsy and females (82%). These patients are also less likely to have spontaneous remission (Baykan, et al., 2005).

1.1.3 General Tonic-Clonic Seizures Only

Idiopathic generalized epilepsy with general tonic-clonic seizures only is a separate entity from other IGE syndromes, however an inability to recognize a history of absences or mild myoclonic jerks may lead to an over-diagnosis of IGE with GTCS only. Diagnostic criteria for this syndrome include the mandate that an individual has suffered at least one GTCS (with no criteria stipulating the timing or frequency of such seizures), general slow wave discharges on EEG and no other history of seizures including absences or myoclonia (Fisher, et al., 2005).

The cohort of patients that experiences only GTCS shares similar phenomenology of their seizures. Typically GTCS occur mainly within the first one to two hours after waking (Janz, 2000). There is no gender predominance. A comparison of IGE-GTCS only and IGE with GTCS and absences showed that GTCS were twice as frequent in the tonic-clonic only group (Koutroumanidis, et al., 2008). Also, this group tended to show GTCS more in the morning while GTCS timing was more variable when co-morbid with other seizures. There is
also a stronger genetic background contributing to the GTCS syndrome (Unterberger, Trinka, Luef, & Bauer, 2001).

1.1.4 Conclusions

The shared EEG signature, namely the spontaneous and synchronous 3 Hz discharge throughout the brain and normal appearing MRI link all subtypes of IGE. The propensity for multiple seizure types to co-exist suggests that a common underlying pathology contributes to the several manifestations of IGE. Furthermore, it is not always clear that a diagnosis of a single type of IGE is accurate, as a history of absences or myoclonic jerks may go unnoticed or unappreciated as seizure activity. In the MRI analyses described here, all subtypes of IGE are studied together in an attempt to discern cortical and subcortical abnormalities that exist across patients with generalized epilepsy.

1.2 A HISTORY OF THE THALMIC- CORTICAL DEBATE

1.2.1 Early theories and the role of the thalamus

The 3 Hz spike-and-wave discharges seen on EEG in generalized epilepsy are not only synchronous and bilateral, but occur seemingly throughout the brain from their onset. But to what extent are generalized seizures really generalized?
More specifically, does every neuron exhibit 3 Hz oscillations uniformly throughout the brain or are there specific nodes that are responsible for this synchronous activity? Ever since Hans Berger in 1933 first identified the generalized nature of seizure activity in an EEG recording from a single patient during an absence seizure, there has been intense debate over the primary brain structures that contribute to the spike-wave phenomena (Berger, 1933). Berger postulated that these seizures manifest upon withdrawal of thalamic inhibitory influences on the cortex. This hypothesis framed what would become an ongoing debate over the roles of the thalamus and the cortex in generalized seizures, with researchers in each camp presenting evidence pointing to cortical and subcortical pathology. As animal models of absence and general tonic-clonic seizures developed, the ability to study neuroanatomy and physiology progressed (Marcus & Watson, 1966; Prince & Farrell, 1969; van Luijtelaar & Coenen, 1986; Vergnes et al., 1982).

The paroxysmal cerebral dysrhythmia described by Gibbs in 1937 over the entire brain established a cortical-centric view of generalized epilepsy (Gibbs, Gibb, & Lennox, 1937). In an era when discrete areas of the neuroanatomical architecture were being assigned to specific functions, Gibbs’ view established a de-localizing and diffuse theory of these seizures. The notion of a localizing understanding of brain function resurfaced when Jasper and Penfield (1947) put forth their ‘centrencephalic’ view of seizures (Penfield & Jasper, 1947). They
argued, again, that the primary abnormality lies in thalamic generation of spike and wave activity. Jasper and Droogleever-Fortuyn showed that disrupting activity in parts of the thalamus could produce seizure-like activity in the cortex (Jasper & Droogleever-Fortuyn, 1946). Shortly thereafter, Williams highlighted thalamic involvement during seizures by demonstrating that oscillations start there before signs of seizure appear on EEG (D. Williams, 1953). Additionally, Weir showed that by applying a 3 Hz stimulus to the midbrain, he could elicit the same oscillatory behavior in the cortex of cats (Weir, 1965).

1.2.2 Evidence emerges that supports a primary cortical abnormality

The discovery of animal models of generalized epilepsy created a platform to study both in vitro and in vivo mechanisms that contribute to slow, whole-brain oscillations. The feline generalized penicillin epilepsy (FGPE) model described by Prince and Farrell had EEG features similar to a 9 year-old boy with childhood absence epilepsy (Prince & Farrell, 1969). 3 Hz spike-and-wave activity could be recorded from the cortex and the thalamus, and this model would later be used to show both cortical and thalamic abnormalities in IGE. Marcus and Watson (1966) were able to show that applying convulsants to the frontal lobes of cats produces cortical spike-wave discharges, even when subcortical structures are removed but with the corpus callosum intact (Marcus & Watson, 1966). The baboon species, papio-papio, that experiences general tonic-clonic seizures upon
Photic stimulations was used by Fischer-Williams and his colleagues to show that SW discharges in GTCS are initiated in the frontal lobe before spreading to other cortical and subcortical structures (Fischer-Williams, Poncet, Riche, & Naquet, 1968). They found that these baboons have a hyperexcitable frontal cortex and that the corpus callosum must be intact for bilateral, synchronous activity. In humans, bilaterally synchronous slow wave discharges were induced by stimulating the mesial frontal lobe in patients with absence seizures (Bancaud et al., 1974). Evidence from these studies in animals contributed considerable attention to the cortex as the primary site of abnormalities in IGE.

1.2.3 The corticoreticular hypothesis

Further studies of the thalamus and cortex shifted to new paradigms to explain generalized epilepsy. Pierre Gloor put forth the term ‘corticoreticular’ to include both cortical and subcortical features in the way we think about the pathogenesis of spike-and-wave features. He proposed that a breakdown of inhibition regulating cortical-reticular interaction contributes to the bilateral asynchronous discharges (Gloor, 1968). Manipulations of the cortex or ablations of reticular nuclei of the thalamus were shown to abolish slow-wave oscillations in genetic rat models of epilepsy (Vergnes & Marescaux, 1992). Data from Avoli and Gloor demonstrated that both cortical and thalamic neurons participate in the
SW firing pattern in FGPE cats by undergoing periods of mutually phase-locked cyclic alternations of excitation and inhibition at the frequency of the EEG SW rhythm (Avoli, Gloor, Kostopoulos, & Gotman, 1983). Further studies on this cat model show that the ‘spike’ is associated with increased firing and action potentials, while the ‘wave’ is a function of Cl– mediated inhibition (Fisher & Prince, 1977). Anatomic integrity is required between a rhythmogenic thalamus and hyperexcitable cortex for SW generalized discharges.

1.2.4 The volley of the thalamic and cortical debate

With the discovery of T-type Ca2+ channels, the focus of study again shifted back to the role of the thalamus (Jahnsen & Llinás, 1984). This calcium channel is de-inactivated by a hyperpolarizing current, leading to less polarization and bursting action potentials. The bursting action potentials affect thalamic relay neurons, eliciting a GABA-mediated inhibition and creating an oscillatory feature of firing generated in the thalamus (Steriade, McCormick, & Sejnowski, 1993). Genetic mutations in a subunit of the GABA_A channel, found only in thalamic reticular cells, greatly reduces its inhibition causing more hyperpolarizing-induced Ca2+ current from the T-type Ca2+ channel, more action potential bursting, and increased oscillatory synchrony. GABA antagonists in the thalamus were shown to abolish SW discharges (Liu, Vergnes, Depaulis, & Marescaux, 1992). Furthermore, Coulter showed that ethosuximide, an effective drug for
absence seizures, acts on thalamic T-type Ca\(^{2+}\) channels and GABA-ergic neurons reducing bursting in thalamocortical relay cells (Coulter, Huguenard, & Prince, 1990a, 1990b).

Steriade highlighted again the role of the cortex by suggesting that the 3 Hz phenomena is cortical in origin which activates thalamic reticular cells and inhibitory postsynaptic potentials (IPSPs) in thalamocortical relay cells (Steriade, 1997). Two studies further pointed to cortical excitability and its role in regulating the oscillatory nature of thalamic cells. By adding a stimulus to corticothalamic cells in phase with thalamic relay cells, spindle rhythms transformed to SW discharge (Blumenfeld & McCormick, 2000). A proponent of cortical origin theories, Meeren showed that recording from the Wistar Absence Glaxo from Rijswik (WAG/Rij) mouse model of absence seizures pointed to a focal cortical area where SW discharges start first and that cortical bursting even preceded thalamic bursting (Meeren, Pijn, Van Luijtelaar, Coenen, & Lopes da Silva, 2002).

Evidence for a primary thalamic abnormality in generalized epilepsy comes from studies of the genetic absence epilepsy rats from Strasbourg (GAERS) animal model. Here, GABA receptor currents are specifically abnormal in thalamic reticular neurons, not corticothalamic neurons, and disinhibition of reticular cells may promote SW discharges, alluding to a primary thalamic abnormality. In 2009, Cope et al., suggested that a lack of GABA uptake, and
hence tonic inhibition of thalamocortical relay cells, is a common feature of these seizures (Cope et al., 2009).

1.2.5 Conclusions from the thalamic-cortical debate in animal models

Because patients with IGE are not currently candidates for neurosurgery and thus are not candidates for the placement of intracranial electrodes, electrophysiology studies in humans are not possible. Animal models approximate the human condition but basic differences exist. Spike-wave discharges in rat models occur at 5-10 Hz rather than the 3-4 Hz frequency seen in humans (Danover, Deransart, Depaulis, Vergnes, & Marescaux, 1998). However, these animals provide a substrate to study how abnormal and intense firing in a region of the cortex or thalamus converts the oscillatory behavior of the entire network. And only in these models can we study the mechanism responsible for this transformation. Progression of accepted views of the pathogenesis of generalized seizures is highlighted by examining the history of manipulations and physiology studies in rats, cats, monkeys and baboons. Furthermore, brain slices from these animals lend themselves to examination of pathology at the cellular level. But still the thalamic-cortical debate continues as new evidence emerges. Whether the primary pathology in humans is thalamic or cortical in nature, or truly an abnormality of thalamo-cortical network, remains unanswered.
2. IMAGING

2.1 The Role of MRI in Focal and Network Imaging

The ability to study neuropathology non-invasively using imaging techniques is of great importance. While animal models can provide information to elucidate the neurobiological processes underlying seizures, the use of MRI in people can identify focal and network pathology specific to human anatomy.

Neuronal activity that gives rise to cognition, behavior, motor output and homeostatic functions, does not exist independently from widely distributed networks in the brain. The central nervous system consists of numerous distinct structures yet it is the integration of diverse activity from these varied regions that gives rise to brain function. Conventional MRI provides information about anatomy and anatomical pathology but does not well characterize function of the brain. Clinically, a network perspective of brain function points to physiological effects that are widely distributed and more thoroughly characterizes normal from pathological function.

Recent developments in MRI techniques have the potential for clinical utility but such tools are underutilized in the clinical setting. Functional
architecture, microstructural environments and their disturbances in neuro-pathology can be studied using quantitative morphometric analyses, diffusion tensor imaging (DTI), and resting state fMRI. The ability to apply these MRI techniques to patients suffering from psychiatric illness and neurologic diseases not only informs the study of network interactions throughout the brain but also has implications for diagnosis, prognosis, neurosurgical planning, response to therapeutics and recovery after neurologic insult.

2.2 The Basis of MR Image Formation

Nuclear magnetic resonance imaging is based on the energy and spin properties of individual atoms, usually hydrogen atoms. Generation of the MR signal is dependent upon a set of physical principles, primarily that of nuclear spin. In an external magnetic field, atomic nuclei will exhibit a precessional spin, much like that of a gyroscope. Spins parallel to the magnetic field are in a low-energy state while those whose longitudinal plane of spin is anti-parallel to the magnetic field assume a higher energy state. Spins can be excited to the higher energy state when an electromagnetic pulse is applied. The Larmor frequency is the frequency of a pulse that oscillates at the same frequency as the spins of the atoms in question. Because hydrogen atoms are ubiquitous in all tissues, MR machines are tuned to their precessional frequency at any given magnetic field
strength. The precessional frequency or Larmor frequency, $v$, is given by the equation:

$$v = \frac{\gamma}{2\pi} B_0$$

For any given nucleus, $\gamma/2\pi$ is constant. The gyromagnetic ratio ($\gamma$) is the ratio between the charge and mass of a spin and for hydrogen is $2.67 \times 10^8$. 

