I, Benjamin P Kay, hereby submit this original work as part of the requirements for the degree of Doctor of Philosophy in Neuroscience/Medical Science Scholars Interdisciplinary.

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Resting-State Functional Connectivity in Treatment-Resistant Idiopathic Generalized Epilepsy

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Abstract

Three million Americans live with epilepsy at an annual cost of over $12 billion per year. Idiopathic generalized epilepsy (IGE) is a primary seizure disorder that accounts for 15-20% of all epilepsies and is associated with high rates of cognitive and psychiatric comorbidities in addition to seizures. Approximately 20% of IGE patients experience refractory seizures despite appropriate therapy. Uncontrolled seizures are a significant cause of poor quality of life, morbidity, and mortality. Although treatment-resistance is common and associated with poor clinical outcome, there are few reliable biomarkers for it and its neuronal correlates are not well understood. Therefore, the aim of this study was to investigate the neuronal correlates of treatment-resistance using data collected from 100 IGE patients and 40 healthy control subjects.

We used simultaneous electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) to measure resting-state functional connectivity, a recent technique for detecting abnormal neuronal connectivity in epilepsy. We found increased connectivity in a thalamocortical circuit thought to give rise to epileptic discharges in IGE patients vs. healthy controls. A separate circuit involving cerebellum, thalamus, basal ganglia, and cortex was found to have reduced connectivity in treatment-resistant vs. responsive IGE patients. This previously unknown cerebellar circuit was not significantly different between IGE patients vs. healthy controls and may be altered specifically in treatment-resistant patients.

We also found reduced connectivity in the default-mode network (DMN), a set of brain regions thought to be important for maintaining consciousness. The DMN is deactivated by epileptic discharges, and its inhibition may cause loss of consciousness during seizures. DMN connectivity was found to be reduced in IGE patients vs. healthy controls, and it was further reduced in treatment resistant vs. responsive IGE patients. Loss of DMN connectivity was also correlated with disease duration. Therefore, DMN connectivity may be a biomarker for treatment-resistance. Patients with a high frequency of epileptic discharges were found to have increased connectivity between a motor, seizure-related network and the DMN. This finding provides a plausible mechanism by which the DMN is disrupted in IGE.

The discovery of biomarkers for treatment-resistant IGE is clinically important and adds to the scientific understanding of epilepsy. Future studies may further elucidate the mechanisms underlying these altered neuronal circuits, establish a causal relationship between these biomarkers and clinical outcome, and investigate new pharmaceutical agents for the treatment of refractory IGE.
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Nomenclature

AAL  automated anatomical labelling
ACC  anterior cingulate cortex
AED  anti-epileptic drug
AFNI analysis of functional neuroimages
BG   basal ganglia
BOLD blood oxygenation level-dependent
CAE  childhood absence epilepsy
CCHIPS Cincinnati Children’s Hospital image processing software
DLPFC dorsolateral prefrontal cortex
DMN  default mode network
DNA  deoxyribonucleic acid
DTI  diffusion tensor imaging
ECG  electrocardiogram
EEG  electroencephalogram
EEG/fMRI EEG recorded simultaneously with fMRI
fMRI functional magnetic resonance imaging
FSL  FMRIB software library
FWHM full width at half maximum
GABA γ-aminobutyric
GICA3 group ICA [backprojection algorithm version] 3
GIFT group ICA fMRI toolbox
GSWD generalized spike and wave discharge
GTC  generalized tonic-clonic [seizure]
HFO  high frequency oscillation
HRF  hemodynamic response function
IC   independent component
ICA  independent component analysis
IGE  idiopathic generalized epilepsy
JME  juvenile myoclonic epilepsy
MDEFT modified driven equilibrium Fourier transform
MEG  magnetoencephalography
MELODIC multivariate exploratory linear optimized decomposition into independent components
MERS multi-echo reference scan
MFG  medial frontal gyrus
MRS  magnetic resonance spectroscopy
PAG  periacqueductal grey
PC   principal component
PCC  posterior cingulate cortex
PET  postron emission tomography
PSC  partial spectral coherence
PSD  power spectral density
RF   radiofrequency
ROI  region of interest
RSFC resting-state functional connectivity
RSN  resting-state network
SC   spectral coherence
Seizures+ patients w/at least 1 seizure (absence or GTC) during the 3 months preceeding scanning
Seizures- patients w/out any seizures (absence or GTC) during the 3 months preceeding scanning
STR  spatiotemporal regression
<table>
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<tr>
<td>SVD</td>
<td>singular value decomposition</td>
</tr>
<tr>
<td>TA</td>
<td>[fMRI] acquisition time</td>
</tr>
<tr>
<td>TE</td>
<td>[MRI] echo time</td>
</tr>
<tr>
<td>TH</td>
<td>thalamus</td>
</tr>
<tr>
<td>TR</td>
<td>[fMRI] repetition time</td>
</tr>
<tr>
<td>VBM</td>
<td>voxel based morphometry</td>
</tr>
<tr>
<td>VEOG</td>
<td>vertical electro-oculogram</td>
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<tr>
<td>VPA</td>
<td>valproic acid (valproate)</td>
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</tbody>
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Chapter 1

Background & Introduction

1.1 Idiopathic Generalized Epilepsy

Idiopathic generalized epilepsy (IGE) is a superset of generalized seizure disorders with presumed genetic etiologies [Berg et al., 2010] that affect all age groups [Commission on Classification and Terminology of the International League Against Epilepsy, 1989] and account for 15-20% of all epilepsies [Jallon & Latour, 2005]. Although IGE is subclassified into several subtypes (i.e. childhood absence, juvenile absence, and juvenile myoclonic) [Rudzinski & Shih, 2010; Sullivan & Dlugos, 2004], these are sufficiently similar to suggest a final common mechanism that justifies their study as a single entity [Benbadis, 2005; Reutens & Berkovic, 1995]. IGE is formally a diagnosis of exclusion after symptomatic (structural) epilepsy, cryptogenic (metabolic) epilepsy, and epilepsy secondary to another condition (e.g. fragile-X syndrome), have been ruled out [Commission on Classification and Terminology of the International League Against Epilepsy, 1989]. However, several known mutations are associated with IGE and may aid in diagnosis, see Section 1.1.4 for examples.

1.1.1 Significance

There are 3 million Americans living with epilepsy at an annual cost in excess of $12 billion [Begley et al., 2000]. Epilepsy is associated with social stigma, lack of independence, and unemployment in adults [Arunkumar et al., 2000; Devinsky et al., 1995; Szaflarski & Szaflarski, 2004]. For example, all
50 states limit driving in epilepsy patients with uncontrolled seizures [Krauss et al., 2001; Krumholz, 2009]. Children with IGE are at risk for poor academic achievement, even if their seizures are well-controlled [Henkin et al., 2005; Sturniolo & Galletti, 1994]. Clinical complications of IGE include status epilepticus [Shorvon & Walker, 2005], physical injury [Ficker, 2000], and death [Nashef et al., 1998, 2007]. IGE patients have high rates of cognitive and psychiatric comorbidities as discussed in Section 1.1.5.

1.1.2 Semiology

Seizures are brief (seconds to minutes) episodes of abnormal, synchronous brain activity accompanied by outward changes in behavior – typically, convulsions. In the clinic, brain activity is monitored using an electroencephalogram (EEG) to detect neuronal electrical potentials on the surface of the scalp. An abnormal electrical event during a seizure is termed an epileptiform discharge. Generalized spike and wave discharges (GSWDs) are one such electrographic hallmark of seizures in IGE, and they are discussed at length in Section 1.2.

Seizures in IGE are generalized (epileptiform discharges involve the entire brain at onset), more likely to occur after awakening or when drowsy, and characterized by the presence of GSWD. It is possible to distinguish between three main types of seizure based on clinical presentation, or semiology. These seizure types and the subtypes of IGE with which they are typically associated are described in the literature [Commission on Classification and Terminology of the International League Against Epilepsy, 1989; Janz, 1997; Lüders et al., 1998; Noachtar & Peters, 2009; Rudzinski & Shih, 2010] and briefly summarized here.

Absence

Absence, or “petit-mal” seizures are abrupt episodes of unconsciousness that typically occur without warning or post-ictal confusion. They are brief (typically < 10 seconds) but can progress to nonconvulsive status epilepticus. Absence seizures are not usually accompanied by myoclonus or loss of muscle tone such that falls are rare, however eye fluttering and oral automatisms are not uncommon. Described as “blank sells” or “mental pauses”, absence seizures may go undetected –
even by the patient, who will often resume whatever task was in progress before the seizure began.

**Tonic-Clonic**

Generalized tonic-clonic (GTC), or “grand-mal” seizures begin with abrupt loss of consciousness followed by tonic contraction of muscles throughout the body. Falls and tongue-biting are common injuries during this tonic phase. The tonic phase is followed by clonus that is initially rapid but then slows. Injury may occur during the clonic phase due to striking nearby objects. Cyanosis and urinary incontinence may occur due to involvement of the associated muscles. The entire seizure typically lasts minutes but can progress to status epilepticus. Post-ictal confusion is typical, and the patient may feel drowsy and complain of muscle soreness.

**Myoclonic**

Myoclonic seizures, or “jerks” are brief episodes of myoclonus that occur without impairment of consciousness. They typically occur with praxis and involve the arms.

**1.1.3 Treatment**

Pharmacotherapy with anticonvulsants or anti-epileptic drugs (AEDs) is the first line of treatment in IGE [Benbadis, 2005; Mantoan & Walker, 2011; Sullivan & Dlugos, 2004; Wolf & Inoue, 1984]. Seizure remission is achieved in 80% of patients by means of one or more AEDs [Faught, 2004; French, 2007; Holland et al., 2010], although adverse drug effects represent a significant source of iatrogenic morbidity [Arunkumar et al., 2000; Greenwood, 2000; Perucca & Gilliam, 2012] that includes exacerbation of seizures [Benbadis, 2005; Genton et al., 2000]. Pharmacoresistant epilepsy is generally treated with resective surgery [Boon et al., 1996; Engel, 2003], but this treatment modality is contraindicated in IGE because of generalized seizure onset and presumed genetic etiology [Engel, 1993]. Vagus nerve stimulation [Kostov et al., 2007; Labar et al., 1999; Ng & Devinsky, 2004] and a ketogenic diet [Groomes et al., 2011] are effective adjuvant therapies for IGE that ameliorate seizure frequency and severity, but they do not typically result in complete seizure remission. Therefore, pharmacoresistant IGE is a clinically significant antecedent of uncontrolled
seizures and treatment refractory epilepsy.