$B_0$ is the magnetic field generated by the MRI scanner. The Larmor frequency of hydrogen in a 3 Tesla (T) scanner is 127.6 MHz.

By applying a pulse at the Larmor frequency, the magnetization vector of precessing hydrogen atoms is tipped from the longitudinal plane to the transverse plane, assuming a higher energy state and an MR signal. As spins relax back to a lower energy state, there is loss of the MR signal. Longitudinal relaxation is the recovery of the net magnetization along the longitudinal direction as spins return to the parallel state. The time constant associated with the longitudinal relaxation process is $T_1$. The amount of longitudinal relaxation $M_z$ at time, $t$, is a function of the original magnetization, $M_0$, and $T_1$.

$$M_z(t) = M_0(1 - e^{-t/T_1})$$
Transverse relaxation is the loss of coherence of all spins tipped into the transverse plane. Spin-spin interactions contribute to signal loss, which is referred to as T2 decay. The transverse magnetic signal, \( M_{xy} \), is a function of the time constant \( T_2 \).

\[
M_{xy} = M_0 e^{-t/T_2}
\]

The MR signal measured is the combination of the transverse magnetization from all voxels in a sample. By applying a slight magnetic gradient along the bore of the magnet, an excitation pulse allows for selection of a defined slice within the imaging volume, enabling one to collect information from parallel planes over time that together make a three dimensional volume. The repetition time, TR, is the time interval between successive excitation pulses. The echo time, TE, is the time interval from the excitation pulse to data acquisition. Varying the TR allows distinct tissues with different inherent T1 relaxation times to recover longitudinal magnetization to various extents. The tissue that has a shorter T1 value recovers more rapidly and therefore generates greater MR signal. This is the basis of image formation of T1 – weighted images (Figure 1). Cerebral spinal fluid has the longest T1 and therefore appears very dark on T1 MR
images. White matter has the shortest $T_1$, causing it to appear very bright while gray matter, with a slightly longer $T_1$, appears darker. These properties allow the user to discern the cortical ribbon, white matter, subcortical structures and ventricles by MRI.

![Figure 1](image.png)

**Figure 1:** An axial view of a brain shows cerebral spinal fluid (CSF) in the ventricles as dark, white matter as bright and the gray matter as less bright. Image intensities are based on the $T_1$ properties of different tissue. CSF, with a long $T_1$, will have a hypointense signal strength compared to tissue with a short $T_1$, such as fat in white matter.

### 2.3 Structural Analysis

Automated analysis of both cortical thickness and gray/white blurring at the boundary of the cortex and white matter allows for quantitative measurement of focal cortical differences on MRI. Based on the signal intensity from the
cortical mantle, which contains cell bodies in a laminated array, and the underlying white matter, composed of myelinated neuronal axons and glial cells, both measurements can point to potential pathology that may go unseen by passive viewing. Gray/white blurring occurs with age but it can also be present in focal cortical dysplasia (Salat et al., 2009; Thesen et al., 2011). Subtleties in cortical structural abnormalities, caused by neuronal migration problems, the presence of balloon cells and decreased myelination can all result in blurring of the boundary of the gray and white matter on T1-weighted MRI (Bernasconi, Bernasconi, Bernhardt, & Schrader, 2011).

In the clinical setting, judgment of cortical thickness differences or blurring of the gray/white junction has been used to identify potential areas of abnormalities. In patients with medically intractable seizures undergoing resective surgery, the ability to identify gray-white blurring on a pre-operative MRI that points to abnormal and potentially epileptogenic tissue yields better outcomes in terms of seizure freedom after resection (Chang et al., 2011). Loss of gray-white differentiation after abusive head trauma in a pediatric population is also associated with the occurrence of seizures (Goldstein et al., 2011). But, up to 80% of small, focal lesions can go undetected by visual inspection alone, highlighting the importance of quantitative analysis for diagnostics and surgical planning (Besson, Andermann, Dubeau, & Bernasconi, 2008). Analysis of MRIs from patients with neurologic abnormalities but normal appearing imaging using
quantitative measures improves our understanding of morphometric differences that point to underlying pathology.

One can take advantage of the differences in longitudinal relaxation times to measure signal intensities at specific distances from the border between the gray and white matter in the brain and to discern where differences in these signals lie (Figure 2). Gray-white contrast is calculated as signal intensity at 0.5 mm from the gray/white junction: \([\text{white-gray}/(\text{white + gray})]\). Increased blurring, or decreased contrast between gray and white matter suggests underlying cellular pathology such as the presence of balloon cells, or small, focal cortical dysplasias that might contribute to abnormal neuronal function or seizure activity (Thesen, et al., 2011).

**Figure 2:** Computing GWC. A, Sampling points on T1-weighted MPRAGE image with gray–white (GW) junction surface (pink line) and pial surface (turquoise). Signal intensity is measured in the white matter, (blue dot) and gray matter (green dot) that are 0.5 mm from the gray/white junctions (red dot). Gray white contrast is calculated as signal intensity at 0.5 mm from the gray/white junction: \([\text{white-gray}/(\text{white + gray})]\]. (Blackmon et al., 2011)
Advances in imaging analysis have also made it possible to detect differences in cortical thickness from T1-weighted images (Figure 3; Fischl and Dale, 2000) The thickness of gray matter ranges from 1.5 to 4.5 mm and reflects normal cortical organization and lamination. Measurement of cortical thickness specifically addresses focal differences in regional cell architecture without confounds that might include increased gyrification, global brain atrophy or increased ventricular size. Differences in cortical thickness can be seen in several pathologies including schizophrenia, ADHD, depression and TLE, among others (Butler et al., 2012; Makris et al., 2012; Tu et al., 2012; M. R. Williams et al., 2012).
While these measures of gray-white contrast and cortical thickness point to areas of focal abnormalities, correlations with other measures of altered structure or function can also elucidate the extent to which anatomically distinct changes alter networks throughout the brain. For example, concomitant increases in the volume of a subcortical structure and specific cortical areas suggest that

**Figure 3:** Cortical Thickness. Freesurfer measurements of cortical thickness at every vertex over an inflated brain are shown in the left hemisphere. Cortical thickness is estimated by measuring distances perpendicular from the gray/white interface to the pial surface, or outer edge of gray matter, and from the pial surface to the gray/white surface. (FreeSurfer: surfer.nmr.mgh.harvard.edu)
these brain structures are part of the same network that are affected by reciprocal inputs (Bernhardt et al., 2009).

2.4 Diffusion Tensor Imaging

Diffusion tensor imaging, introduced in 1994, measures the diffusion, or translational displacement of water molecules in vivo (Basser, Mattiello, & LeBihan, 1994). DTI can be used to discern the white matter fiber tracts in the brain and the integrity of such tracts at any voxel (Basser & Pierpaoli, 1996; Mori, Crain, Chacko, & van Zijl, 1999). Image acquisition is based on b value diffusion weightings and depends on the gradient strength and diffusion time (De Santis, Gabrielli, Palombo, Maraviglia, & Capuani, 2011). Diffusion metrics must be obtained in at least six noncollinear spatial directions.

DTI is based on diffusion-weighted imaging (DWI) which characterizes the scalar and isotropic properties of apparent diffusion coefficients (ADC). ADC measures the magnitude of diffusion of water molecules within tissues. Highly structured tissue has lower ADC whereas tissue with less cellularity and cerebral spinal fluid in ventricles have higher ADC. A Gaussian distribution describes the probability of finding a molecule in a position, r, at time, t, proportional to the motion propagator. Because water diffusion occurs within microstructures, the Gaussian distribution of anisotropic diffusivity is
characterized by three perpendicular eigenvectors ($\varepsilon_1$, $\varepsilon_2$, $\varepsilon_3$) that provide information about that specific environment. In the brain, myelinated axons allow for fastest diffusion in the axial direction ($\lambda_1$), while motion is more restricted in the radial ($\lambda_2$ and $\lambda_3$) direction (Figure 4). The tensor, $\mathbf{D}$, describes the movement of water along each axis and correlates these directions:

$$
\mathbf{D} = \begin{bmatrix}
D_{xx} & D_{xy} & D_{xz} \\
D_{yx} & D_{yy} & D_{yz} \\
D_{zx} & D_{zy} & D_{zz}
\end{bmatrix}
$$

The mean diffusivity is calculated by averaging the diffusion in axial and radial directions: $\text{MD} = (\lambda_1 + \lambda_2 + \lambda_3)/3$. This parameter provides information about the restriction of water movement and is independent of the directionality of water diffusion (Wu & Cheung, 2010).

**Figure 4:** Diffusivity in an elliptical environment. Here axial ($\lambda_1$) and radial ($\lambda_2$, $\lambda_3$) diffusivity and the associated eigenvectors ($\varepsilon_1$, $\varepsilon_2$, and $\varepsilon_3$) in an elliptical environment are demonstrated.
An important parameter garnered by DTI data is Fractional Anisotropy (FA), and like MD, can be used to study intrinsic microstructural changes.

\[
FA = \sqrt{\frac{1}{2} \left( \frac{1}{(\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_2 - \lambda_3)^2} \right)} \sqrt{\frac{1}{(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}}
\]

FA takes on values between 0 and 1 and describes the degree of anisotropy, or the likelihood that water diffuses fastest in a specific direction. It is used as a measure of axonal integrity and can be set as a threshold to define tracts in a network. In white matter, FA is high because diffusivity is fastest along fibers and slower perpendicular to them. FA approaches 0 in gray matter and cerebral spinal fluid (Pierpaoli & Basser, 1996). Oligodendrocytes are cells in the brain that make up myelin sheaths. It is the myelination that insulates neurons and accelerates the transmission of electrical signals along axons. Changes in myelination affect the restricted diffusion of water in white matter and FA values (Horsfield, 2008; Mukherjee et al., 2001). Disorders that affect myelination will show decreased FA values throughout the white matter because water can diffuse
more easily perpendicular to the length of the axon as opposed to axial diffusivity along the axon.

At the beginning of the 21st Century, DTI started to be used to visualize trajectories of white matter bundles (Basser, Pajevic, Pierpaoli, Duda, & Aldroubi, 2000). Maps along adjacent voxels could be visualized based on the largest principal diffusivity and DTI was used to map known tracts throughout the brain (Catani, Howard, Pajevic, & Jones, 2002; Mori, et al., 1999).

Anatomical connectivity, until this point, was previously limited to histological tracer systems in post-mortem studies. Pajevic and Pierpaoli

**Figure 5:** Tractography Schematic. This schematic shows how white matter tracts are inferred from diffusion tensor images. At each voxel, the direction of fastest water diffusion is shown with a vector arrow. Starting at any voxel, one can trace these vectors across adjacent voxels to map neuronal projections.
introduced a color system to show 3-dimensional tractography with red representing fibers projecting in the medial-lateral direction, green representing fibers situated anterior-posterior and blue denotes superior-inferior tracts (Pajevic & Pierpaoli, 2000). It is important to note that while DTI elicits information about the orientation of neurons, the direction of information transfer or action potential propagation remains ambiguous.

Changes in the DTI MRI signal may be attributed to a number of factors including demyelination, edema, gliosis, inflammation, fiber-crossing or a disorganization of anatomical networks throughout the brain (Cruz & Sorensen,
2006; Kobari, Meyer, Ichijo, & Oravez, 1990; Sotak, 2002). DTI is being employed more widely in the clinical setting primarily for neurosurgical cases. It can be used to map white matter tracts, or viable tissue, around brain lesions (Witwer et al., 2002). Diffusion imaging is also used to study demyelinating diseases such as multiple sclerosis and psychiatric conditions with normal appearing white matter such as schizophrenia (Boos et al., 2012; Tillema, Leach, & Pirko, 2012). Its application in epilepsy is in the interrogation of the integrity of networks that potentially cause or spread abnormal neuronal firing synchronously throughout the brain.