**Valproate**

Valproic acid (valproate, Depakote, VPA) is an archetype AED for the treatment of IGE. It is described as a first-line treatment [Faught, 2004; Mantoan & Walker, 2011; Peterson & Naunton, 2005; Sullivan & Dlugos, 2004; Wolf & Inoue, 1984], but a review of our patient cohort revealed that physicians delayed the use of VPA ($p = 0.02$ controlling for patient age), presumably in favor of newer AEDs [Mantoan & Walker, 2011]. We believe this is because newer AEDs offer more favorable adverse effect profiles compared to VPA, especially in females, in whom VPA can cause polycystic ovarian disease [Mintzer, 2010] and is teratogenic during pregnancy [Peterson & Naunton, 2005]. Nevertheless, response to VPA is highly predictive of pharmacoresistance. 90% of patients whose seizures are controlled by VPA monotherapy can also achieve seizure remission on other AEDs, but less than half of patients in whom an appropriate dose of VPA does not control seizures will benefit from addition of other AEDs [Holland et al., 2010]. VPA’s mechanisms of action include inhibition of excitatory voltage-gated sodium channels and T-type calcium channels, increased concentration of the inhibitory neurotransmitter $\gamma$-aminobutyric acid (GABA), and decreased concentration of the excitatory neurotransmitter aspartate [Kwan et al., 2001; Löscher, 1999].

1.1.4 Genetics

The heritable nature of epilepsy has been recognized since antiquity, and its scientific investigation predates the discovery of DNA [Lennox, 1951]. However, it was not until the sequencing of the human genome that the genetic etiology of IGE was fully appreciated [Durner et al., 2001]. A growing number of genetic mutations have been associated with idiopathic generalized epilepsy [Gardiner, 2005; Lucarini et al., 2007], and it has been suggested that the term “idiopathic” (meaning “unknown cause”) is inappropriate. The name “primary generalized epilepsy” might best describe the disease’s etiology, however “IGE” remains in use for now [Berg et al., 2010].

The T-type calcium channel provides a representative example of how genetics has contributed to a fuller understanding of IGE etiology. A mutation in this ion channel is associated with
childhood absence epilepsy (CAE) in humans and also in several rodent models of IGE [Crunelli & Leresche, 2002]. The succinimides, a class of AED that includes ethosuximide, block T-type calcium channels in *in vitro* human and *in vivo* animal models and are recommended for the treatment of human CAE [Manning et al., 2003; Sullivan & Dlugos, 2004]. Mutations in the T-type calcium channel are mechanistically related to ictogenic brain circuits [Khosravani & Zamponi, 2006; Vitko et al., 2005]. Thus, the discovery of mutant T-type calcium channels elucidates the ictogenic mechanism in a population of IGE patients and predicts treatment response.

Many of the known mutations associated with IGE affect ion channels, which initially led to the classification of IGE as a channelopathy [Heron et al., 2007; Mulley et al., 2003]. However, not all mutations associated with IGE are monogenic, affect ion channels, or have an obvious effect on ictogenic circuits. Some mutations affect neurotransmitter receptors [Baulac et al., 2001], glucose transporters [Arsov et al., 2012; Striano et al., 2012], plasma binding factors [Saccucci et al., 2004; Sadrzadeh et al., 2004], or microtubular proteins [Suzuki et al., 2004, 2009; Wong, 2010]. They may have incomplete penetrance, relying on environmental or other genetic factors for ictogenesis [Durner et al., 2001; Heron et al., 2007]. The genetics of IGE, taken together with inconclusive [Opeskin et al., 2000] evidence of microdysgenesis [Meencke & Janz, 1984; Woermann et al., 1999], suggest a complex, developmental disease [Wong, 2010] involving multiple neuronal networks.

### 1.1.5 Supratentorial Effects

Intelligence in IGE is defined as normal [Commission on Classification and Terminology of the International League Against Epilepsy, 1989], but a strong association of IGE with specific cognitive deficits and personality traits has been noted [Koepp, 2005; Wandschneider et al., 2012], so much so that juvenile myoclonic epilepsy (JME), a subtype of IGE, was once referred to as “impulsive petit mal” [Janz, 1985]. With respect to cognition, IGE patients have difficulty with tasks involving predominantly the frontal lobes including working memory [Swartz et al., 1996], verbal fluency [Kim et al., 2007], mental flexibility, abstract reasoning [Devinsky et al., 1997], and attention [Killory et al., 2011; Maneshi et al., 2012]. Changes in the frontal lobes associated with
IGE are observed using positron emission tomography (PET) [Swartz et al., 1996], voxel based morphometry (VBM) [Betting et al., 2006; Pulsipher et al., 2011; Vulliemoz et al., 2011; Wörmann et al., 1999], magnetic resonance spectroscopy (MRS) [Savic et al., 2000; Simister et al., 2003], magnetoencephalography (MEG) [Sakurai et al., 2010; Stefan et al., 2009; Westmijse et al., 2009], diffusion tensor imaging (DTI) [Deppe et al., 2008; O’Muircheartaigh et al., 2011; Vulliemoz et al., 2011], and functional magnetic resonance imaging (fMRI) [Killory et al., 2011; Maneshi et al., 2012]; the degree of change correlates with the degree of cognitive deficit [Killory et al., 2011; Swartz et al., 1996; Vulliemoz et al., 2011] and may increase with duration of epilepsy [Kim et al., 2007; Maneshi et al., 2012; Pulsipher et al., 2011].

IGE patients also suffer from a high rate of psychiatric comorbidity ranging from 26.1% for IGE as a whole [Akanuma et al., 2008] to 47% for JME [Trinka et al., 2006]. Mood, anxiety, and personality disorders are the most common, and the risk is higher in patients whose seizures are not well-controlled [Akanuma et al., 2008]. Both psychiatric and cognitive symptoms may precede a patient’s first seizure [Hermann & Seidenberg, 2007]. Elevated anxiety and depressive-like behavior are also observed in a rat model of IGE, suggesting that IGE and its psychiatric comorbidities share a common etiology [Jones et al., 2008].

1.2 Anatomy of the Generalized Spike and Wave Discharge

The electroencephalogram (EEG) is a non-invasive method for recording electrical brain activity by means of electrodes placed on the scalp [Berger, 1929]. It was quickly realized that the EEG of epilepsy patients differed from healthy individuals [Gibbs et al., 1935]. Epileptics manifested “brainstorms” described as “brain waves... at the rate of about three per second and in a strange pattern of large round waves with a spiky wave between the round ones.” [Stafford, 1935] Such a waveform is shown in Figure 1.1. Note the alternating “spiky” and “round” wave-like components of the discharge, and note its general presence, i.e. it is observed simultaneously in all leads. It is a generalized spike and wave discharge (GSWD).
Figure 1.1: Normal 11 Hz alpha background rhythm (left) followed by generalized spike and wave discharges (GSWDs) at 3 Hz (right). Note the sharp, downward spike followed by a slow, round wave. Note how the GSWDs appears simultaneously in all leads (i.e. generalized onset). This EEG was recorded from an IGE patient simultaneous with fMRI and subsequently denoised as described in Appendix C.1. Ten seconds of data are shown in a bipolar, double-banana montage.

1.2.1 Mechanism

The GSWD waveform is observed during absence and generalized tonic-clonic seizures (GTCs) in IGE, and it may also appear interictally (i.e. between seizures) [Lüders et al., 1998; Pedersen & Petersen, 1998; Smith, 2005; Yenjun et al., 2003]. The waveform manifests everywhere at once, and it was thus reasoned that the GSWD must originate in some “centrencephalic” structure such as the thalamus, which is approximately equidistant from the cortical surface [Jasper & Kershman, 1941]. Mounting evidence of cortical involvement in the manifestation of GSWD eventually led to a “corticoreticular” hypothesis in which GSWD arose from interactions between cortical and subcortical tissues [Gloor, 1968]. This has evolved into the current consensus that reentrant thalamocortical circuits are the substrate of GSWD [Blumenfeld, 2002; Contreras et al., 1996; Destexhe et al., 1999; Moeller et al., 2008a], possibly via a mechanism shared with sleep spindles [Blumenfeld & McCormick, 2000; Blumenfeld, 2005; Destexhe, 1998].
Figure 1.2: Spike-related activation (red) and deactivation (blue), controlling for number of spikes, are overlayed on the standard MNI anatomic brain in radiological orientation. Spike times obtained from a simultaneous EEG recording were convolved with a canonical gamma variate hemodynamic response function (HRF) with its peak 4.7 s post onset using AFNI. Group responses from all subjects (left) and a contrast between valproate (VPA)-resistant and VPA-responsive subjects (right) were obtained. Significant ($\alpha = 0.05$) clusters of $\geq 36$ suprathreshold voxels (left: two-sided $t > 2.3$, right: one-sided $t > 2$) are shown. Note the widespread cortical activation that includes thalamus, frontal (paracingulate), and motor regions (Section 1.2.2). Note the specific deactivation in default mode network (DMN) regions (Section 1.4.3). Paracingulate cortex activates more strongly with GSWD in VPA-resistant IGE patients. The original version of this figure may be found in [Szaflarski et al., 2013].

1.2.2 Sources of Evidence

Animal models of murine [Coenen & Van Luijtelaar, 2003] and feline [Avoli, 1995] epilepsies have provided abundant information about GSWD through invasive experimentation. Some human data from invasive subdural and depth electrodes are available [Hayne et al., 1949; Niedermeyer et al., 1969; Williams, 1953], but since IGE patients are not candidates for epilepsy surgery [Engel, 1993], these data are rare. Neuroimaging provides a non-invasive source of data about IGE. MRI [Betting et al., 2006; Pulsipher et al., 2011], MRS [Bernasconi et al., 2003], MEG [Sakurai et al., 2010; Stefan et al., 2009], and DTI [Deppe et al., 2008] studies have shown subtle differences in brain anatomy and metabolism consistent with the corticoreticular hypothesis. More recently, simultaneous EEG/fMRI has provided a means of measuring GSWD-related brain activity directly [Gotman et al., 2006] (See Appendix C.1). These studies show thalamic and diffuse cortical activation during GSWD [Aghakhani et al., 2004; Gotman et al., 2005; Hamandi et al., 2006; Laufs et al., 2006; Moeller et al., 2008b; Szaflarski et al., 2010, 2013; Tyvaert et al., 2009], see Figure 1.2. EEG/fMRI also makes it possible to observe resting-state brain activity while controlling for the effects of GSWD [Gotman et al., 2006] as discussed in Section 1.4.
1.2.3 Further Questions

Although the immediate process by which the GSWD waveform is produced is now somewhat better understood, many aspects of this process remain unsolved. It is not known how seizures start [Arroyo & Uematsu, 1992; Wood, 2011], how they stop [Lado & Moshé, 2008], nor how interictal GSWD affect the brain [Staley et al., 2005]. The variable semiology of GSWD is also not explained, although the network inhibition hypothesis discussed in Sections 1.4.3 and 5.2 may explain why GSWD can occur interictally without causing an absence seizure. It is not known if all of cortex or thalamus participates equally in the reentrant thalamocortical circuits. In the genetic rodent models of IGE, the orofacial region of somatosensory cortex appears to initiate GSWD [Polack et al., 2007]. The medial frontal lobes are suspected of playing such a role in humans [Meeren et al., 2005; Niedermeyer, 1996; Sakurai et al., 2010]; this hypothesis is consistent with the supratentorial, frontal lobe deficits discussed in Section 1.1.5. Using EEG/fMRI, it was recently shown that this region activates more strongly during GSWD in IGE patients whose seizures are resistant to treatment with VPA [Szafarski et al., 2013].