2.5 Functional Imaging

2.5.1 Functional MRI

Functional MRI (fMRI) has become a mainstay in cognitive research and is typically used to identify active brain regions in task paradigms. Since its inception in 1992, fMRI has become indispensable to neuroscience research. The blood oxygen level dependent (BOLD) signal that is measured in fMRI reflects an indirect correlate of neuronal activity. Deoxyhemoglobin is treated as an endogenous tracer because of its paramagnetic properties, which allows for mapping of brain activation with echo-planar imaging (Mansfield, 1977). The
increase in cellular metabolism and extraction of oxygen from hemoglobin in the blood causes an increase in deoxyhemoglobin, however the increase in cerebral blood flow from neurovascular coupling dominates and yields a proportionally larger concentration of oxygenated hemoglobin, which is diamagnetic (Buxton, Uludağ, Dubowitz, & Liu, 2004). The relative increase in the BOLD signal reflects postsynaptic events related to neural activity (Logothetis, 2010).

**Figure 7:** The BOLD Signal. The panel on the left is a cartoon of the vascular response to neuronal activity. In active states, or when metabolic demands increase in a specific area over baseline, there is increased oxygen extraction from hemoglobin in the blood, leaving deoxygenated hemoglobin. There is also an increase in blood flow, resulting in higher amounts of oxygenated hemoglobin. The panel on the right shows the change in relative concentrations of oxygenated and deoxygenated hemoglobin in response to localized increase in neural activity. (Huettel, Song, & McCarthy, 2009)
2.5.2 Resting State Functional Connectivity

Recently, BOLD fMRI methodology has been applied to imaging the brain during a ‘resting state,’ or when the patient is not engaged in any task-based activity. Such resting state fMRI is thought to reflect the brain’s *intrinsic* activity and can be used to study normal brain function and neuropathology.

It has long been known that the brain accounts for 20% of total body energy utilized, yet it consumes no less oxygen at rest than during cognitive or physical tasks (SOKOLOFF, WECHSLER, MANGOLD, BALLS, & KETY, 1953; Swaminathan, 2008). While at rest, the brain maintains connections between anatomically distinct and distant areas. These are networks that sustain a functional organization of activity between cortical and subcortical nodes. In 1995, Biswal and his colleagues were the first to describe a resting state network that reflected brain activation patterns during a task (Biswal, Yetkin, Haughton, & Hyde, 1995). Low frequency oscillations in the range from 0.01-0.08 Hz were extracted from the entire BOLD timecourse by Fast Fourier Transform (Figure 8).
The coherent spontaneous low-frequency fluctuations in the 0.01-0.08 Hz range during resting conditions that are thought to reflect cyclic modulation of gross cortical excitability and long-distance neuronal synchronization (Biswal, et al., 1995). These spontaneous low frequency oscillations can be used to map the spatial distribution of temporal correlations in resting state fMRI studies.

**Figure 8:** Time Series Fast Fourier Transform. A BOLD time series shows the percent change in signal intensity over the course of the scanning session. This signal is composed of several oscillations of different frequencies superimposed on each other. The cartoon is a schematic of a Fast Fourier Transform (FFT) that segregates component oscillations. FFT is used to discern fluctuations that occur at low frequencies. The bandpass of oscillations between 0.01-0.08 Hz is used to study resting state functional connectivity.
Specifically, areas of the brain that show correlated BOLD changes, seen as increases and decreases in low frequency fluctuations over a time course from the entire scanning period, are said to be functionally integrated. That is, these distant areas belong to an established network of neurons. Biswal and colleagues were the first to show that low frequency oscillations extracted from voxels in the motor cortex had a similar BOLD signature to other areas of the motor cortex. These correlated low frequency oscillations mapped onto a motor network that showed increased activity when engaged in a physical task. The figure below shows distinct cortical areas that have highly correlated low frequency oscillations (Figure 9).

![Figure 9: Temporal Correlations in a Resting State Network. Time courses from the posterior cingulate cortex (yellow) and the medial prefrontal cortex (orange) show significant temporal correlations in BOLD signaling oscillating at frequencies between 0.01-0.1 Hz. (Raichle & Snyder, 2007)]
Distant neuronal assemblies that fire synchronously at very low frequencies maintain large-scale networks throughout the brain. Since Biswal first showed functional connectivity from LFOs that reflects networks called into action to perform cognitive of physical tasks, many other resting state networks have been described.

Resting state scans have several advantages over conventional MRI and task-based fMRI. This imaging technique provides information about the function of the brain and disturbances in various psychological and neurological diseases, including epilepsy. Furthermore, the testing conditions are reproducible, as they are not limited by task paradigms. Children and patients with cognitive constraints can more easily undergo resting state scans than other fMRI scans because they are not required to follow specific instructions beyond what is normally required for anatomic MRI scans.

2.5.3 Default Mode Network

Resting state fMRI (R-fMRI) elicits maps of functional connectivity throughout the brain and allows for visualization of networks that create a functional architecture. Among the functional networks interrogated using R-fMRI approaches, the “default mode network” (DMN) is one of the most commonly studied. It is composed of a set of functionally connected brain areas
that typically show high metabolic activity during rest but relatively reduced activity during the performance of cognitive or sensorimotor tasks (Esposito et al., 2006; Fransson, 2005; Greicius, Krasnow, Reiss, & Menon, 2003), leading to the hypothesis that this network and its subsystems subserve a variety of cognitive processes including episodic memory, imagination of the future and self-referential thought (Andrews-Hanna, Reidler, Sepulcre, Poulin, & Buckner, 2010; Greicius, et al., 2003). The DMN includes several distant brain regions, including the precuneus, posterior cingulate cortex (PCC), angular gyrus, medial prefrontal cortex (MPFC), and ventral anterior cingulate cortex (vACC) (Raichle & Snyder, 2007; Uddin, Kelly, Biswal, Xavier Castellanos, & Milham, 2009). Though the DMN is one of the most commonly studied networks, resting state functional connectivity (RSFC) has been used to identify several other established networks and this analysis technique can be implemented to identify areas of the brain that are functionally connected. To this end, the study of functional connectivity MRI (fcMRI) can be used in epilepsy to study established networks throughout the brain, such as the DMN, that contribute to the cortical architecture, and it can also be used to study networks whose nodes are directly affected by focal epileptogenic abnormalities.

The DMN is referred to as the ‘task-negative’ network because its nodes show decreased activity during specific cognitive demands while the ‘task-positive’ network, comprised of the dorsolateral prefrontal cortex, inferior parietal
cortex and supplementary motor area, shows increased activity in response to preparation (Fox et al., 2005; Sonuga-Barke & Castellanos, 2007). Though the alternating synchrony of the spontaneous activity of these two networks suggests they must be organized in some manner into one well-orchestrated unit, we refer to functional integration as temporally correlated activity in distinct nodes while segregation is the network of regions in which the time series of activation is anti-correlated (Fair et al., 2007). The organization of the integration and segregation is well-preserved in healthy individuals, but has been shown to break down in some neurological populations.

2.5.4 Fractional Amplitude of Low Frequency Fluctuations

While the spatial distribution of temporally synchronous oscillations in the low frequency range has been studied to determine resting state functional connectivity, other aspects of the BOLD signal, such as the amplitude, also contribute to the understanding of spontaneous fluctuations (Biswal, et al., 1995). The amplitude of low frequency oscillations (ALFF) are reliably reproduced across subjects and within individuals at different scan times (Zuo et al., 2010). Amplitude measures are higher in gray matter than white matter, which suggests a neuronal basis for this signal, and do not change with breathholding. This also
supports the notion that neuronal processes are the underlying mechanism for such measurements (Zuo, et al., 2010).

ALFF measurements are the square root of the amplitude of the power spectrum (since this measure is derived by squaring the amplitude). A better measure of the amplitude of this frequency range is the fractional ALFF, or fALFF, which is the amplitude of the signal in the LFO range of interest divided by the amplitude of the signal in the entire 0-0.25 Hz band. (This range is naturally limited by the sampling rate during MRI scanning.) ALFF has higher measures in perivascular and periventricular areas but the amplitude of the entire detectable signal is also higher because of increased blood flow to these areas (Zou et al., 2008). For this reason, the fractional amplitude of the LFOs is a better measure and provides more physiological data pertaining to the spontaneous fluctuations that contribute to the stable functional architecture of the brain.

Neuronal oscillation classes are arrayed linearly when plotted on the natural logarithmic scale (Buzsáki & Draguhn, 2004). In the signal range detected by fMRI, these classes are: slow 5 (0.01 – 0.027 Hz), slow-4 (0.027-0.073 Hz), slow 3 (0.073 – 0.198 Hz), and slow-2 (0.198-0.25 Hz). These fALFF measures can be used to study normal and disease states. Differences in fALFF have been noted in neuronal pathologies including depression (Guo et al., 2012), ADHD (Zang et al., 2007), and TLE (Zhang, Lu, et al., 2010b).
Because temporal synchrony of spontaneous activity at low frequency fluctuations is used to identify established brain networks, fALFF provides further information to help elucidate focal abnormal activity that exists at baseline and may contribute to aberrant networks. fALFF abnormalities in IGE point to focal abnormalities that have implications for network pathology since it is derived from an oscillation class whose fluctuations are used to study resting state functional connectivity.

Figure 10: Oscillatory classes. For each band, the range of frequencies is shown, together with its commonly used term. From (Buzsáki & Draguhn, 2004).
3. MRI in IGE

3.1 Introduction

Primary brain abnormalities in IGE are clearly evident on EEG and in clinical manifestations. The spontaneous 3 Hz spike and wave synchronous waves seen throughout the brain suspend normal brain activity. However, in routine clinical work-up for this diagnosis, no brain abnormalities can be detected on standardized imaging including MRI. The inability to gain access to brain...
tissue or employ electrophysiologic studies in vivo further confounds the ability to study this neurologic disorder in humans. Given the advances in MRI image acquisition and analysis described above, the studies presented here are aimed at determining how magnetic resonance capabilities can be used to study both focal and network changes in IGE and how these abnormalities can be described using morphometric, diffusion and functional metrics.

**Publication 1:**

The work presented here on the default mode network abnormalities in IGE was previously published. The full reference is:


The sections below that are included in that publication are (Introduction - 3.2) Imaging the DMN in IGE; (Methods – 4.1 – 4.3) Participants, Image acquisition and DMN in IGE; (Results – 5.1) Default Mode Network in IGE; and (Discussion – 6.1) Functional Cortical Abnormalities. Please note that an addendum has been added to the discussion here that was not included in the original publication titled, Limitations addendum: Micromovements Discussion.
Contributions:

I am primarily responsible for the data collection and analysis in this study in addition to writing the paper. The study would not have been made possible without the following contributions: Jonathan Dubois and Jonathan Young aided with patient recruitment and data acquisition; Clare Kelly, Michael Milham and F. Xavier Castellanos provided the scripts for analysis, taught me how to perform the analysis, and contributed significantly to the editing of the manuscript; Orrin Devinsky and Thomas Thesen oversaw all aspects of this projects from patient recruitment to manuscript submission and contributed greatly to the editing of the paper; Chad Carlson, Jacqueline French, Ruben Kuzniecky and Eric Halgren helped with patient recruitment and financial support for this project.

Publication 2:

The remaining sections of this thesis reflect the work that is in preparation for submission. These studies combine structural and functional data to characterize focal and network abnormalities in IGE. The reference is:

Contributions:

I am responsible for all aspects of this manuscript preparation including data acquisition, analyses and authoring the paper. Brian Quinn and Hugh Wang provided valuable guidance with the analyses; Orrin Devinsky and Thomas Thesen aided with study conceptualization and editing; Chad Carlson, Jacqueline French and Ruben Kuzniecky helped with patient recruitment and valuable guidance.

3.2 Imaging the DMN in IGE

3.2.1 Specific Aim 1

To identify functional cortical abnormalities characterized by altered resting state functional connectivity of the default mode network in people with IGE.

3.2.2 Background

IGE is characterized by widespread cortical hyperexcitability. Abnormal neuronal activity at a distinct anatomic location that is part of a larger cortical network may be the basis for rapid propagation of aberrant neuronal firing that contributes to generalized seizures. Because synchronous spike and wave discharges appear spontaneously throughout the cortex simultaneously, large cortical networks may be disrupted at rest in generalized epilepsy.
Healthy individuals reliably exhibit robust, positive correlations between regions of the DMN and negative correlations between DMN regions and other cortical areas. Abnormal DMN functional connectivity occurs in several brain disorders, including Alzheimer’s disease (Greicius, Srivastava, Reiss, & Menon, 2004), schizophrenia (Garrity et al., 2007), ADHD (Tian et al., 2006), Parkinson’s (van Eimeren, Monchi, Ballanger, & Strafella, 2009), and depression (Greicius et al., 2007). Decreased functional connectivity within the DMN has also been found in people with temporal lobe epilepsy (Zhang, Lu, Zhong, Tan, Liao, et al., 2010).

There is conflicting evidence that people with IGE show differences in resting state functional connectivity (RSFC). Both Luo et al. and Song et al. demonstrated decreases in DMN integration in people with absence and general tonic-clonic epilepsy, respectively (Luo et al., 2011; Song et al., 2011). However, Moeller et al. showed no differences in functional connectivity in areas that deactivate the most during general spike and wave discharges, including nodes of the DMN (Moeller et al., 2011). Given the spontaneous, deviant neuronal activity that spreads throughout the brain in people with IGE, we aim to identify abnormal regions in the DMN, and the extent to which these pathologic areas are a part of a larger network.

3.2.3 Hypothesis 1:

We predict disruptions of the default mode network in the frontal lobes, consistent with previous studies showing frontal lobe abnormalities in IGE.
3.3 Morphometry studies in IGE

3.3.1 Specific Aim 2

To identify, using quantitative MRI measures, morphometric differences that may contribute to seizure pathology in generalized epilepsy.

3.3.2 Background

Emerging quantitative MRI analysis techniques used to measure differences of cortex and subcortical structures between patients and controls have revealed subtle structural changes, but there is disagreement amongst studies with regard to which, if any, structural brain abnormalities exist (Bernhardt, et al., 2009; Chan et al., 2006; Helms, Ciumas, Kyaga, & Savic, 2006; Kim et al., 2007). Decreases in thalamic volume (Bernhardt, et al., 2009; Chan, et al., 2006; Ciumas & Savic, 2006; Kim, et al., 2007; Lin et al., 2009), increases in thalamic regions (Betting et al., 2006) and normal thalamic volumes (Bernasconi et al., 2003; Natsume, Bernasconi, Andermann, & Bernasconi, 2003; Seeck et al., 2005) have been reported. Similarly, studies of cortical thickness or volume in IGE identify increased gray matter (Kim, et al., 2007; Lin, et al., 2009; Woermann, Sisodiya, Free, & Duncan, 1998), cortical atrophy (Bernhardt, et al., 2009; Woermann, et al., 1998) and no differences (Bernasconi, et al., 2003; Natsume, et al., 2003; Seeck, et al., 2005). Many studies were limited by sample size or differing
methodology techniques such a voxel based morphometry (VBM) or measuring thalamic subregions that were discerned by independent raters.

3.3.3 Hypothesis 2

In contrast to functional measures discussed below, we do not expect to detect morphometric differences between patients with IGE and controls in the thalamus or prefrontal cortex (PFC), despite evidence that suggests primary functional abnormalities in these areas of the brain are responsible for generalized seizures.

3.4 Diffusion tensor imaging in IGE

3.4.1 Specific Aim 3

To characterize the integrity of white matter tracts throughout the brain, specifically focusing on tracts between the thalamus and PFC.

3.4.2 Background

While EEG and morphologic studies have been inconclusive in determining where the primary pathology exists in IGE, the study of specific brain networks may shed light on pathogenic circuits that allow seizure spread.
MRI can be used to characterize both functional and anatomic differences in neurologic disorders, and identify abnormalities that may be either focal or network in nature. Structural, volumetric, cortical thickness and gray-white contrast differences have been used to identify focal, morphometric abnormalities (MacDonald, Kabani, Avis, & Evans, 2000). DTI identifies structural differences in the integrity of structural networks between distant, connected areas (Smith et al., 2004). There have been few reports of DTI abnormalities in IGE. Decreased FA in the anterior corpus callosum of rat models of IGE and of the anterior thalamic radiation in people with JME have been shown (Chahboune et al., 2009; Deppe et al., 2008). However, the small sample size and age disparities between patients and normal controls in the latter study suggest further research is needed to support these findings.

3.4.3 Hypothesis 3

The integrity of white matter tracts, determined by FA values, between the thalamus and PFC will be compromised, contributing to a dysfunctional network of cortical-subcortical activity in IGE.

3.5 Thalamic Functional Differences in IGE

3.5.1 Specific Aim 4

To identify functional differences in specific subregions of the thalamus.
3.5.2 Background

Abnormal thalamic functioning has long been thought to contribute to seizure initiation or propagation of generalized seizure activity (Gloor, 1968). To identify where focal functional differences may exist, we use fALFF to discern abnormalities in the thalamus of patients with IGE.

Though specific thalamic nuclei cannot be discerned by 3T MRI, atlases showing probabilistic cortical connections can be used to study distinct subregions of the thalamus, a structure that controls many regulatory functions and acts as a ‘gate-keeper’ for cortical-cortical and cortical – subcortical interactions. Here we study thalamic subregions that have been previously defined based on DTI tractography to specific cortical areas (Behrens et al., 2003). Because these subregions are defined by anatomical connections with the cortex, they provide a means for defining and studying areas of the thalamus that have implications for specific thalamo-cortical connections (Figure 12).
Given previous evidence that PFC abnormalities or fronto-thalamic interactions might subserve seizure activity in IGE, we sought to identify abnormalities in the thalamus that are related to this circuitry that can be characterized by MRI. This study employs functional data to characterize focal and network abnormalities in IGE.
3.5.3 Hypothesis 4

We hypothesize that functional abnormalities, seen in fALFF differences between patients with IGE and normal controls, will be seen in the subregion of the thalamus that is structurally related to the PFC.

3.6 Summary

Given evidence that PFC abnormalities or fronto-thalamic interactions might subserve seizure activity in IGE, we aim to identify abnormalities in the prefrontal-thalamic network and default mode network that can be characterized by MRI. This study combines structural and functional data to characterize focal and network abnormalities in IGE. We hypothesize that morphologic abnormalities will be restricted to the frontal lobes, specifically the amPFC, while abnormal anatomic connections characterized by decreased integrity of white matter tracts on DTI will exist between the frontal lobes and the thalamus. Finally, we expect to see functional differences, characterized by decreased fALFF in parts of the thalamus implicated in dysregulated thalamo-cortical circuitry and abnormal integration of the prefrontal cortex in the default mode network in IGE.

The study of pathology contributing to seizure activity in patients with IGE is limited by restricted access to the brain and few opportunities for post-mortem studies. With emerging medical and research technology, MRI provides
a unique opportunity to measure correlates of neuronal function, diffusion properties across space and morphometry differences in brains that seem to show no abnormalities on diagnostic imaging. However, since abnormal manifestations of anomalous brain activity is present in discrete intervals, seen as clinical seizures and synchronous activity on EEG, the question addressed here is whether emerging MRI methods can be used to identify discrete abnormalities during interictal periods. The aim of this thesis is to shed light on advanced applications of MRI technology, potential underpinnings of primary pathology in IGE and targets for treatment in this disorder.

4. METHODS

4.1 Participants

Twenty-seven people with IGE were recruited from the patient population at New York University Medical Center, Comprehensive Epilepsy Center (12 women, age range 19.9 – 49.6 years, mean age 32.2 years) and were age- and sex-matched with 27 normal control subjects recruited from the general population 11 women, age range 20.9-48.6, mean age 32.1 years). Patients met criteria for IGE and had to have a history of seizures with no history of developmental delay or structural brain abnormalities. Standard, diagnostic structural imaging studies
were normal. Electrophysiologic evaluation with interictal, and in most patients, ictal EEG demonstrated typical generalized epileptiform spikes; patients with focal epileptiform discharges or focal slowing on EEG were not eligible. People with IGE were classified according to the ILAE classification as having absence seizures (37%), myoclonic seizures (59%), or general tonic clonic seizures (78%) (Table 1). All people diagnosed with IGE were under medical treatment at the time of study. All subjects gave their written informed consent to participate in this study, which was approved by the Institutional Review Board of NYU Langone School of Medicine.
Table 1: IGE patients

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4.2 Image acquisition

Subjects underwent scanning on a Siemens Allegra 3T scanner at New York University Center for Brain Imaging. All participants had a T1-weighted MRI sequence optimized for gray-white matter contrast. (TR = 2530 ms, TE = 3.25 ms, T1 = 1100 ms, flip angle = 7 deg, field of view (FOV) = 256 mm, matrix = 256x256x192, voxel size = 1x1.33x1.33 mm) Images were corrected for nonlinear warping caused by non-uniform fields created by the gradient coils. Image intensities were further normalized and made uniform with the FreeSurfer (4.0.2) software package. Fifteen people with IGE (8 women, mean age 30.1 years) and 15 age- and sex-matched control subjects (8 women, mean age 29.8 years) underwent resting state scans. We collected 197 contiguous echo planar imaging functional volumes for each subject (TR= 2000ms; TE = 25 ms; flip angle = 90, 39 slices, matrix = 64x64; FOV = 192 mm; acquisition voxel size = 3x3x3 mm). All participants were instructed to lie as still as possible with their eyes closed for the duration of the 6 min, 38 second scan. Eighteen people with IGE (8 women, mean age 31.7 years) and 18 age- and sex-matched normal control subjects (8 women, mean age 31.6 years) also had DTI scans. Diffusion-weighted echo-planar MRI were acquired by applying diffusion gradients along 64 directions (b value = 1000 s/mm²) with the following parameters during the 6-min, 3 second scan (TR = 5500 ms, TE = 86 ms, FOV = 240mm, slice thickness = 2.5mm, voxel size = 2.5 x 2.5 x 2.5mm).
4.3 DMN in IGE

The aim is to identify functional cortical abnormalities characterized by altered resting state functional connectivity of the default mode network in people with IGE.

4.3.1 fMRI data preprocessing

AFNI (Cox, 1996) was used to perform slice timing correction, motion correction, and detection and reduction of extreme time series outliers. The first 5 volumes of each participant’s scan were discarded. All other data processing was done with FSL (FMRI Software Library; www.fmrib.ox.ac.uk). Further processing included spatial smoothing using a Gaussian kernel (FWHM = 6mm), mean-based intensity normalization (each subject’s 4-D dataset was scaled by its global mean), and temporal bandpass filtering (0.01 – 0.1 Hz). To control for the effects of motion, as well as normal physiologic processes such as cardiac and respiratory rhythms, each participant’s 4-dimensional (4-D) preprocessed volume was regressed on 9 predictors that modeled nuisance signals from white matter, cerebrospinal fluid and the global signal, and 6 motion parameters. Correction for time series autocorrelation (prewhitening) was performed. Each voxel’s time series was then scaled by its standard deviation, and the volume was spatially normalized to MNI152 standard space using linear registration.
4.3.2 DMN identification with anatomically distinct regions

Spherical seed regions of interest (ROIs) with radii of 6 mm were placed in areas consistently implicated in the DMN: the PCC (BA 31) and MPFC (BA 10), centered at MNI coordinates x = 3, y = -57, z = 26 (PCC), and x = 8, y = 59, z = 19 (MPFC) (Uddin, et al., 2009). Although there are many distinct nodes in the DMN, these two structures were chosen as seed regions because they robustly elicit DMN maps, are not lateralized, and are distant anatomic regions that belong to a well-characterized network (Fair, et al., 2007). The mean time series of each seed was obtained by applying these seed ROIs to each participant’s 4-D residuals standard space volume and averaging across the time series of each voxel within the ROI (see Figure 14A). Subject-level RSFC maps of all voxels that were positively and negatively correlated with the seed ROI were generated by regressing each participant’s 4-D residual volume on the seeds’ time series. Group level RSFC analyses were carried out using an ordinary least squares model implemented in FSL, which generated Z-score maps (“networks”) of positive and negative RSFC for each seed. Correlation coefficients of each voxel were normalized to Z-scores with Fisher’s r-to-Z transformation. Maps of correlated networks were thresholded at Z > 2.3, corrected for multiple comparisons with a FDR criterion cluster level significance of p < 0.05.
4.3.3 Differences in DMN functional connectivity

To examine differences between positively and negatively correlated networks with the seed regions, a direct voxel-wise comparison was performed using a mixed-effects ordinary least-squares model implemented in FSL, thresholded at Z > 2.3. Group difference maps were generated using both the PCC and MPFC seed maps. Individual parameter estimates for each participant were generated for degree of correlation between the average time series in the voxels comprising the seed and all voxels included in the group difference ROI. This was done for each seed region and plotted with average values and standard error of the mean calculated.