1.3 Causes of Treatment Resistance

The 20% of IGE patients resistant to pharmacotherapy are otherwise clinically indistinguishable from their treatment-responsive counterparts [Holland et al., 2010; Kwan & Brodie, 2000]. The absence of biomarkers for treatment-resistance [Berg, 2009] makes it difficult for the clinician to anticipate which patients will remit and difficult for the scientist to devise novel treatment strategies.

1.3.1 Adverse Effects

A subset of patients responds to pharmacotherapy but they are unable to tolerate the coincident adverse effects, rendering them effectively treatment-resistant. Somatic adverse effects range from weight gain to rash to liver toxicity [Greenwood, 2000; Perucca & Gilliam, 2012]. Exacerbation of seizures may occur if the wrong AED is used for the wrong type of epilepsy (i.e. due to misdiagnosis) [Benbadis, 2005; Gentry et al., 2000]. Cognitive adverse effects are not uncom-
mon [Mula & Trimble, 2009; Reynolds, 1983] and are associated with measurable changes in brain function [Szaflarski & Allendorfer, 2012; Yasuda et al., 2013]. Fortunately, 41-90% of patients in this category will remit when switched to a better-tolerated AED [Holland et al., 2010; Kwan & Brodie, 2000].

1.3.2 Bioavailability

Studies of the cancers have led to the observation that individual differences in pharmacokinetics related to genetic polymorphisms, such as higher rates of drug metabolism and clearance, can prevent an otherwise effective drug from acting at its target [Persidis, 1999]. Similar mechanisms of drug resistance exist in epilepsy; for example, drug resistance genes may limit AED access to the blood brain barrier [Basic et al., 2008; Dombrowski et al., 2001; Sisodiya et al., 2002]. Nevertheless, the pharmacopeia for IGE contains many chemically dissimilar AEDs with overlapping mechanisms of action [Kwan et al., 2001]. Therefore, while genetic factors affecting bioavailability and metabolism may account for resistance to an AED or class of AEDs [French, 2007], these factors cannot fully account for the phenomenon of treatment-resistance.

1.3.3 Mechanisms of Drug Action

Whereas 41-90% of patients who discontinue an AED due to adverse effects will achieve seizure remission on other AEDs, only 11-45% of patients who discontinue an AED due to ineffectiveness will do so [Holland et al., 2010; Kwan & Brodie, 2000]. This observation suggests that the pharmacological properties of AEDs are relatively unimportant determinants of treatment-resistance compared to their mechanisms of action. Many different chemical classes of AED exist for the treatment of IGE. However, all are thought act through common mechanisms of either inhibiting excitatory neurotransmission (via sodium channels, calcium channels, and glutamate receptors), potentiating inhibitory neurotransmission (via potassium channels and GABA receptors), or both [Kwan et al., 2001]. For example, even though VPA has broad-spectrum action on both excitatory and inhibitory neurotransmission, it is not very effective in cortical-onset epilepsies [Jeavons & Clark, 1974] and thus might not be effective in an IGE patient with significant cortical involvement. AEDs with
novel mechanisms of action targeting specific ictogenic or anti-ictogenic neuronal networks could prove to be useful adjuvant treatments [Holtkamp & Meierkord, 2007].

1.4 Resting-State Functional Connectivity

This section introduces the concept of resting-state networks (RSNs), brain regions in which activity is correlated at low frequencies even in the absence of an experimental task [Biswal et al., 1995]. The default mode network (DMN) is a robust resting-state network that is observed under conditions of awake relaxation and is thought to play a role in consciousness [Raichle et al., 2001].

1.4.1 Interpretation of Low Frequency Oscillations

Neuroimaging with fMRI has conventionally examined changes in brain activity associated with an experimental task, but it is also possible to observe spontaneous, low-frequency oscillations in a brain at “rest” [Biswal et al., 1995]. Although referred to as the “resting-state,” subjects are not actually “resting” in the sense of being asleep. Rather, subjects are placed supine in the scanner with eyes closed or with a fixation cross and not given any explicit task to perform [Yan et al., 2009]. Low-frequency oscillations on the order of 0.1 Hz are observed in the fMRI signal [Achard et al., 2006; Biswal et al., 1995; Cordes et al., 2001]. These oscillations are spectrally similar to physiological sources of signal such as heartbeat and breathing, which are aliased into the recorded data at typical fMRI acquisition (TR) intervals of 2-3 seconds [Lowe et al., 1998]. Although physiological aliasing does pose a challenge during data processing, it has been shown that low frequency resting-state oscillations are localized primarily to cerebral cortex and reflect neuronal activity [Chang & Glover, 2009; De Luca et al., 2006]. The neuronal origin of these oscillations is confirmed by PET [Raichle et al., 2001], EEG [Laufs, 2008], MEG [de Pasquale et al., 2010], and subdural electroencephalographic recording [Miller et al., 2009].

Temporally distinct low frequency oscillations involve different sets of brain regions, or resting-state networks (RSNs), which are thought to represent groups of cognitive modules that may be recruited during specific cognitive tasks [Cordes et al., 2000; De Luca et al., 2006; Fox et al., 2005]. Within-network correlation is thought to reflect the strength of interaction, or resting-state
functional connectivity (RSFC), between the associated brain regions [De Luca et al., 2006]. RSFC reflects underlying anatomical connectivity, i.e. DTI white matter integrity, but is more sensitive to rapid changes [Greicius et al., 2009]. RSFC is sensitive to developmental and pathological changes between subjects and groups [Biswal et al., 2010].

1.4.2 Relevance in Epilepsy

Epilepsy is thought to be a disorder of brain synchrony and organization [Garcia Dominguez et al., 2005; Zhang et al., 2011]. Many IGE studies have shown changes in anatomical, or structural connectivity using DTI [Deppe et al., 2008; Hutchinson et al., 2010; O’Muircheartaigh et al., 2011; Vulliemoz et al., 2011], and RSFC may be even more sensitive to changes associated with IGE than DTI is [Zhang et al., 2011]. Thus, RSFC is well suited to the investigation of epilepsy. A growing number of resting-state studies have demonstrated altered RSFC associated with IGE and its cognitive effects [Bai et al., 2011; Killory et al., 2011; Luo et al., 2011, 2012; Maneshi et al., 2012; McGill et al., 2012; Moeller et al., 2011; O’Muircheartaigh et al., 2012; Sakurai et al., 2010; Wang et al., 2011; Zhang et al., 2011].

1.4.3 The Default Mode Network

The default mode network (DMN) is a resting-state network involving, chiefly (and in addition to other regions), lateral parietal/occipital cortex, frontal cortex, and cuneus [Raichle et al., 2001; Raichle & Snyder, 2007], with its center, or “hub” in posterior cinculate cortex (PCC) [Greicius et al., 2003]. Although modulated by age [Ferreira & Busatto, 2013; Sambataro et al., 2010], it is robust and reproducible across subjects [Buckner et al., 2008; Calhoun et al., 2008; Damoiseaux et al., 2006; Morgan et al., 2008; van de Ven et al., 2004], and may even be observed in preterm human infants [Doria et al., 2010] and rodents [Lu et al., 2012; Upadhyay et al., 2011]. The DMN is therefore a reliable network to investigate in neurological disorders.

Although a resting-state network, the DMN may also be observed during the performance of cognitive tasks [Calhoun et al., 2008; Fair et al., 2007; Gordon et al., 2012], during which its RSFC is transiency reduced [Greicius & Menon, 2004]. The strong anticorrelation between the DMN and
cognitively demanding tasks has earned it the pseudonym of “task-negative network” [Fox et al., 2005]. Unlike most RSNs, the DMN is thought to directly support cognitive activities in the resting brain including introspection [Gusnard et al., 2001], memory [Greicius et al., 2004], spontaneous cognition [Raichle & Snyder, 2007], and consciousness [Fox et al., 2005; Morgan et al., 2008; Raichle et al., 2001; Vanhaudenhuyse et al., 2010].

Network Inhibition Hypothesis

In addition to GSWD-related activation, EEG/fMRI studies of IGE have also revealed GSWD-related deactivation (i.e. decreases in fMRI signal) in specific brain regions corresponding to the DMN [Aghakhani et al., 2004; Gotman et al., 2005; Hamandi et al., 2006; Laufs et al., 2006; Moeller et al., 2008b; Salek-Haddadi et al., 2003; Szafarski et al., 2010, 2013], see Figure 1.2. DMN involvement in GSWD is also observed using MEG [Sakurai et al., 2010]. It is probably not a coincidence that DMN deactivation accompanies absence seizure semiology, discussed in Section 1.1.2, which involves selective impairment of consciousness. Rather, it is thought that inhibition of the DMN leads to loss of consciousness during absence seizures via the network inhibition hypothesis [Danielson et al., 2011; Vaudano et al., 2009]. Several recent resting-state fMRI studies of IGE have observed reduced DMN connectivity in patients with IGE compared to healthy controls where the degree of reduction is correlated with disease duration [McGill et al., 2012; Luo et al., 2011; Wang et al., 2011].