Finally, to fully characterize all the brain regions showing a difference in connectivity with the abnormally integrated region elicited in the previous analysis, a 4 mm seed was centered on the point of maximum difference in connectivity. Using the mixed-effect ordinary least squares model, a direct voxel-wise comparison between the people with IGE and normal controls was performed.

4.3.4 Correlation Analysis

The effects of seizure duration, age of seizure onset and age at time of scan on RSFC were examined by using these measures as covariates of positive and negative connectivity with each of seeds. Seizure duration is defined as the
time in years from first seizure to time of scan. With each of these measures used as regression coefficients implemented in FSL’s general linear model, maps were generated that show clusters that co-vary positively and negatively with RSFC of both the PCC and MPFC seed. The parameter estimates between the clusters elicited and seed voxels were correlated with each parameter mentioned above to determine the correlation coefficient.

4.4 Morphometric Differences

The aim of these studies is to determine, using quantitative MRI measures, morphometric differences that may contribute to seizure pathology in generalized epilepsy.

4.4.1 Volumetric Analysis

FreeSurfer (http://surfer.nmr.mgh.harvard.edu) software package was used to segment subcortical structures and generate volumes for these structures. Each voxel is assigned a neuroanatomical label based on probabilistic information from an atlas (Fischl et al., 2002). Assigning each point to the class for which the probability is greatest generates an initial segmentation. Labels are generated based on the prior probability of a given tissue class occurring at a specific atlas location, the likelihood of the image intensity given that tissue class, and the probability of the local spatial configuration of labels given the tissue class.
Given this segmentation, the neighborhood function is used to recompute the class probabilities. FreeSurfer measures of right and left thalamic volumes were collected from each subject, as was intracranial volume (ICV) to control for differences in head size. The statistical software package SPSS was used to compare volumetric differences between people with IGE and normal controls. Thalamic volume was modeled as the dependent variable using multiple linear regression and ICV and subject status (ie, IGE or normal control) were used as independent variables.

4.4.2 Prefrontal Cortex Thickness and Gray-White Blurring

FreeSurfer was also used to reconstruct surface models of each subject’s cortex using T1-weighted anatomic MRI scans. The automated steps facilitate quantitative cortical measurements include identification and tessellation of both the gray/white boundary and pial surface of the brain. The folded surfaces are inflated and topological defects corrected. These steps are described in detail elsewhere (Dale, Fischl, & Sereno, 1999; Fischl, Sereno, & Dale, 1999). Estimates of cortical thickness are made by averaging the distance perpendicular from each point on the white matter surface to the pial surface, and the distance from each point on the pial surface to the white matter surface. Maps are smoothed with a Gaussian kernel (10 mm FWHM) across the surface and
averaged across participants using a spherical averaging technique (Fischl, et al., 1999). Gray/white matter contrasts are calculated by measuring the image intensity contrast ([white-gray]/[white+gray]) at 0.5 mm from the white matter surface. Values at each voxel indicate contrast measures, with values closer to zero indicating increased blurring.

4.4.3 Group Analysis

A region of interest (ROI) in the amPFC was identified in the previous study that showed abnormal functional integration with a cortical network, the Default Mode Network (DMN). Given the abnormal resting state functional connectivity of all voxels in this amPFC ROI, and previous research showing that the PFC may contribute to the pathogenesis of generalized epilepsy, we specifically interrogated structural and anatomical network properties of this ROI. Cortical thickness was averaged over a region of interest (ROI) in the amPFC in each subject and subsequent t-tests, controlling for age and intracranial volume were employed to detect differences between groups. Cortical thickness and gray-white contrast was measured across the entire cortex and compared between the IGE group and normal control group, controlling for age and intracranial volume. Thalamic volumes and each of these cortical measures were correlated in each subject to determine if thalamo-cortical morphometric variations differ
between each group. All results were corrected for multiple comparisons using Monte Carlo Simulations.

4.5 Diffusion Tensor Imaging

The aim of these studies is to characterize the integrity of white matter tracts throughout the brain, specifically focusing on tracts between the thalamus and PFC.

4.5.1 Tractography

DTI analysis is performed using FMRIB’s Diffusion Toolbox (FDT) (http://www.fmrib.ox.ac.uk/fsl/fdt/index.html) implemented in FSL. Preprocessing of data included correction of both eddy current distortion induced by the gradient coils and simple head motion. Diffusion data were co-registered with T1 images to create brain masks. Vectors were normalized at every voxel and probabilistic tractography is created by modeling crossing fibers using Bayesian estimation of diffusion parameters obtained using sampling techniques. Probabilistic tracts are generated between the thalamus and the amPFC. FA values at all voxels along the tract are determined. Additionally, FA values along the anterior thalamic radiation provided by the JHU DTI-based white matter atlas in FSL were generated (Hua et al., 2008). Analysis of variance of average FA
values across both the anterior thalamic radiation tract and tracts between the thalamus and amPFC in each group were calculated between the IGE and normal control group.

4.5.2 Tract Based Spatial Statistics

Voxelwise statistical analysis of the FA data over the entire brain was carried out using tract based spatial statistics (TBSS) (Smith et al., 2006), part of FSL (Smith, et al., 2004). First, FA images were created by fitting a tensor model to the raw diffusion data using FDT, and then brain-extracted using BET (Smith, 2002). All subjects' FA data were then aligned into a common space using the nonlinear registration tool FNIRT, which uses a b-spline representation of the registration warp field. Next, the mean FA image was created and thinned to create a mean FA skeleton, which represents the centers of all tracts common to the group. Each subject's aligned FA data was then projected onto this skeleton and the resulting data subjected to voxelwise cross-subject statistics. A permutation algorithm (randomize, within FSL) was used for inference testing, and a total of 500 permutations were conducted. Both uncorrected and family-wise error (FWE)–corrected p-value images were generated using a threshold-free cluster enhancement (TFCE) approach.
4.6 Functional imaging of the thalamus

The aim of this study is to identify functional differences in specific subregions of the thalamus.

4.6.1 Preprocessing and analysis

Preprocessing steps were carried out using SPM8, (http://www.fil.ion.ucl.ac.uk/spm) and Data Processing Assistant for Resting-State fMRI (DPARSF) V2.0 Basic Edition (Yan & Zang, 2010). The first 10 of 197 time points were removed and slice-timing correction for interleaved acquisition was performed. The volumes were all realigned and linearly normalized to their standard MNI template in 3mm space. The images were smoothed with a full-width half maximum Gaussian kernel of 4mm. The data were then detrended. The time series was transformed to the frequency domain with a fast Fourier transform to obtain the power spectrum. The fractional amplitude of low frequency bandpasses at each voxel were calculated by dividing the amplitude of the BOLD signal at the specified bandpass by the amplitude of the entire BOLD signal at every voxel.

We compared the amplitude of the BOLD signal in the bandpass of the power spectrum between 0.01-0.08 Hz, in addition to the slow-5, slow-4, slow-3 and slow-2 bandpass frequencies in patients with IGE vs normal control subjects. The bandpass between 0.01-0.08 Hz is often used to look at spontaneous neural
activity that, when in temporal synchrony with other distinct regions, suggests functional connectivity. To determine if specific subregions of the thalamus show differences in spontaneous activity at this frequency, we compared the fractional amplitude of the low frequency fluctuations at each of the bandpasses mentioned above in each of the thalamic subregions and entire thalami. Thalamic subregions were determined from the Behrens thalamic atlas in FSL, thresholded at 25%. The average fALFF values across all voxels within each identified region were averaged and two-sided t-tests implemented in SPSS were used to determine the subregions that show abnormal functional activity.

5. RESULTS

5.1 Default Mode Network in IGE

5.1.1 Integrated and Segregated Networks with MPFC and PCC Seeds

Connectivity maps for each group and for each node of the DMN were generated. Both groups exhibited functional connectivity within areas of the DMN. Regions exhibiting positive RSFC with the MPFC seed in both groups included medial prefrontal cortex, extending to the paracingulate gyrus, superior frontal gyrus, middle frontal gyrus (superiorly and laterally), ventral ACC, precuneus/PCC, angular gyrus extending to the lateral occipital cortex, and
middle temporal gyrus. In both groups, negative correlations were observed in the supramarginal gyrus extending to the superior occipital cortex, and insular cortex. However, there were differences seen between the groups on visual inspection. Normal controls had greater negative correlations with the planum temporale extending to the parietal operculum, the lateral edge of the thalamus on the right and areas in the cerebellum (Figure 13B).
Figure 13: Positively and Negatively Correlated Time Series with Seed Regions.

A. Voxel-wise correlation map in a single control subject with a seed region in medial prefrontal cortex (MPFC) indicated by the circle in green. Brain regions in orange/yellow, such as the angular gyrus (AG), are positively correlated with the seed region, brain regions in blue, such as the supramarginal gyrus (SMG) are anti-correlated with the seed region. Timecourses of the average BOLD signal from the seed region (green trace), positively correlated AG (orange trace) and negatively correlated SMG (blue trace) are shown below.

B. Red/yellow colors show brain regions that are positively correlated with the seed ROI (green circle) in the MPFC in controls and people with IGE. Blue colors show areas that are negatively correlated. C. Areas positively and negatively correlated with the seed in the PCC are shown in controls and people with IGE.

PCC/Pr, posterior cingulate cortex/precuneus; vACC, ventral anterior cingulate cortex; SFG, superior frontal gyrus; MPFC, medial prefrontal cortex; AG, angular gyrus; IPS, intraparietal sulcus; SMC, supplementary motor cortex; SMG, supramarginal gyrus; LPFC, lateral prefrontal cortex.
Figure 13C shows the areas positively and negatively correlated with the seed placed in the PCC in healthy control subjects and in people with IGE. Regions exhibiting positive RSFC with the PCC in both healthy controls and people with IGE included proximal areas of PCC and precuneus, angular gyrus, MPFC, ACC, paracingulate gyrus, and inferior temporal poles extending to the middle temporal gyrus. In both groups, negative correlations were observed in dorsolateral prefrontal cortex, insular cortex, planum polare, supramarginal gyrus, left intraparietal sulcus and dorsal anterior cingulate extending to the supplementary motor cortex. People with IGE failed to show negative correlations in the precentral gyrus, extending rostrally to the superior frontal gyrus and paracingulate gyrus, central opercular cortex, posterior inferior temporal gyrus, antero-superior aspects of the temporal lobe, left precentral gyrus, and cerebellum.