1.5 Specific Aims

Idiopathic generalized epilepsy (IGE) is a primary [Berg et al., 2010] seizure disorder associated with high rates of cognitive [Devinsky et al., 1997; Killory et al., 2011; Kim et al., 2007; Maneshi et al., 2012; Swartz et al., 1996] and psychiatric comorbidity [Akanuma et al., 2008; Trinka et al., 2006] that is thought to be caused by abnormal neuronal circuits [Garcia Dominguez et al., 2005; Engel et al., 2013; Zhang et al., 2011]. IGE accounts for 15-20% of the three million Americans who live with epilepsy [Jallon & Latour, 2005] at an annual cost in excess of $12 billion [Begley et al., 2000]. Anti-epileptic drugs (AEDs) are the primary mode of treatment in IGE [Benbadis,
2005; Mantoan & Walker, 2011; Sullivan & Dlugos, 2004; Wolf & Inoue, 1984], and surgery is
contraindicated [Engel, 1993]. Approximately 20% of IGE patients experience uncontrolled seizures
despite appropriate treatment with AEDs [Holland et al., 2010; Kwan & Brodie, 2000]. Uncontrolled
seizures are a significant cause of poor quality of life [Arunkumar et al., 2000; Devinsky et al., 1995;
Szaflarski & Szaflarski, 2004] (e.g. driving restrictions [Krauss et al., 2001; Krumholz, 2009]),
morbidity [Akanuma et al., 2008; Ficker, 2000; Shorvon & Walker, 2005; Trinka et al., 2006] (also
due to AED adverse effects [Greenwood, 2000; Mula & Trimble, 2009; Reynolds, 1983; Szaflarski &
Allendorfer, 2012; Yasuda et al., 2013; Perucca & Gilliam, 2012]), and mortality [Nashef et al., 1998,
2007] in epilepsy patients. Although the clinical occurrence of treatment-resistant IGE is common,
its neuronal correlates are not well understood [Berg, 2009]. Resting-state functional connectivity
(RSFC) is a recent technique for the investigation of neuronal circuits in IGE using simultaneous
electroencephalography and functional magnetic resonance imaging (EEG/fMRI) [Gotman et al.,
2006; Zhang et al., 2011].

1.5.1 Cortical-Subcortical Connectivity

IGE ictogenesis is thought to involve reentrant cortical-subcortical circuits [Gloor, 1968], thala-
mocortical circuits in particular [Blumenfeld, 2002; Contreras et al., 1996; Destexhe et al., 1999;
Moeller et al., 2008a]. In this aim we tested the hypothesis of altered cortical-subcortical RSFC
in IGE with special attention given to thalamocortical RSFC (Chapter 2). We used seed-based
voxel correlation to measure RSFC in resting-state EEG/fMRI data after excluding time points
associated with epileptiform discharges. Beginning with a functionally-relevant \textit{a priori} seed in
paracingulate cortex (part of medial frontal cortex) that was previously shown to be associated
with treatment-resistance [Szaflarski et al., 2013], we identified regions exhibiting altered RSFC
with the seed in (1) IGE patients vs. healthy controls and (2) treatment-resistant vs. treatment-
responsive IGE patients. We then created \textit{a posteriori} seeds in regions exhibiting significantly
altered RSFC with the paracingulate seed and used these to investigate (3) reciprocally altered
RSFC with the paracingulate seed and (4) the possible involvement of other brain regions.
1.5.2 Default Mode Network Connectivity

Epileptic discharges in IGE are associated with specific deactivation in default mode network (DMN) brain regions [Aghakhani et al., 2004; Gotman et al., 2005; Hamandi et al., 2006; Laufs et al., 2006; Moeller et al., 2008b; Salek-Haddadi et al., 2003; Szaflarski et al., 2010, 2013], and DMN RSFC is reduced in IGE patients compared to healthy controls [McGill et al., 2012; Luo et al., 2011; Wang et al., 2011]. In this aim we test the hypothesis of reduced DMN RSFC in treatment-resistant vs. treatment-responsive IGE (Chapter 3) and explore possible mechanisms by which GSWD could affect the DMN (Chapter 4). We used independent component analysis (ICA) and dual regression to identify the DMN and measure RSFC in resting-state EEG/fMRI data after excluding scans containing epileptiform discharges. We compared DMN RSFC in (1) IGE patients vs. healthy controls and (2) treatment-resistant vs. treatment-responsive IGE patients. Using seed-based voxel correlation, we performed confirmatory analysis of DMN RSFC with a seed in posterior cingulate cortex (PCC). We then used the paracingulate seed for DMN to explore (3) the correlation between RSFC and discharge frequency.
Chapter 2

Uncontrolled Seizures are Associated with Changes in Cortical-Subcortical Connectivity

This chapter and Chapter 4 contain portions of a manuscript under review [Kay et al., 2013b].

2.1 Introduction

Approximately 20% of IGE patients are treatment-resistant and experience uncontrolled seizures despite appropriate treatment with AEDs [Holland et al., 2010; Kwan & Brodie, 2000]. Uncontrolled seizures contribute to poor quality of life [Arunkumar et al., 2000; Devinsky et al., 1995; Szaflarski & Szaflarski, 2004] and an unfavorable clinical outcome [Akanuma et al., 2008; Ficker, 2000; Shorvon & Walker, 2005; Trinka et al., 2006; Nashef et al., 1998, 2007] (Section 1.1.3). Whereas EEG alone is not sufficient to reliably identify treatment-resistant patients [Berg, 2009], a combination of EEG and fMRI provides sufficient spatiotemporal localizing power to elucidate ictogenic regions associated with treatment-resistance [Szaflarski et al., 2013] (Section C.1). Resting-state EEG/fMRI is a recent technique that is sensitive to IGE-related changes in neuronal circuits [Zhang et al., 2011] (Section 1.4). In this study we use resting-state EEG/fMRI to investigate neuronal circuits
associated with uncontrolled seizures.

### 2.1.1 GSWDs and the Paracingulate Cortex

As discussed in Section 1.2, the GSWD is an EEG waveform associated with IGE [Lüders et al., 1998; Pedersen & Petersen, 1998; Smith, 2005; Yenjun et al., 2003]. GSWDs are thought to arise from reentrant thalamocortical circuits [Blumenfeld, 2002; Contreras et al., 1996; Destexhe et al., 1999; Moeller et al., 2008a]. Simultaneous EEG/fMRI recordings (see Appendix C.1) reveal thalamic and cortical fMRI activation coincident with GSWD timing [Gotman et al., 2006]. Although GSWDs involve the entire cortex, it is thought that medial frontal cortex in particular may be involved in the initiation and maintenance of GSWDs [Meeren et al., 2005; Niedermeyer, 1996; Sakurai et al., 2010]. This hypothesis is consistent with the finding of cognitive [Swartz et al., 1996; Kim et al., 2007; Devinsky et al., 1997; Killory et al., 2011; Maneshi et al., 2012] and psychiatric [Akanuma et al., 2008; Trinka et al., 2006] deficits associated with frontal lobe abnormalities [Betting et al., 2006; Deppe et al., 2008; O’Muircheartaigh et al., 2011; Pulsipher et al., 2011; Savic et al., 2000; Simister et al., 2003; Stefan et al., 2009; Vulliemoz et al., 2011; Westmijse et al., 2009; Woermann et al., 1999] in IGE (Section 1.1.5). A recent EEG/fMRI study found that GSWD-related activation in paracingulate cortex, which is a part of medial frontal cortex, is associated with uncontrolled seizures and treatment-resistance in IGE (specifically resistance to VPA; see Section 1.1.3) [Szaflarski et al., 2013].

### 2.1.2 Hypotheses

In this resting-state EEG/fMRI study we used seed-based voxel correlation to investigate RSFC in thalamofrontal circuits. Previous resting-state studies [Moeller et al., 2011] have demonstrated the difficulty of obtaining an optimal thalamic seed for this purpose, so in this study we chose to use the region of paracingulate cortex associated with treatment-resistance [Szaflarski et al., 2013] (Figure 1.2) as an *a priori* seed. We hypothesized (1) that paracingulate RSFC in IGE patients with uncontrolled seizures (Seizures+) would be different from that of IGE patients in remission (Seizures-), and (2) that thalamocortical RSFC with the paracingulate seed would be increased in
IGE consistent with a thalamocortical mechanism of GSWD ictogenesis.

2.2 Subjects & Methods

This study examined a previously described cohort of 100 epilepsy patients and 40 healthy control subjects [Szaflarski et al., 2013]. Epilepsy patients who satisfied published criteria for the diagnosis of IGE [Commission on Classification and Terminology of the International League Against Epilepsy, 1989] were enrolled after evaluation at the Cincinnati Epilepsy Center. Diagnosis and treatment were directed by an epilepsy specialist [Szaflarski et al., 2008] with specific inclusion and exclusion criteria published previously [Szaflarski et al., 2013]. All participants in the study provided written informed consent for a protocol approved by the Institutional Review Boards of the University of Cincinnati and the Cincinnati Children’s Hospital Medical Center. Each IGE patient underwent 1-3 consecutive 20-minute EEG/fMRI scans, and each healthy control subject underwent 1-2 consecutive scans. All patients and 20/40 control subjects listened to self-selected music during scanning to increase comfort and compliance. The effect of music-listening on resting-state data is discussed in Appendix A and was included as a covariate in analyses [Kay et al., 2012]. Seizure freedom (Seizures-) was defined as no seizures in the 3 months preceding the scanning session while patients with any seizures (absence or GTC) were included in the uncontrolled group (Seizures+).

Eleven IGE patients failed to complete at least one scan due to claustrophobia ($n = 3$), metallic artifacts ($n = 1$), or not wanting to continue the procedure ($n = 7$). Of the 89 IGE patients who completed scanning, 15 patients were excluded due to poor quality data and an additional 2 were excluded because a high number of GSWD led to an ill-conditioned design matrix. All control subjects completed scanning, but 2 were excluded due to poor quality data. Thirty scans (28 IGE and 2 healthy controls) were excluded in total. Resting-state analysis was carried out on 231 scans from 72 IGE patients (152 scans) and 38 healthy controls (67 scans), see Table 2.1.

2.2.1 EEG Acquisition & Processing

Acquisition and processing of EEG data simultaneous with fMRI was carried out as described previously [DiFrancesco et al., 2008; Espay et al., 2008; Szaflarski et al., 2013] using Scan 4.3.5 software...
Table 2.1: Demographics of idiopathic generalized epilepsy (IGE) patients, subdivided by clinical feature, and healthy controls. Mean age and duration of epilepsy at scanning (±standard deviation) are given in years. Seizures- = epilepsy patients who were seizure-free during the three months leading up to scanning. Seizures+ = IGE patients who experienced at least one seizure during the three months leading up to scanning. JME = juvenile myoclonic epilepsy.

(Compumedics U.S.A., Ltd., El Paso, TX, U.S.A.). (Also, see Appendix C.1.) Subjects were fitted with an MRI-compatible EEG cap with electrodes arranged according to the international 10/20 system, braided carbon cabling, and current-limiting in-line resistors (Compumedics USA, Ltd., El Paso, TX). Conductive gel (Quik-Gel; Compumedics Neuromedia Supplies, Charlotte, NC, U.S.A.) was used to establish low impedance (confirmed as < 20 kΩ) between each electrode and the scalp. 64-channels of data, including two mastoid channels, one vertical electro-oculographic (VEOG) channel, and one electrocardiographic (ECG) channel, were recorded at 10 kHz concurrent with fMRI using an MRI compatible system. Time marks generated by the scanner were automatically inserted into the datastream at the onset of each volume acquisition.

Data were low-pass filtered at 30 Hz and gradient artifacts were removed using an average artifact subtraction technique [Allen et al., 2000] based on the time marks inserted by the scanner. A moving average template of five volumes was used to account for changes in the artifact gradient over time. The fidelity of this technique was improved by optimizing the temporal alignment between the average gradient waveform and the raw data using cross-correlation with a shift limit of 25 samples. Reduction of ballistocardiographic (BCG) artifacts was performed using a linear spatial filtering technique [Lagerlund et al., 1997]. For each subject, a component of the QRS complex was manually identified in the ECG channel and used to epoch a majority of heartbeat events in the first functional scan. These epochs were detrended, demeaned, and reviewed (BPK) for additional
artifact contamination. An average artifact waveform excluding contaminated epochs was obtained. Spatial singular value decomposition (SVD) was used to obtain the principal components of the average waveform. Those components accounting for 99% of signal variance were used to construct a spatial filter, which was applied to EEG data post gradient artifact removal and decimation to a 1000 Hz sampling rate.

All EEG data were reviewed by a board certified epilepsy specialist (JPS), and GSWD timings were marked to within 10 ms precision.

2.2.2 MRI Acquisition & Processing

Acquisition of MRI and fMRI data was carried out on a 4 Tesla, 61.5 cm bore Varian Unity INOVA system (Varian, Inc., Palo Alto, CA) equipped with a standard head coil [DiFrancesco et al., 2008; Szaflarski et al., 2013]. T1-weighted structural images were acquired for use as an anatomical reference. A modified driven equilibrium Fourier transform (MDEFT) method [Duewell et al., 1996; Uğurbil et al., 1993] was used with an 1100 millisecond inversion delay, $256 \times 196 \times 196$ mm field of view, $256 \times 196 \times 196$ voxel matrix, $22^\circ$ flip angle, and $\text{TR/TE} = 13.1/6.0$ ms. T2*-weighted echo-planar functional images with blood oxygenation level-dependent (BOLD) contrast, were acquired with an $256 \times 256$ mm field of view, $64 \times 64$ voxel matrix, $90^\circ$ flip angle, and $5$ mm slice thickness in axial orientation without gap. 400 volumes consisting of 30 slices each and $\text{TR/TA} = 3000/2000$ ms were collected during each scan.

Data were reconstructed and corrected for geometric distortion and Nyquist ghosting with the aid of multi-echo reference scans (MERS) [Schmithorst et al., 2001]. Functional scans underwent slice timing correction, motion correction [Jenkinson et al., 2002], rigid-body registration to a high-resolution anatomical scan [Jenkinson & Smith, 2001], and non-linear registration [Andersson et al., 2007a,b] to an MNI152 standard using FSL [Smith et al., 2004]. Data were spatially blurred in-mask with a gaussian kernel of $\text{FWHM} = 6$ mm using AFNI [Cox, 1996]. The quality of functional to anatomical registration was measured using the mutual information cost function [Jenkinson & Smith, 2001]. Twelve functional scans with an outlying cost indicating unsatisfactory registration were excluded from the study. The quality of motion correction was measured using the normalized
correlation ratio cost function [Jenkinson & Smith, 2001] of each timepoint to the reference volume. Thirteen additional scans with outlying costs indicating excessive motion were excluded from the study.

\subsection*{2.2.3 Seed-Based Voxel Correlation}

A previous EEG/fMRI study at 3 T [Moeller et al., 2011] demonstrated the difficulty of obtaining optimal thalamic seed regions from GSVD-related EEG/fMRI. Thus, in this study, we used a cortical, paracingulate seed region previously identified in the same cohort of patients [Szaflarski et al., 2013] (Figure 1.2) to explore corticothalamic connectivity, and we then used the RSFC results from this paracingulate seed to generate a thalamic seed region for investigation of reciprocal thalamocortical connectivity. The paracingulate cluster used as an initial seed region had its centroid at MNI coordinates $X = 2.0$, $Y = 13.6$, $Z = 45.9$. Two \textit{a posteriori} seeds were manually generated from the results of seed-based voxel correlation with the initial seed by selecting highly correlated voxels at the centers of clusters of correlation. These were bilateral seeds located in dorsal anterior thalamus ($X = \pm 9.2$, $Y = -15.6$, $Z = 13.6$) and cerebellum ($X = \pm 31.6$, $Y = -56.4$, $Z = -28.8$).

The method of seed-based voxel correlation is further discussed in Appendix C.2. The mean timecourse of voxels within each seed region was extracted from each subject’s fMRI data prior to spatial blurring. This timecourse was used as the regressor of interest in a voxelwise general linear model for each subject (3dDeconvolve tool in AFNI). The output of 3dDeconvolve was submitted to the 3dREMLfit tool in AFNI to achieve temporal prewhitening via an autoregressive (AR) model. Baseline drift was modeled using a first-order polynomial as no physiologic regressors were available. No global or tissue regressors were used because these may introduce an unwanted bias [Fox et al., 2009; Weissenbacher et al., 2009]. However, motion has been shown to have an artifactual effect on resting-state connectivity [Power et al., 2012; Van Dijk et al., 2012]. Therefore, we included the six-rigid body motion parameters generated by FSL as nuisance regressors in the model. In addition, timepoints associated with high motion measured as the normalized correlation ratio cost function [Jenkinson & Smith, 2001] to the reference volume were excluded from analysis. Three
timepoints were excluded: those preceeding, including, and following each high-motion volume.

We included subjects and scans containing interictal GSWD in our analysis, but timepoints associated with GSWD were excluded so as to avoid possible contamination of the resting-state by GSWD. GSWD exclusion was analogous to the exclusion of high-motion timepoints. A total of 19 timepoints comprising 57 seconds were excluded for each GSWD: the 9 preceeding, 9 following, and 1 including the GSWD. The exclusion of timepoints from the general linear model due to GSWD and motion resulted in ill-conditioned design matrices for 2 IGE patients (5 scans) with very frequent GSWD who were therefore excluded from the study.

2.2.4 Voxelwise Analysis

The Pearson correlation coefficient of each voxel with the seed timecourse was converted to a $z$-value using the Fisher transformation. Voxelwise analysis of the resultant $z$-values was carried out using R [R Development Core Team, 2013; Whitcher et al., 2011]. IGE patients were divided into two groups: those who had experienced at least one seizure during the 3 months leading up to scanning (Seizures+) and those who were seizure-free (Seizures-). $T$-maps of connectivity for all IGE patients vs. controls and for IGE patients who were Seizures+ vs. IGE patients who were Seizures- were computed with age [Ferreira & Busatto, 2013; Sambataro et al., 2010] and music-listening [Kay et al., 2012] as covariates.

Cluster-based correction for multiple comparisons was carried out using the AlphaSim and 3dmerge tools in AFNI at a significance level of $\alpha = 0.05$. Ventricular and white matter masks were generated from the Harvard-Oxford subcortical probabilistic atlas ($p > 50\%$) distributed with FSL [Desikan et al., 2006]. Observations within these regions were assumed to be artifactual, therefore $t$-maps were masked prior to cluster-based thresholding to avoid inflation of cluster sizes by spurious correlations.
Figure 2.1: Significant ($\alpha = 0.05$, $|t| > 2.24$, $p < 0.028$, # voxels $\geq 27$) decreases (blue) in connectivity for epilepsy patients with uncontrolled seizures (Seizures+) vs. seizure-free patients (Seizures-) are overlayed on the MNI152 standard brain in radiological orientation. Seed regions are shown in green for paracingulate cortex (A) and cerebellum (B).

### 2.3 Results

#### 2.3.1 Seizures+ vs. Seizures- Patients

IGE patients with uncontrolled seizures (Seizures+) exhibited significantly reduced RSFC between the paracingulate seed and bilateral cerebellum when compared to the Seizures- IGE patients (Figure 2.1A). A manually-created seed in the cerebellum exhibited significantly reduced reciprocal RSFC with the paracingulate seed region in Seizures+ vs. Seizures- (Figure 2.1B). The cerebellar seed also exhibited significantly reduced connectivity with thalamus, basal ganglia, and cortex diffusely; ventromedial frontal cortex, including the anterior cingulate cortex (ACC), was relatively spared.

#### 2.3.2 IGE Patients vs. Healthy Controls

No significant differences in RSFC were observed between the paracingulate seed and thalamus in IGE patients vs. healthy controls (Figure 2.2A). However, examination of corticothalamic connec-
Corticothalamic Connectivity (Epilepsy vs. Control)

Figure 2.2: Significant ($\alpha = 0.05$, $|t| > 2.24$, $p < 0.027$, # voxels \(\geq 26\)) increases (red) and decreases (blue) in connectivity for epilepsy patients vs. healthy controls are overlayed on the MNI152 standard brain in radiological orientation. Seed regions are shown in green for paracingulate cortex (A) and thalamus (B).

Figure 2.3: IGE patients exhibited greater connectivity between the parcingulate seed and dorsal anterior thalamus than did healthy controls, but they exhibited reduced RSFC between the parcingulate seed and ventral posterior thalamus. A manually-created seed in dorsal anterior thalamus exhibited significantly increased reciprocal RSFC with the parcingulate seed region in IGE patients vs. controls (Figure 2.2B). The thalamic seed also exhibited significantly reduced RSFC with posterior cingulate cortex (PCC).