5.1.2 Direct group comparisons of RSFC

The direct comparison functional connectivity, that was cluster-based thresholded at Z>2.3 to control for FDR, between controls and people with IGE identified a group difference in the integrated network between each of the seeds in the PCC and MPFC and a cluster elicited more ventrally in the MPFC (Figure 14). This area is consistently shown to be integrated in the DMN but people with IGE lacked extensive positive RSFC in this area of the DMN compared to healthy
controls and failed to show strong positive correlations (mean $r = 0.07$, SEM 0.02) compared to controls (mean $r = 0.29$, SEM 0.04). Similarly, DMN connectivity based on the seed in the MPFC exhibited a group difference within the same area of the prefrontal cortex. People with IGE did not reliably show strong positive correlations between these areas (IGE group mean $r = 0.22$, SEM 0.03; control group mean $r = 0.41$, SEM 0.03). Scatter plots in both panels of Figure 14 show the correlation values for each participant.
Figure 14: Group differences in functional connectivity between patients and controls.
The cortical cluster shown in red in both panels is the cluster that exhibit group differences between normal controls and people with IGE upon direct comparison with cluster-based thresholding at $Z>2.3$. Both differences between the functional connectivity of the seed regions in the MPFC (A) and the posterior cingulate cortex (PCC) (B) show differences in the same prefrontal cortex region (red cluster in both A and B). Scatter plots show the parameter estimate, or correlation of the low frequency oscillations of the BOLD signal, between the seed regions and the entire ROI (red) in the prefrontal cortex. The scatter plot in (A) shows that normal controls (NC) tend to show stronger correlations in resting state functional connectivity (RSFC) between both the MPFC seed (green) and the PFC cluster (red). Similarly, the scatter plot in (B) shows NC subjects have greater RSFC between the PCC seed (green) and PFC (red) than people with IGE.
To further examine the group differences in RSFC in prefrontal cortex areas, we performed an additional analysis to investigate the connectivity network of the discordant area in the prefrontal cortex seen in warm colors in Figure 14. A seed with a radius of 4mm was placed where differences in PCC RSFC between controls and people with IGE were maximal in the prefrontal cortex cluster at MNI coordinates -2, 44, 0 (Figure 15). The difference from the PCC RSFC was the most robust, though the point of maximal difference elicited from the MPFC seed was adjacent. Maps of significant group differences in RSFC were generated in the same manner as for the primary PCC and MPFC seed analyses. The comparison map (normal controls v. people with IGE) thresholded at Z>2.3 and corrected for multiple comparisons showed robust group differences in both positive and negative RSFC between the disrupted node in the prefrontal cortex and areas typically integrated in the DMN and segregated from the DMN. Specifically, patients did not consistently show positive correlations with areas included in the ‘task-negative’ network, namely the dorsal-medial prefrontal cortex, extending from the superior frontal gyrus to the paracingulate gyrus and ventral ACC, and the PCC/Precuneus (control group mean r=0.20, SEM = 0.02 IGE group mean r= 0.01, SEM= 0.02).
**Figure 15**: Group differences in functional connectivity from abnormal region in the prefrontal cortex.

**A.** When a seed was placed in the abnormal PFC region (green), the cortical maps of group differences upon direct comparison with cluster-based thresholding at $Z>2.3$ show that normal controls show greater positive RSFC than people with IGE in areas typically included in the DMN (red areas), and greater negative RSFC in areas typically included in the ‘task-positive’ networks (blue).

**B.** The scatter plot shows the degree of RSFC (the parameter estimate) between the green seed region and all red clusters in both the normal controls (NC) and people with IGE.

**C.** The scatter plot shows the parameter estimate of the green seed region and all blue clusters in NC and IGE. Yellow bars show the mean and standard error of the mean in each plot.
Group differences also appeared in areas typically negatively correlated with the DMN, in the ‘task-positive’ network. All controls showed negative correlations between the seed in the PFC and the areas shown in blue on the group difference map, such as the supramarginal gyrus, superior parietal lobule, and intraparietal sulcus, while the majority of IGE patients showed positive correlations between these areas (control group $r= -0.15$, SEM 0.02; IGE group $r= 0.02$, SEM 0.01). Scatter plots show that all normal controls exhibit positive RSFC between the seed in the PFC (green) and areas typically integrated in the DMN (red) while people with IGE exhibit overall decreased or negative functional connectivity between these areas. Similarly, the negative connectivity scatter plot illustrates that normal controls consistently show negative RSFC between the seed and areas in the task-negative network (blue) though our cohort of people with epilepsy fail to show strong segregation in these areas.

5.1.3 Correlation Analyses

When parameters such as seizure onset age, age at time of scan and seizure duration were used as regressors with the correlation analysis for functional connectivity of both the PCC and MPFC seed, only seizure duration correlated with network differences from the PCC node (Figure 16). Seizure duration was positively correlated with increased RSFC between the PCC and
bilateral anterior temporal lobes, primarily in the parahippocampal cortex ($R^2 = 0.71, p < 0.005$). Seizure duration was also negatively correlated with decreased RSFC between the PCC and a cluster in the frontal cortex ($R^2 = 0.64, p < 0.005$), dorsal to the cluster in the DMN that showed decreased integration compared to controls (Figure 16). These areas failed to show any correlation with onset age or age at time of scan. None of the parameters used as regressors showed any differences with network integration or segregation from the MPFC seed.
**Figure 16:** Functional connectivity with PCC seed regressed with seizure duration.

**A.** Seizure duration positively varies with the correlations between the average time series of the seed in the PCC (green) and clusters bilaterally in the anterior temporal lobes (yellow). The scatter plot shows the relationship between seizure duration and parameter estimates between the regions specified (R²=0.71, p<0.005).

**B.** Seizure duration negatively varies with the parameter estimates between the PCC and a cluster near the anterior cingulate cortex in the frontal lobe. The scatter plot shows the relationship between seizure duration and parameter estimates between the regions specified (R²=0.62, p<0.005). Each green point on the scatter plots represents a person with IGE’s RSFC between the seed and ROI and their seizure duration.
5.2 Morphometry

5.2.1 Thalamic volumes

Left and right thalamic volumes were not different between people with IGE and age- and sex-matched controls. Left thalamic volumes for those with IGE and normal controls were 6940 and 6744 mm$^3$, respectively (p = .30). Right thalamic volumes for IGE and normal controls 6982 and 7027 mm$^3$, respectively (p = .81). ICV was a predictor of both left and right thalamic volumes (p< 0.001), but a history of epilepsy was not predictive of left thalamus volume (p = 0.60) or right thalamus volume (p = 0.23). Figure 17 depicts the distribution of thalamic volumes in patients with IGE and normal controls.

Figure 17 shows box plots of right and left thalamic volumes in IGE subjects and normal controls. There are no significant volumetric differences between the two study groups.
5.2.2 Cortical thickness and gray-white contrast

Average cortical thickness in the amPFC ROI showed no difference between subjects with IGE and normal controls (Figure 18). The average cortical thickness in the left amPFC in normal controls was 2.765 mm and in patients with IGE it was 2.763 (p = 0.97). In the right thalamus, cortical thickness was 2.525 mm in controls and 2.473 in IGEs (p=0.45). To explore possible cortical differences located elsewhere in the brain, a whole-brain analysis of cortical thickness and gray-white contrast measures was performed. Correlations between thalamic volumes and cortical thickness also revealed no differences between the two groups when corrected for multiple comparisons using Monte Carlo simulations.

No differences between the patient and control groups were seen in the cortical gray-white contrast throughout the cortex after corrected for multiple comparisons, and again controlling for age. The gray-white contrast measure correlated with either left or right thalamic volumes, controlling for age, did show a region in the anterior prefrontal cortex of difference (Figure 19), however there were no differences throughout the cortex when corrected for multiple comparisons.
Figure 18: amPFC and Cortical Thickness.
The amPFC ROI, identified as having abnormal resting state functional connectivity within the DMN, is shown in an axial slice in volumetric space. This same ROI, projected onto the pial surface of inflated brains, is shown above, in both left and right hemispheres. There were no differences in cortical thickness in this ROI between patients with IGE and normal controls shown in the box plots.
Figure 19: Gray-white contrast correlation differences with left thalamic volumes.
The inflated left hemisphere shown here shows cortical areas where correlations between the gray-white contrast and left thalamic volumes differed between patients with IGE and normal controls. All areas showing a difference, including the large cluster in the medial prefrontal cortex where the correlation between gray-white contrast and left thalamus was decreased in the IGE group did not survive corrections for multiple comparisons using Monte Carlo simulations.
5.3 DTI

Both the JHU anterior thalamic radiation (shown on an MNI152 standardized brain in Figure 20) and the tracts generated from the thalamic prefrontal subregion (Behrens, et al., 2003) to the amPFC (Figure 21) are shown. FA values along the each entire tract were averaged for each subject. Average FA values from all normal controls and patients with IGE are shown in scatter plots, with mean and standard error of the mean depicted. No significant differences in the tracts extending from the thalamus to the prefrontal cortex were seen.
Figure 20: Anterior Thalamic Radiation and Fractional Anisotropy. The left anterior thalamic radiation from the Johns Hopkins University Atlas is shown here on an MNI standard brain. Scatter plots depict the distribution of average FA values from all normal controls and IGEs within the tracts with mean and standard error the mean illustrated. No differences in mean FA values, averaged across left and right tracts each, between patients with IGE and normal controls were seen.
Figure 21: Thalamic Tracts to amPFC with Fractional Anisotropy. Thalamic tracts from prefrontal subregion of the left thalamus to the amPFC in a representative subject is shown here in patient diffusion space. Scatter plots depict the distribution of average FA values from all normal controls and IGEs within the tracts with mean and standard error of the mean illustrated. No differences in tract integrity across all IGE subjects and normal controls were significant.
Figure 22 shows both an uncorrected, unthresholded map and a group comparison between IGEs and normal controls FWE – corrected thresholded at p<0.05 map projected onto a MNI standard brain in diffusion space. Analysis using tract based spatial statistics showed no differences between FA values along any of the skeletonized tracks throughout the brain, using threshold-free cluster enhancement to correct for family-wise errors.

Figure 22: Tract Based Spatial Statistics.
FA maps are projected onto skeletonized white matter tracts in standard diffusion brains. A, Differences in FA values between normal controls and IGE patients (not thresholded) are shown. Red indicates greater FA in normal controls, blue indicates greater FA in IGE. B, when thresholded at p<0.05 and family-wise error corrected, there are no differences between controls and patients in all tracts throughout the brain.
5.4 Thalamic fALFF

In the thalamic subregions, all fALFF values in the 0.01-0.08 Hz frequency range were lower in IGE patients than normal controls. The subregions that are connected structurally to the Prefrontal Cortex (PFC subregion), PreMotor cortex (PMC subregion) and the entire thalami showed significant decreases (p< 0.05) in the fALFF values from the subjects with IGE. When other bandpasses were examined, only the PMC subregion showed a significantly smaller fALFF in the Slow 4 bandpass (0.027-0.073 Hz). There were no differences seen in the Slow 5 (0.01-0.027), Slow 3 (0.073 – 0.198), or Slow 2 (0.198-0.25) frequencies in any of the thalamic subregions (Figure 23).

![Graph showing average fALFF values in the 0.01-0.08 Hz bandpass in normal controls and patients with IGE.](image)

**Figure 23:** Average fALFF values in the 0.01-0.08 Hz bandpass in normal controls and patients with IGE are shown in this graph. In the PreFrontal subregion, PreMotor subregion and entire thalamus, patients with IGE showed reduced fALFF values compared to controls.
6. DISCUSSION

6.1 Functional Cortical Abnormalities

Using R-fMRI, we examined the integrity of the DMN at rest in people with IGE and found abnormalities in interictal RSFC. Specifically, those with IGE have diminished RSFC between nodes of the DMN and a cluster in the PFC, relative to healthy controls. People with IGE exhibit both disrupted functional network integration (positive RSFC between nodes of the DMN) and functional segregation (negative RSFC between areas of the DMN and “task-positive” regions) in IGE, supporting aberrant functional network organization in people with IGE.

6.1.1 Decreased Positive Functional Connectivity in the Frontal Cortex

Collectively, the participants with IGE showed abnormal functional connectivity within the DMN. They exhibited decreased positive RSFC between areas in the frontal lobe and the rest of the DMN. These areas serve various cognitive and emotional functions, including “mentalizing,” (i.e., understanding the mental states of one’s self and others) (Amodio & Frith, 2006; Gusnard, Akbudak, Shulman, & Raichle, 2001; Simpson, Drevets, Snyder, Gusnard, & Raichle, 2001). Patients with IGE show difficulties in social processing similar to those exhibited by patients with frontal lobe lesions (Damasio, Grabowski, Frank, Galaburda, & Damasio, 1994), including limited self-control, suggestibility,
distractibility, and indifference to physical needs (Bech, Pedersen, Simonsen, & Lund, 1976; de Araujo Filho et al., 2009). Such deficits may be the result of impaired mentalizing abilities such as concept formation, abstract reasoning, mental flexibility, cognitive speed and planning (Devinsky et al., 1997). Furthermore, the cluster in the frontal lobe showed abnormal segregation from regions typically considered to be part of ‘task positive’ networks including lateral parietal and occipital regions. Though the cognitive implications of decreased segregation amongst these areas would be speculative, these group differences strengthen the argument that an abnormal area in the frontal cortex is neither well integrated into the default mode network nor segregated from task-positive networks. Future studies should investigate whether the decreased frontal DMN RSFC observed in this study is related to these IGE-related social-cognitive impairments.

Physiological recordings in rat models support our findings of frontal lobe dysfunction, where aberrant firing rates and patterns have been observed in MPFC neurons during spike-and-wave discharges (Lorincz, Olah, Baracskay, Szilagy, & Juhasz, 2007). Source localization utilizing MEG has identified frontal lobe localizations in people with IGE (Santiago-Rodriguez et al., 2002; Stefan, Paulini-Ruf, Hopfengärtner, & Rampp, 2009). In people with juvenile myoclonic epilepsy, spike-and-slow wave discharges were modeled to the medial prefrontal region (Santiago-Rodriguez, et al., 2002). An EEG-fMRI study of a patient with
IGE revealed frontal deactivation during a generalized spike-wave discharge (Laufs, Lengler, Hamandi, Kleinschmidt, & Krakow, 2006). These studies, together with our results, suggest that frontal deactivations may disrupt the DMN and contribute to the cognitive and behavioral deficits in IGE.

Differences in RSFC with the PCC that correlate with seizure duration are not limited to the MPFC and are specific to the PCC seed only. These abnormalities extend beyond the DMN, but we address them here because the PCC is a prominent node of the DMN. The frontal lobe cluster that shows decreased RSFC with the PCC seed negatively correlates with seizure duration. This suggests that the chronic effects of seizures, epileptiform activity, and the underlying abnormalities disrupt the functional integration between medial posterior and frontal regions. fMRI studies show negative BOLD responses (NBR) in frontal, parietal and posterior cingulate cortices during GSW discharges (Hamandi et al., 2006). Surprisingly, we found seizure duration significantly correlated with increased connectivity of both parahippocampal gyri to the PCC seed. Though frontal-thalamic circuits are implicated in IGE, temporal lobe function may also be affected in generalized epilepsies. Frontal and temporal volumes are decreased in childhood absence epilepsy (Caplan et al., 2009) and perirhinal kindling increases discharges in a rat model of absence epilepsy (Akman, Karson, Aker, Ates, & Onat, 2010). Our finding of increased parahippocampal connectivity to the PCC seed may reflect either 1) increased
synchronization of these limbic regions in people with IGE, 2) a disinhibition reflecting loss of frontal inhibitory input on this circuit, or 3) other processes.

IGE-related disruption in DMN RSFC may reflect chronic disorganization of the functional architecture due to neurophysiologic dysfunction (e.g., channelopathy), or intermittent disruptions to that functional architecture that perturb cortical networks (e.g., frequent abnormal discharges). Differences between the RSFC of a region in the PFC to the DMN and task-positive areas suggest the disruptions affect the DMN as well as extensive cortical networks. Further work combining R-fMRI with electrophysiological recordings providing access to high-frequency neuronal signals may help adjudicate between these alternatives.

6.1.2 Limitations and Future Directions

In our study, healthy subjects expectedly and uniformly showed either strong positive or negative correlations within the DMN. In turn, people with IGE showed both a decrease and greater variability in connectivity strength, potentially identifying a feature of the population or a potential effect of other disease-related variables. As this study utilized a cross-sectional sample of controls as well as patients, it is not known whether the network differences we found manifested before seizure onset, result from seizures, medications, spike-and-wave discharges, or other disease-related factors. Longitudinal studies would
confirm that seizure duration correlates with changes in DMN RSFC over time. Future studies should use simultaneous EEG-fMRI to determine whether periodic epileptiform discharges contribute to the observed group differences in DMN functional connectivity. Further studies would also benefit from investigating the relationship of the specific cognitive deficits seen in this patient population and its relation to DMN integrity.

6.1.3 Limitations addendum: Micromovements Discussion

Since the publication of resting state functional connectivity abnormalities of the DMN in IGE, the neuroimaging community has increasingly become concerned about the effects of motion artifacts on resting state fMRI data. Functional MRI data acquisition is dependent upon both the position and temporal sequence of BOLD responses. Traditional displacements of image voxels can be described by a combination of six movements: linearly along x, y and z axes and rotational movements of the head designated as pitch, yaw and roll. Traditional pre-processing of fMRI data consists of realigning the data by motion correction to a median image with 6 degrees of freedom (Jenkinson, Bannister, Brady, & Smith, 2002). However, because the gradients established along the bore of the magnet establish the basis of the acquired BOLD signal, movements that disrupt the established gradient foundation also affect the intensity of the signal readout (Power, et al., 2012).
There is ongoing debate over how to take into account and adequately address the motion artifacts’ affect on the BOLD readout. Traditional processing involves head realignment estimates regressed from the data. Yet recent studies have questioned how larger changes in the BOLD signal resulting from significant movement and consequential frame displacement (FD) should be handled (Figure 24).

**Figure 24:** Movement and changes in the BOLD signal.  
**A.** rs-fcMRI timecourses are shown from 3 ROIs in a single subject.  **B.** Head movement are indicated by translations (solid line) and rotational (dotted line).  **C.** Absolute differences in the timecourses from (A) are calculated at each time point here. **D.** The framewise displacement (FD) of head position is shown here. There is a high correspondence between FD (D) and change in BOLD signal (C). (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012)
Power et al. (2012) introduced a technique they refer to as ‘scrubbing’ to account for these spurious signal changes. They apply a temporal max that identifies motion-induced spikes in their data and subsequently remove affected volumes of the affected time series from further analysis. Specifically, they identify volumes with FD > 0.2mm and censor that time point along with time points 1 before and 2 after the offending movement. The resulting time series is then concatenated (Power, et al., 2012). This approach removes most of the motion-induced artifacts, thereby theoretically improving the quality of the data for further analysis, but it significantly reduces the quantity of the resulting time series. At least 4-5 minutes of data is needed in order to adequately draw conclusions about spontaneous low frequency activity that is the basis of resting state functional connectivity (Van Dijk, Sabuncu, & Buckner, 2012).

Furthermore, the resulting gaps in the temporal sequence by censoring large frames of the data may destroy the continuity that is needed to establish regular oscillations of the BOLD signal over time. The large censoring of data and disruption may have effects on subsequent bandpass filtering and therefore there are questions about during which point in pre-processing of data scrubbing should be performed (ie, before or after bandpass filtering) (Carp, 2011).

Another approach is to model spikes in the data resulting from motion with individual regressors, thereby removing offending data from the timeseries (Lemieux, Salek-Haddadi, Lund, Laufs, & Carmichael, 2007). Group-level
regression with individual average motion estimates also reduces spurious artifacts (Satterthwaite, Wolf, et al., 2012). Still, there remains no clear method for reducing artifacts while retaining variability in the BOLD signal that is due to differences between patient populations, especially when the ‘ground truth’ remains unknown in any specified data set.

Even if scrubbing is performed, the parameters dictating what data should be removed remains controversial. Motion reduces the BOLD signal but removing a frame of data that covers four time points (one before the offending time point, motion time point with FD > 0.2mm, and two after) creates large holes in the data. Satterthwaite et al. (2012) studied the effects of signal change at time points surrounding significant motion and showed that the offending time point with significant FD and the point after are most significantly affected by the motion (Figure 25) (Satterthwaite, Elliott, et al., 2012).

**Figure 25:** Motion Volume Changes. Motion produces a large reduction in BOLD signal that is maximal in the volume during which the subject moved and just following subject movement. The magnitude of signal reduction increases as motion amplitude increases (red, >.3 mm FD; green, >.5 FD; blue >.7 FD). (Satterthwaite, Elliott, et al., 2012)
Though no proscriptive terms for addressing potential motion artifacts have been established for future analyses, it has been shown in three studies with large datasets that motion tends to increase short-range connectivity while diminishing apparent long-range connectivity (Power, et al., 2012; Satterthwaite, Wolf, et al., 2012; Van Dijk, et al., 2012). In the resting state functional connectivity study presented above, both the short-range connectivity between the vACC seed and amPFC ROI, and the long-range connectivity between the PCC seed and the same amPFC ROI were affected in the same direction, showing decreased functional connectivity in the IGE group compared to the control group. Such results would not be seen if they could be attributed to motion artifacts. Scrubbing should not be performed prior to fALFF analysis because this is computed in the frequency domain via fourier transform for which a primary assumption is that the time series is continuous.

6.2 Functional, not morphometric, differences in IGE

The structural and functional studies presented here, aimed at examining the integrity of thalamic-prefrontal networks in patients with IGE, show that functional, but not structural, differences in nodes of this network can be detected
with MRI. There were no differences in the thalamic volumes or cortical measures between patients and normal controls. Axonal integrity between these areas as determined by DTI was not compromised in the patient group, suggesting that cellular tracts are intact in IGE. While cortical and thalamic structures in IGE are morphometrically normal, abnormal rest function (i.e., decreased fALFF of the BOLD signal) was detected in thalamic subregions that are structurally connected with the prefrontal cortex and premotor cortex.

6.2.1 Morphometry – Thalamic Volumes, Cortical Thickness and Gray-White Contrast

IGE is characterized by normal MRI on visual inspection and there are no commonly accepted cellular changes attributable to generalized epilepsy (Engelborghs, D'Hooge, & De Deyn, 2000). The lack of structural changes that can be quantified by cortical thickness and gray-white blurring measures, in addition to normal thalamic volumes compared to controls in our study population, suggest that cell structures remain intact in IGE, though only histopathological studies would confirm this. In other types of focal epilepsy, these imaging measures can be used to identify focal cortical dysplasia or epileptogenic tissue (Bernasconi, et al., 2011; Thesen, et al., 2011). We specifically examined the morphometry measures in a ROI in the amPFC because
atypical PFC signaling with the thalamus is implicated in IGE and this area is abnormally integrated with the DMN (McGill et al., 2012). However, there were no structural differences in the amPFC, and further whole-brain analyses failed to show morphometric differences throughout the brain.

Our study found no volumetric abnormalities of the thalamus in IGE. While cortical thinning and thalamic volume loss would support the concept that thalamo-cortical networks are abnormal in IGE (Duncan, 2005), other studies have failed to identify consistent thalamic structural abnormalities in IGE (Bernasconi, et al., 2003; Natsume, et al., 2003; Seeck, et al., 2005). The findings presented here suggest that morphometric changes may not correlate with the brain abnormalities that are clearly present on functional and electroencephalographic recordings in IGE.

6.2.2 Thalamic-Prefrontal Network

Despite normal morphometry of the cortex and thalamus in IGE, there is evidence that abnormal prefrontal-thalamic networks may underlie seizure generation in this type of epilepsy. During spike-and-wave bursts, EEG-fMRI shows an association between increased thalamic activity and decreased BOLD fluctuations in frontal regions (Aghakhani et al., 2004; Gotman et al., 2005; Hamandi, et al., 2006). Stimulation of both the thalamus (GUERRERO-FIGUEROA, BARROS, DE VERSTER, & HEATH, 1963) and frontal cortex
(Bancaud, 1965) elicit spike-wave phenomena suggesting the connection between these two areas are essential for initiation or propagation of epileptiform activity.