2.4 Conclusions

In this study we investigated the effects of uncontrolled seizures on RSFC in cortical-subcortical circuits using a paracingulate seed located in the brain area previously shown to be associated with decreased response to valproate (VPA) [Szafarski et al., 2013]. We found significant differences in RSFC of the paracingulate seed with cerebellum and thalamus in patients with uncontrolled
Subthreshold Corticothalamic Connectivity

\[ X = 10 \quad Y = -18 \]

MNI Coordinate of Slice

Z = 0 \quad Z = 4 \quad Z = 8 \quad Z = 12 \quad Z = 16

Figure 2.3: Increases (red) and decreases (blue) in connectivity between the paracingulate seed region and thalamus for epilepsy patients vs. healthy controls are overlayed on the MNI152 standard brain in radiological orientation. The paracingulate seed region is not shown but is labeled in green in Figures 2.1A and 2.2A. The manually-generated thalamic seed region is shown in green. All voxels with \(|t| > 0.25\) are shown, including those below the threshold for statistical significance (\(\alpha = 0.05\)).

seizures (Seizures+) vs. patients who were seizure free (Seizures-) and in patients with IGE vs. healthy controls. These results are consistent with the thalamocortical hypothesis of GSWD and suggest that an additional corticocerebellar mechanism of ictogenesis is at work in patients with uncontrolled seizures.

2.4.1 Seizures+ vs. Seizures- Patients

At least two previous EEG/fMRI studies have discussed inconclusive cerebellar findings in IGE [Blumental, 2005; Moeller et al., 2011]. The cerebellum shares reciprocal connections with thalamus, cortex [Ramnani, 2006], and basal ganglia [Bostan et al., 2010] through which it could, theoretically, modulate ictogenic activity throughout the brain. Paracingulate RSFC in Seizures+ vs. Seizures- was significantly reduced exclusively with cerebellum (Figure 2.1A), however the same relationship was not observed for IGE patients vs. controls (Figure 2.2A). Reciprocal cerebellar RSFC was reduced in thalamus, basal ganglia, and most of cortex for Seizures+ vs. Seizures- (Figure 2.1B). These data suggest that although cerebellum may not be a primary cause of ictogenesis in IGE, the loss of generalized cerebellar connectivity with thalamus, basal ganglia, and cortex may contribute
to the expression of seizures in treatment-resistant patients.

2.4.2 IGE Patients vs. Healthy Controls

The thalamocortical hypothesis of GSWD is well supported, yet changes in thalamocortical connectivity are not consistently observed [Moeller et al., 2011]. As expected, we observed increased RSFC between the paracingulate seed and thalamus, but these results did not meet our threshold for statistical significance. Upon examining subthreshold RSFC between the paracingulate seed and thalamus in Figure 2.3, we found that thalamus was functionally divided into at least two regions: a ventral posterior region of decreased RSFC and a dorsal anterior region of increased RSFC. Although not statistically significant, these results suggest that adjacent thalamic nuclei may be affected differently by epilepsy [Betting et al., 2006]; exhibit different, and even opposite, connectivity changes in epilepsy [O’Muircheartaigh et al., 2012]; or play different roles in the initiation vs. maintenance of seizure activity [Tyvaert et al., 2009]. This may explain why some previous studies did not detect IGE-associated changes in thalamocortical connectivity.

A manually created seed placed in thalamus based on regions of increased RSFC in Figure 2.3 exhibited significantly increased reciprocal RSFC with cortex in the region of the paracingulate seed (Figure 2.2B) consistent with our hypothesis of increased thalamocortical connectivity in IGE. Nevertheless, we were not able to find widespread increases in cortical connectivity with the thalamic seed. Although GSWD are a generalized phenomenon, it is possible that ictogenic thalamocortical circuits preferentially involve specific regions of cortex such as the paracingulate seed region [Meeren et al., 2005; Niedermeyer, 1996; Szafarski et al., 2013].

RSFC between the thalamic seed and PCC was decreased, consistent with findings from a previous study of the same patient cohort in which we used a seed in PCC to investigate the DMN [Kay et al., 2013a]. Thus, decreased RSFC between thalamus and PCC in IGE patients vs. controls may be explained by reduced DMN RSFC. Decreased connectivity between the PCC and thalamus has been found to be associated with mild cognitive impairment (MCI) in Alzheimer’s disease [Wang et al., 2012].
Chapter 3

Reduced Default Mode Network Connectivity is a Biomarker for Treatment-Resistance

This chapter contains portions of a published paper [Kay et al., 2013a].

3.1 Introduction

Pharmacotherapy is the first line of treatment for IGE, but approximately 20% of IGE patients do not achieve complete seizure remission on AEDs [Holland et al., 2010; Kwan & Brodie, 2000]. As discussed in Section 1.1.3, failure of the archetype AED valproate (VPA) to control seizures is highly predictive of overall pharmacoresistance [Holland et al., 2010]. Thus, the presence of uncontrolled seizures and resistance of seizures to treatment with VPA are both indicators of a clinically treatment-resistant state. Treatment-resistance is associated with poor quality of life [Arunkumar et al., 2000; Devinsky et al., 1995; Szaflarski & Szaflarski, 2004] and an unfavorable clinical outcome [Akanuma et al., 2008; Ficker, 2000; Shorvon & Walker, 2005; Trinka et al., 2006; Nashef et al., 1998, 2007]. Although treatment-resistance is a common, adverse phenomenon, no reliable biomarkers exist for its detection [Berg, 2009]. In this study we use EEG/fMRI to investigate
default mode network (DMN) resting-state functional connectivity (RSFC) as a possible biomarker for treatment-resistant IGE.

### 3.1.1 DMN Activation is Modulated by GSWDs

The absence seizure is characterized by abrupt loss of consciousness without convulsions or post-ictal confusion [Janz, 1997; Lüders et al., 1998; Noachtar & Peters, 2009; Rudzinski & Shih, 2010] (Section 1.1.2). Absence seizures are accompanied by GSWDs, an EEG waveform associated with IGE [Lüders et al., 1998; Pedersen & Petersen, 1998; Smith, 2005; Yenjun et al., 2003] (Section 1.2). EEG/fMRI [Aghakhani et al., 2004; Gotman et al., 2005; Hamandi et al., 2006; Laufs et al., 2006; Moeller et al., 2008b; Salek-Haddadi et al., 2003; Szafarski et al., 2010, 2013] and MEG [Sakurai et al., 2010] studies of IGE show specific deactivation in regions associated with the DMN during GSWDs. As discussed in Section 1.4.3, The DMN is a resting-state network thought to support consciousness [Fox et al., 2005; Morgan et al., 2008; Raichle et al., 2001; Vanhaudenhuyse et al., 2010]. It is probably not an accident that loss of consciousness during absence seizures coincides with deactivation in DMN regions during GSWDs. Rather, it is thought that inhibition of the DMN by GSWDs during absence seizures results in unconsciousness via the network inhibition hypothesis [Danielson et al., 2011; Vaudano et al., 2009] (Section 1.4.3).

### 3.1.2 Hypotheses

In this resting-state EEG/fMRI study we used independent component analysis (ICA) to investigate RSFC in the DMN. Confirmatory analysis was performed using seed-based voxel correlation. Previous resting-state fMRI studies have shown that DMN connectivity is reduced in IGE patients vs. healthy controls and that this reduction is correlated with disease duration [McGill et al., 2012; Luo et al., 2011; Wang et al., 2011]. At least one such study used EEG/fMRI to exclude the effects of GSWD on the resting-state [Luo et al., 2011]. However, the effect of treatment-resistance on DMN connectivity in IGE patients has not been previously investigated. We hypothesized (1) that DMN RSFC would be reduced in IGE patients who were experiencing uncontrolled seizures or were resistant to VPA, and (2) that DMN RSFC would be more strongly anticorrelated with disease
duration in this treatment-resistant cohort.

### 3.2 Subjects & Methods

This study examined a previously described cohort of 100 IGE patients and 40 healthy control subjects [Szaflarski et al., 2013], refer to Section 2.2. Subjects underwent 1-3 consecutive 20-minute EEG/fMRI scans. Some control subjects listened to self-selected music during scanning; the effect of music listening is discussed in Appendix A and was included as a covariate in analyses [Kay et al., 2012]. Eleven IGE patients were unable to complete at least one scan. Of the 89 IGE patients and 40 healthy controls who completed scanning, 15 IGE patients (23 scans) and 2 healthy controls (2 scans) were excluded due to poor quality data. 37 scans containing GSWD were excluded, resulting in the complete exclusion of an additional 12 IGE patients. Resting-state analysis was carried out on 189 scans from 60 IGE patients (122 scans) and 38 healthy controls (67 scans), see Table 3.1.

IGE patients were categorized as treatment-resistant or responsive on the basis of their recent seizure history and prior experience with VPA. As in Section 2.2, seizure freedom (Seizures-) was defined as no seizures in the 3 months preceding the scanning session while patients with any seizures (absence or GTC) were included in the uncontrolled group (Seizures+). Response to VPA (VPA+) was defined as seizure freedom lasting at least three months during treatment with VPA with at least one VPA level documented as therapeutic. Patients who did not achieve seizure control for three months while receiving VPA with consistently therapeutic VPA levels were considered VPA-resistant (VPA-). Patients who were not treated with VPA or whose treatment with VPA lasted less than three months due to intolerable side effects were categorized as VPA-unknown (VPA?). Most \( n = 9 \) patients with uncontrolled seizures also satisfied our criteria for VPA-resistance, but some did not either because they had never tried VPA \( (n=3) \) or because they had been treated with and responded to VPA in the past but were not being treated with VPA in the months leading up to scanning \( (n=4) \). Refer to Table 3.1 and also to the Venn diagram in Figure 3.1.
Figure 3.1: Venn diagram of subject demographics (by group) showing sex and age (years ± standard deviation). Seizures+ (yellow) = IGE patients who experienced at least one seizure during the three months leading up to scanning. VPA+ (cyan) = patients who could achieve seizures control on valproate. VPA- (magenta) = patients who could not achieve seizure control on valproate. Note that patients who had previously achieved seizure control on valproate may have discontinued the drug and resumed seizures at the time of scanning. Note that 19 patients had never tried valproate.
Table 3.1: Demographics of idiopathic generalized epilepsy (IGE) patients, subdivided by clinical feature, and healthy controls. Mean age and duration of epilepsy at scanning (±standard deviation) are given in years. The number of anti-epileptic drugs (AEDs) being taken at time of scanning and number of AEDs that previously failed to produce seizure control are reported. Seizures- = epilepsy patients whose seizures are controlled. Seizures+ = uncontrolled seizures. VPA+ = epilepsy patients who respond to the drug valproate. VPA- = valproate resistant patients. VPA? = epilepsy patients whose response to valproate is unknown. JME = juvenile myoclonic epilepsy.