The anterior thalamic radiation carries nerve fibers between the thalamus and prefrontal cortex. It is part of the anterior limb of the internal capsule. Decreased integrity of this tract, attributed to decreased fractional anisotropy, has been noted in other neuropathologies that also show signs of frontal lobe dysfunction (Mamah et al., 2010). Given the concomitant aberrant functioning in the thalamus and PFC in IGE, we examined the integrity of this tract in IGE but found no differences in FA values in the anterior thalamic radiation between patients and controls. Because our patients also showed abnormal functional connectivity in the amPFC, we examined the tracts specifically connecting this region and the subregion of the thalamus that has high probability of being structurally connected to the prefrontal cortex. The lack of differences in this anatomic network suggests the integrity of tracts is not a contributing factor to network abnormalities in IGE. Throughout the brain, skeletonized tracts showed no differences in FA values suggesting tract integrity is maintained throughout the brain in these patients.

The lack of morphometric differences and differences in tractography confirms that seizure activity in IGE does not result from pathology that typically causes morphometric differences seen on MRI. Abnormal migration, a difference in structural organization of the brain, atrophic neurons, and compromised
myelination can all result in differences in cortical thickness of the blurring of the
boundary between gray and white matter (Bernasconi, et al., 2011; Salat, et al.,
2009; Thesen, et al., 2011). The lack of these findings in patients with IGE
suggests that the primary pathology underlying generalized seizures is not one
that is consistent with morphometric and macroscopic changes.

6.2.3 Focal Functional Differences

Decreases in the fALFF in specific thalamic subregions provide initial
evidence that there are functional abnormalities in this subcortical structure at
rest. Differences in the amplitude of the BOLD signal have been found in several
neuropathologies (Han et al., 2011; Hoptman et al., 2010; Jiao et al., 2011; Zhang,
Lu, et al., 2010a). While these low frequency oscillations are used to identify
resting state networks, they may also correlate or entrain faster EEG bands
(Buzsáki & Draguhn, 2004). The slow oscillations (<0.1 Hz) are correlated with
changes in the amplitude of EEG alpha rhythms (Mantini, Perrucci, Del Gratta,
Romani, & Corbetta, 2007). Abnormalities in these slow oscillations may
precipitate certain types of seizures (Vanhatalo et al., 2004).

Low frequency fluctuations are an integral component of activity in the
thalamus (Filippov, Williams, Krebs, & Pugachev, 2008). They are present in
thalamic firing during the generation of cyclic paroxysms, experimental
electrographic seizures seen in cats (Steriade & Contreras, 1995). The decrease in fALFF in specific thalamic subregions suggest that this cyclic, spontaneous activity in either intrathalamic or thalamocortical neurons is abnormal. Though the thalamus is commonly viewed as the gatekeeper of all neuronal activity spread to the rest of the cortex, the ability to find differences in specific thalamic regions that reflect a disorganization of spontaneous, oscillatory firing points to specific functional pathology that may underlie spike-and-wave discharges that spread throughout the entire cortex.

Individual thalamic nuclei cannot be discerned by 3T MR imaging. Based on the tractography projections done by Behrens et al., the thalamic subregions that showed decreased fALFF values correspond to specific nuclei. The mediodorsal nucleus is connected to the prefrontal cortex via the anterior thalamic peduncle (D. Tanaka, 1976). The ventral lateral and ventral anterior nuclei project neurons to the premotor cortex (Jones, Wise, & Coulter, 1979). Given previous implications of the role of the prefrontal cortex in the generation of spike-and-wave activity in conjunction with the centrencephalic theory put forth by Jasper and Penfield, the ability to find functional aberrations at rest in thalamic nuclei that have reciprocal connections with the prefrontal cortex further strengthens the notion that this cortico-thalamic circuitry is involved in generalized seizure activity.
The decreased fALFF in the premotor subregion of the thalamus was not expected. This cluster of voxels that has reciprocal connections with the Premotor Cortex (PMC) is thought to include ventral lateral and ventral anterior thalamic nuclei (Behrens, et al., 2003). Whether a PMC-thalamic network is specifically engaged in generalized seizure activity is difficult to conclude. Both the proximity of this thalamic subregion to the PFC subregion and the premotor cortex’s location adjacent to the prefrontal cortex may render these networks less distinguishable from each other. Further study that examines spontaneous activity in the mediodorsal, ventral anterior and ventral lateral nuclei is needed to identify abnormal function that contributes to seizures in IGE.

Evidence from studies of voltage gated channels in thalamic neurons provide potential substrates for the underlying neuropathology that contributes to thalamic functional differences seen on MRI (Steriade & Contreras, 1995). GABA-ergic neurons of the reticular nucleus and T-type Ca\(^{2+}\) currents in thalamocortical relay cells are among several channelopathies implicated in cortical spike-and-wave phenomena seen in generalized seizures. (Couter et al. 1989, 1990; Leresche 1998). The cyclic gating of these channels, specifically the de-inactivation of the T-type Ca\(^{2+}\) channel and feedback inhibition of the GABA-ergic neurons, may contribute to the coordinated firing of spike-wave discharges propagated to the cortex. Given that low frequency oscillations reflect network coupling and cortical inputs to the thalamus mediate the cyclic nature of thalamic
activity, decreased fALFF in the prefrontal subregion of the thalamus suggest that a fronto-thalamic network contributes to uncontrolled gating of neuronal activity that causes epileptic activity. The inhibitory effects of deep brain stimulation on the dorsomedial thalamus specifically have been shown to arrest status epilepticus in a rat model (Urino et al., 2010) which also points to the epileptogenic significance of this network.

6.2.4 Cellular mechanisms mediating low frequency oscillations in the brain

Low frequency oscillations are present in normal, non-epileptic states and are clearly evident as the 0.5-4 Hz slow waves of sleep (Crunelli & Hughes, 2010). These slow rhythms are essential and entrain and influence faster oscillations such as alpha rhythms (8-13 Hz), gamma (30-80 Hz) and even the very fast oscillations (>100 Hz) pertaining to cognition and attention (Laufs, et al., 2006; Tallon-Baudry, 2009). These slow oscillations that are used to study resting state networks correlate with the faster EEG rhythms in the same spatial distribution (Mantini, et al., 2007). This oscillatory activity at <0.1 Hz is an integral component of cerebral functioning and is especially prevalent in the thalamus (Albrecht & Gabriel, 1994). In our study of generalized epilepsy that
highlight the presence of functional abnormalities, we are particularly interested in the cellular mechanisms underlying the transformation of network activity to massive, synchronous, epileptic discharges.

Recordings from local field potentials in cats have shown that slow oscillations are prevalent in the thalamus (Filippov & Frolov, 2005) and can be associated with cyclic paroxysms (Steriade & Contreras, 1995). Models of excitatory thalamo-cortical and cortico-thalamic neurons with inhibitory influence from thalamic reticular neurons show how feedback mechanisms mediated by GABA_A and GABA_B receptors create 3 Hz oscillatory patterns (Destexhe, 1998).

Thalamocortical (TC) cells have primarily glutamatergic (excitatory) projections, and corticothalamic (CT) neurons are also excitatory, projecting partially to thalamic reticular cells (located along the lateral edges of the thalamus). The firing of these reticular cells creates inhibitory postsynaptic potentials that are GABA mediated, thus suppressing TC activation and creating a natural oscillation of thalamo-cortical circuitry.

Activation of GABA_A ion channels yields oscillatory activity at about 10 Hz but GABA_B mediated activity causes 3 Hz waves (Blumenfeld & McCormick, 2000). Blocking GABA_A receptors leads to seizure activity of the signature EEG pattern while GABA_B agonists cause 3 Hz discharges (Hosford et al., 1992; Snead, 1992). Cortical and thalamic cells fire prolonged discharges in phase with ‘spike’ component but are silent during ‘waves’ of spike-wave discharges.
(Steriade & Yossif, 1974). A potential candidate for the generator of the bursting activity is the low threshold calcium channels that causes a burst of action potentials in TC cells after recovering from an IPSP (Bal, von Krosigk, & McCormick, 1995; Llinás, 1988).

An imbalance of excitation and inhibition in the cortex may result in the strong phasic excitation of thalamic reticular, thalamocortical and GABAergic neurons. On the one hand, the excitatory influence on thalamocortical cells may feed recurrent activation loops. However, the disynaptic inhibition via the thalamic reticular cells may generate low-threshold Ca2+ spikes that may initiate another cycle of paroxysmal activity (Steriade, 1995). A burst of spikes in a thalamocortical neuron activates the cortical network, which generates a strong burst of action potentials through intracortical recurrent excitatory connections. This activity strongly activates both local GABAergic neurons and thalamic reticular neurons. The activation of GABA$_A$ receptors and the inactivation of the depolarizing currents such as the low-threshold Ca2+ spike in thalamocortical cells, results in the cessation of activity in the network. The generation of a rebound Ca2+ spike in the thalamocortical cell, ~300 ms later, initiates the next cycle of the oscillation, accounting for the spike-and-wave phenomena seen in generalized seizures.
This rhythm depends critically on the activation of GABA\textsubscript{B} receptors because this activation provides a prolonged hyperpolarization of thalamocortical cells that keeps these neurons in the range for the generation of rebound low-threshold Ca\textsuperscript{2+} spikes in response to the large GABA\textsubscript{A} receptor–mediated IPSPs. The rhythmic properties of the T-Type calcium current contribute to the 3 Hz paroxysmal events.

6.2.5 Limitations and Future Directions

The primary findings of this study reflect functional differences in subdivisions of the thalamus, suggesting that focal, functional abnormalities contribute to, or result from seizures in IGE. At 3T MR Imaging, individual thalamic nuclei cannot be discerned. The atlas of thalamic subregions, based on the work of Behrens et al., (2003), provides an approximate map of different nuclei but this atlas is based on structural connectivity with different cortical regions and does not reflect functional regions of the thalamus. Furthermore, all images from subjects are co-registered to a standard brain before analyses are performed, yielding it impossible to study functional differences in subregions specific to each subject. Future studies that incorporate higher power imaging, such as 7T might improve identification of thalamic nuclei in individual subjects would lend themselves better to studying functional differences in these areas.
Though no structural differences could be discerned using anatomic measures such as cortical thickness, gray-white contrast and thalamic volumes, in addition to DTI, we are unable to rule out cellular changes that might not affect macroscopic morphometry. Microscopic changes that leave the cortical ribbon and thalamus intact would not be detected with the MRI analysis techniques presented here.

Though the pathogenesis underlying IGE has historically been considered non-localizable, future studies of the thalamic-PFC network may provide a path towards targeting treatment options in this patient population. People with IGE are not candidates for intracranial EEG studies because they are not considered potential surgical patients, so options to potentially localize epileptogenic tissue are limited. Emerging imaging techniques including fMRI analyses, and magnetoencephalography (MEG) will advance the understanding of the primary pathology underlying seizures in generalized epilepsy.

7. CONCLUSIONS

Focal thalamic and cortical network functional abnormalities can be detected by MRI in IGE. Our study of functional connectivity of a widely distributed cortical network, the DMN, and spontaneous fluctuations of specific thalamic subregions both point to altered activity at rest in regions that localize to
thalamo-prefrontal networks. In focal epilepsy and temporal lobe epilepsy, morphologic abnormalities and anatomic lesions are the basis of aberrant neuronal activity and can be detected with morphologic analyses on MRI. The studies presented here suggest that generalized epilepsy is a different entity and that cell structure and physical networks are preserved in IGE. However, the activity of these neural assemblies reflects abnormal spontaneous fluctuations that may disrupt the tightly regulated gating of oscillatory behavior in thalamocortical networks. The ability to localize deviant brain activity using non-invasive techniques narrows the study of pathology in IGE to specific, integrated networks. More broadly, the studies presented here emphasize the power of different imaging acquisition techniques and analysis to identify brain pathology in neurologic populations who not only show preserved anatomic morphology but only transient deviant activity. Further research and clinical applications of MRI in neurologic and psychiatric populations will prove invaluable in diagnosis and treatment of these disorders.

As a future physician-scientist, this is an exciting time to be involved in imaging research. Novel imaging modalities and techniques are rapidly coming to fruition. New ways to analyze data are also emerging and changing constantly in this relatively young field. But I am most interested in the application of the research techniques being developed to clinical populations. The ability to study in vivo processes in humans non-invasively is a great asset to medicine and
clinical practice. Improved diagnostic methods guide advances in therapy and it is a great honor to be part of such an endeavor.
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