<table>
<thead>
<tr>
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<th>♂</th>
<th>♀</th>
<th>Age (±SD)</th>
<th>Duration (±SD)</th>
<th>Curr. AEDs</th>
<th>Failed AEDs</th>
</tr>
</thead>
<tbody>
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<td>25</td>
<td>35</td>
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<td>15.5 ± 12.0</td>
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<td>1.95</td>
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<td>27</td>
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<td>8</td>
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<td>17.4 ± 13.9</td>
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<td>1.83</td>
</tr>
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<td>Control</td>
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<td>16</td>
<td>30.9 ± 10.2</td>
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</tbody>
</table>

3.2.1 EEG/fMRI Acquisition & Processing

Methods for acquisition and processing of EEG data simultaneous with fMRI are given in Sections 2.2.1 and 2.2.2, respectively, and have been described previously [DiFrancesco et al., 2008; Espay et al., 2008; Szafarzski et al., 2013]. Subjects were fitted with an EEG cap with electrodes arranged according to the international 10/20 system, and 64-channels of data including an ECG channel were recorded at 10 kHz concurrent with fMRI using an MRI compatible system (Compumedica U.S.A., Ltd., El Paso, TX, U.S.A.). Time marks generated by the scanner (4 T Varian Unity INOVA, Varian, Inc., Palo Alto, CA) were automatically inserted into the datastream at the onset of each volume acquisition. 400 T2*-weighted echo-planar functional volumes consisting of 30 slices each were collected during each scan with a 256 × 256 mm field of view, 64 × 64 voxel matrix, 90° flip angle, 5 mm slice thickness, and TR/TA = 3000/2000 ms. T1-weighted MDEFT [Duewell et al., 1996; Ügurbil et al., 1993] structural images were acquired for use as an anatomical reference with an 1100 millisecond inversion delay, 256 × 196 × 196 mm field of view, 256 × 196 × 196 voxel matrix, 22° flip angle, and TR/TE = 13.1/6.0 ms.

EEG data were low-pass filtered at 30 Hz, gradient artifacts were reduced with an average artifact subtraction technique using the time marks generated by the scanner [Allen et al., 2000],

31
and BCG artifacts were reduced with a linear spatial filter using pulse events identified in the ECG channel [Lagerlund et al., 1997]. All EEG data were reviewed by a board certified epilepsy specialist (JPS). 37 scans containing GSWD were excluded from further analysis to avoid possible contamination of the resting-state.

Functional MRI data were reconstructed and corrected for geometric distortion and Nyquist ghosting with the aid of MERS [Schmithorst et al., 2001]. Functional scans underwent slice timing correction, motion correction [Jenkinson et al., 2002], rigid-body registration to a high-resolution anatomical scan [Jenkinson & Smith, 2001], and non-linear registration [Andersson et al., 2007a,b] to an MNI152 standard using FSL [Smith et al., 2004]. Data were spatially blurred in-mask with a gaussian kernel of FWHM = 6 mm using AFNI [Cox, 1996].

The quality of functional to anatomical registration was measured using the mutual information cost function [Jenkinson & Smith, 2001]. 12 functional scans with an outlying cost indicating unsatisfactory registration were excluded from the study. The quality of motion correction was measured using the normalized correlation ratio cost function [Jenkinson & Smith, 2001] of each timepoint to the reference volume. 13 additional scans with outlying costs indicating excessive motion were excluded from the study. Since residual motion has been shown to have an artifactual effect on resting-state connectivity [Power et al., 2012; Van Dijk et al., 2012], data were lowpass filtered at 0.1 Hz [Cordes et al., 2001], and the six rigid-body motion parameters were regressed out of the data using AFNI in addition to the motion correction performed using FSL. Unfortunately, data on physiologic regressors such as pulse and breathing were unavailable.

### 3.2.2 Independent Component Analysis

The method of independent component analysis (ICA) is discussed in Appendix C.3. Group ICA [Calhoun et al., 2001; Schmithorst & Holland, 2004] of all scans followed by dual regression [Filippini et al., 2009] was carried out using GIFT [Calhoun et al., 2009]. To make analysis of the large number of scans in the study computationally feasible, PCA reduction was applied in two steps. Each scan was reduced to 75 temporal components, the number suggested by the GIFT software, prior to temporal concatenation, and the concatenated result was reduced to 50 temporal
components prior to ICA. The number of components used in the final reduction was chosen empirically [Schmithorst, 2005] because the number obtained using the minimum description length (MDL) criterion [Li et al., 2007] was very large. This process yielded 50 independent components.

Each independent component is thought to represent either a resting-state network, such as the DMN, or an artifact, such as head motion [Damoiseaux et al., 2006]. The most spatially comprehensive DMN component was identified visually and confirmed [Greicius et al., 2004] via the DMN template distributed with GIFT. Component voxels with intensities greater than 99% of the robust range were used as an DMN region of interest (ROI), where the robust range was defined as the 2nd to 98th percentiles of voxel intensities. Voxels with a 50% or greater probability of being white matter, based on the JHU white matter atlas distributed with FSL, were excluded from the DMN ROI [Hua et al., 2008; Wakana et al., 2007].

Back-projection is the process by which a group-level component is resolved onto a single scan. The back-projection of the group DMN component onto each scan was computed using dual regression [Filippini et al., 2009], a technique that normalizes component intensity across scans so as to allow for direct statistical comparisons between scans and experimental groups. Back-projected component intensities of scans from the same subject were averaged voxelwise to obtain one map of DMN connectivity for each subject in the study. These per-subject connectivity maps were subsequently used in high-level statistical analyses as described in Section 3.2.4.

### 3.2.3 Seed-Based Voxel Correlation

The method of seed-based voxel correlation is discussed in Appendix C.2. The posterior cingulate cortex (PCC) is a major “hub” region in the DMN [Greicius et al., 2003]. We used a previously reported seed region in the PCC to investigate DMN RSFC [McGill et al., 2012]. The spherical seed had a radius of 4 mm and was centered at MNI coordinates $X = 2, Y = -58, Z = 24$. As in Section 2.2.3, the mean timecourse of voxels within the seed was extracted from each subject’s fMRI data prior to spatial blurring. This timecourse was used as the regressor of interest in a voxelwise general linear model for each subject (3dDeconvolve tool in AFNI). The output of 3dDeconvolve was submitted to the 3dREMLfit tool in AFNI to achieve temporal prewhitening via an autoregressive
(AR) model. Baseline drift was modeled using a first-order polynomial as no physiologic regressors were available. No global or tissue regressors were used because these may introduce an unwanted bias [Fox et al., 2009; Weissenbacher et al., 2009].

The average of all subjects’ connectivity maps underwent a voxelwise one-sample \( t \)-test, and the resultant \( t \)-values were used as a group DMN connectivity map. As in Section 3.2.2, voxels in the group DMN connectivity map with intensities greater than 99% of the robust range were used as an DMN ROI, where the robust range was defined as the 2nd to 98th percentiles of voxel intensities. As in Section 3.2.2, voxels with a 50% or greater probability of being white matter, based on the JHU white matter atlas distributed with FSL, were excluded from the DMN ROI [Hua et al., 2008; Wakana et al., 2007].

### 3.2.4 Statistical Analysis

High-level analysis was performed using R [R Development Core Team, 2013; Whitcher et al., 2011]. Each analysis was repeated separately with data from ICA and dual regression and with data from seed-based voxel correlation. Voxelwise analysis using a two-sample \( t \)-test was carried out on subjects’ DMN connectivity maps to compare patients with uncontrolled seizures to healthy controls; age [Ferreira & Busatto, 2013; Sambataro et al., 2010] and music-listening [Kay et al., 2012] were included as co-regressors. Voxelwise regression using a linear model with duration of epilepsy as the explanatory variable was carried out on DMN connectivity maps of patients with uncontrolled seizures. Resultant \( t \)-maps were masked with a dilated DMN ROI and corrected for multiple comparisons at \( \alpha = 0.05 \) with cluster-based thresholding using AFNI as in Section 2.2.4.

For ROI analysis, each subject’s DMN connectivity map was summarized by averaging voxels within the group DMN ROI. The resultant value was used as a measure of DMN RSFC for each subject. Linear models were used to assess the effect of epilepsy, duration of epilepsy, VPA-resistance, and uncontrolled seizures on RSFC.
3.3 Results

3.3.1 Voxelwise Analysis

DMN ROIs are shown in Figure 3.2A,D and correspond to regions of GSWD-related deactivation from the same patient cohort [Szaflarski et al., 2013] (Figure 1.2). A significant (α = 0.05) reduction in voxelwise DMN connectivity was observed in IGE patients with uncontrolled seizures compared to healthy controls (Figure 3.2B,E). No significant increases in DMN connectivity were observed within the DMN ROIs. Affected regions and Brodmann areas (BAs) included lingual gyrus/cuneus (BA 18, 19, 30, and 31), lateral occipital cortex (BA 19 and 39), and dorsolateral prefrontal cortex (DLPFC, BA 6, 8, and 9) for analysis with ICA (Figure 3.2B).

Voxelwise DMN connectivity exhibited a significant (α = 0.05) negative correlation with duration of disease in epilepsy patients with uncontrolled seizures (Figure 3.2C,F). No significant positive correlations between DMN connectivity and duration were observed within the DMN ROIs. Affected regions and BAs included hippocampus (not shown, BA 36 and 37), lingual gyrus/cuneus/posterior cingulate cortex (PCC), and lateral occipital cortex for analysis with ICA (Figure 3.2C). No significant voxelwise negative correlation between DMN connectivity and duration of disease was observed in epilepsy patients whose seizures were controlled (data not shown).

3.3.2 ROI Analysis

Between Groups

The differences in DMN connectivity among IGE patients and between patients and healthy controls are summarized in Figure 3.3, and the differences between epilepsy patients and healthy controls, while controlling for age and music-listening, are summarized in Table 3.2. The difference in connectivity between healthy control subjects and all epilepsy patients was not statistically significant (α = 0.05, p = 0.64). However when epilepsy patients with uncontrolled seizures were considered separately, their connectivity was observed to be significantly reduced when compared to healthy controls using ICA (p = 0.019). VPA-resistant patients also trended toward reduced connectivity (p = 0.094).
Figure 3.2: DMN ROIs and IGE-associated changes in RSFC. (Caption continued on next page.)
Figure 3.2: Default mode network (DMN) regions of interest (ROIs) (A, D) and changes in connectivity related to epilepsy (B, C, E, F) are overlayed on the MNI152 standard brain in radiological orientation. Connectivity is shown in red, and reduced connectivity is shown in blue. MNI coordinates of slices are shown at bottom. Results from independent component analysis (ICA) are at top (A, B, C) and seed-based voxel correlations are at bottom (D, E, F). The posterior cingulate cortex (PCC) seed region is shown in green (D, E, F). A, D: The top 1% of z-value in the group DMN independent component ($z > 0.84$) or t-values in the group ($n = 98$) PCC correlation map ($t > 15.18$). B, E: Clusters (# voxels ≥ 36) of significantly ($\alpha = 0.05$, one-sided $t < -1.68$) reduced connectivity in patients with uncontrolled epilepsy ($n = 16$) vs. healthy controls ($n = 38$). C, F: Clusters (# voxels ≥ 36) of significantly ($\alpha = 0.05$, one-sided $t < -1.77$) reduced connectivity correlated with duration of epilepsy in patients with uncontrolled seizures ($n = 16$).

<table>
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<th>N = 38 vs.</th>
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<th>SE</th>
<th>DF</th>
<th>P-Value</th>
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<td>0.075</td>
<td>94</td>
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<td>0.074</td>
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Table 3.2: Region of interest (ROI) analysis of default mode network (DMN) connectivity in epilepsy patients compared to healthy control subjects, with age and music-listening as covariates. Sample size (N), effect size, standard error (SE), degrees of freedom (DF), and two-sided p-values are shown.
DMN in Epilepsy Patients

<table>
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<th>Method</th>
<th>Condition</th>
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<th>Effect Size</th>
<th>SE</th>
<th>DF</th>
<th>P-Value</th>
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<tbody>
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<td>16 vs. 44</td>
<td>-0.215</td>
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<td>58</td>
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<td>13 vs. 28</td>
<td>-0.281</td>
<td>0.114</td>
<td>39</td>
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Table 3.3: Region of interest (ROI) analysis of default mode network (DMN) connectivity for VPA-resistance (VPA-) compared to response (VPA+) and for uncontrolled seizures (Seizures+) compared to controlled (Seizures-) in epilepsy patients. Sample size (N), effect size, standard error (SE), degrees of freedom (DF), and two-sided p-values are shown.

<table>
<thead>
<tr>
<th>Method</th>
<th>Condition</th>
<th>N</th>
<th>Effect Size</th>
<th>SE</th>
<th>DF</th>
<th>P-Value</th>
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Table 3.4: Region of interest (ROI) analysis of default mode network (DMN) connectivity for VPA-resistance (VPA-) compared to response (VPA+) and for uncontrolled seizures (Seizures+) compared to controlled (Seizures-) in epilepsy patients, with duration of disease as a covariate. Sample size (N), effect size, standard error (SE), degrees of freedom (DF), and two-sided p-values are shown.

The effects of VPA-resistance (vs. responsiveness) and uncontrolled seizures (vs. controlled seizures) on DMN connectivity in IGE patients without controlling for duration of disease are shown in Table 3.3 and when controlling for duration of disease in Table 3.4. A significant (p = 0.024) reduction in connectivity was observed for patients with uncontrolled seizures. VPA-resistance also trended toward reduced connectivity, but this trend was diminished (p = 0.19) when the effect of duration was taken into account.

**Duration**

The correlation between DMN connectivity and duration of disease in IGE patients is summarized in Figure 3.4 and shown in Table 3.5. The difference in the correlation between DMN connectivity and duration of disease among IGE patients is shown in Table 3.6. The correlation between connectivity and duration was observed to be significantly negative in patients with VPA-resistance (p < 0.001) and uncontrolled seizures (p = 0.004) using ICA whereas this correlation was either not significant or, paradoxically, positive in VPA-responders (p = 0.56) and patients whose seizures were controlled
Table 3.5: Region of interest (ROI) analysis of default mode network (DMN) connectivity vs. duration of disease in epilepsy patients. VPA- = valproate non-responders, Seizures+ = uncontrolled seizures. Sample size (N), effect size, standard error (SE), degrees of freedom (DF), and two-sided p-values are shown.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Effect Size</th>
<th>SE</th>
<th>DF</th>
<th>P-Value</th>
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<td></td>
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<tr>
<td>All Epilepsy</td>
<td>60</td>
<td>−0.011</td>
<td>0.003</td>
<td>58</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Seizures-</td>
<td>44</td>
<td>−0.007</td>
<td>0.004</td>
<td>42</td>
<td>0.086</td>
</tr>
<tr>
<td>Seizures+</td>
<td>16</td>
<td>−0.016</td>
<td>0.005</td>
<td>14</td>
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</tr>
<tr>
<td>VPA+</td>
<td>28</td>
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<td>0.009</td>
<td>26</td>
<td>0.556</td>
</tr>
<tr>
<td>VPA-</td>
<td>13</td>
<td>−0.003</td>
<td>0.094</td>
<td>11</td>
<td>&lt;0.001</td>
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<tr>
<th></th>
<th>N</th>
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<th>SE</th>
<th>DF</th>
<th>P-Value</th>
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<td>0.023</td>
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<td>Seizures+</td>
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<td>0.030</td>
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<td>VPA+</td>
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<td>VPA-</td>
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<td>−0.047</td>
<td>0.029</td>
<td>11</td>
<td>0.126</td>
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Table 3.6: Region of interest (ROI) analysis comparing the difference in the slope (effect size from Table 3.5) of the linear relationship between default mode network (DMN) connectivity and duration of epilepsy. The slope is compared between VPA-resistant (VPA-) vs. VPA-responsive (VPA+) patients and between patients with uncontrolled seizures (Seizures+) vs. controlled seizures (Seizures-). Sample size (N), effect size, standard error (SE), degrees of freedom (DF), and two-sided p-values are shown.

<table>
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<th>SE</th>
<th>DF</th>
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<td>0.006</td>
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<td>0.151</td>
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<td>VPA- vs. VPA+</td>
<td>13 vs. 28</td>
<td>−0.024</td>
<td>0.009</td>
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<td><strong>0.013</strong></td>
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<td><strong>Seed-Based Voxel Correlation</strong></td>
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<td>16 vs. 44</td>
<td>−0.108</td>
<td>0.037</td>
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<td><strong>0.005</strong></td>
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<tr>
<td>VPA- vs. VPA+</td>
<td>13 vs. 28</td>
<td>−0.162</td>
<td>0.049</td>
<td>37</td>
<td><strong>0.002</strong></td>
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</table>

(p = 0.086). Using ICA, the difference in the magnitude of correlation (i.e. difference in slopes of the best fit lines) was significant (p = 0.013) for VPA-resistant vs. VPA-responsive patients, and it trended toward significance (p = 0.15) for patients with uncontrolled vs. controlled seizures.

## 3.4 Conclusions

In this study we examined the effect of resistance to VPA and the effect of uncontrolled seizures on resting-state DMN functional connectivity. We found that VPA-resistance and uncontrolled seizures are associated with a greater reduction in DMN resting-state functional connectivity (RSFC) than epilepsy alone. These findings confirm that the DMN [Luo et al., 2011; McGill et al., 2012; Wang...
Figure 3.3: Average connectivity within default mode network (DMN) regions of interest (ROIs, see Figure 3.2) for healthy control subjects (Control, $n = 38$), epilepsy patients whose seizures are controlled (Seizures-, $n = 44$), patients who respond to the drug valproate (VPA+, $n = 28$), patients with uncontrolled seizures (Seizures+, $n = 16$), and patients resistant to valproate (VPA-, $n = 13$). The effects of age and music listening have been regressed out of connectivity in healthy controls. The effect of duration has been regressed out of connectivity in epilepsy patients. The symbols $\star$, $\blacklozenge$, $\spadesuit$, and $\clubsuit$ indicate significant ($\alpha = 0.05$) differences, see Tables 3.2 and 3.4. A: DMN connectivity measured using independent component analysis (ICA). B: DMN connectivity measured using seed-based voxel correlation.
Default Mode Network Connectivity v.s. Duration of Disease in Epilepsy Patients

Figure 3.4: Average connectivity within default mode network (DMN) regions of interest (ROIs, see Figure 3.2) is correlated with duration of epilepsy. The coefficient of determination, \( r^2 \), is given; significant (\( \alpha = 0.05 \)) correlations are indicated with *, see Tables 3.5 and 3.6. The results of independent component analysis (ICA) are shown at top (A, B). The results of seed-based voxel correlation are shown at bottom (C, D). A, C: Patients who responded to the drug valproate (VPA+, \( n = 28 \)) are compared to patients resistant to valproate (VPA-, \( n = 13 \)) at left. B, D: Patients whose seizures are controlled (Seizures-, \( n = 44 \)) are compared to patients with uncontrolled seizures (Seizures+, \( n = 16 \)) at right.
et al., 2011] is among the resting-state features [Bai et al., 2011; Killory et al., 2011; Luo et al., 2012; Maneshi et al., 2012] that differ between IGE patients and healthy controls.

### 3.4.1 Reduced RSFC in Treatment-Resistant IGE

Previous resting-state fMRI studies have found that DMN RSFC is reduced in IGE patients vs. healthy control subjects, and that this reduction is correlated with duration of disease [Luo et al., 2011; McGill et al., 2012; Wang et al., 2011]. However, we are not aware of any previous studies that specifically investigated the effect of treatment-resistance on RSFC in IGE. Our results suggest that reduced DMN RSFC in patients with IGE as a whole is due predominantly to the presence of treatment-resistant patients within the study population. When treatment-resistant and treatment-responsive patients are considered separately, treatment-resistance is associated with significantly lower connectivity whereas, depending on the technique used, it may not be possible to distinguish between treatment-responsive patients and healthy controls; see Figure 3.3 and Tables 3.2-3.4. These findings are consistent with the hypothesis that differences in neuronal networks and their response to AEDs cause treatment-resistance and suggest that reduced DMN connectivity may be useful as a biomarker for treatment-resistance.

### 3.4.2 Reduced RSFC with Disease Duration

It is notable that DMN RSFC declines with duration of epilepsy [Luo et al., 2011; McGill et al., 2012; Wang et al., 2011], and that this diminishing effect is significantly more pronounced in treatment-resistant patients (Figure 3.4 and Tables 3.5-3.6). This finding suggests a cumulative effect of epilepsy on the brain and, if replicated, might weigh in favor of a clinical decision to treat aggressively. It would be interesting to learn if uncontrolled seizures have a uniform effect on connectivity or if seizure or GSWD frequency is negatively correlated with DMN connectivity. Lack of detailed information on seizure load (number of seizures per unit of time) or GSWD frequency during 24 hour monitoring did not allow for these analyses in this study.
3.4.3 Limitations

This study investigated a cross-section of IGE that included newly diagnosed patients as well as patients who had lived with epilepsy for more than 50 years. As such, it was not possible to directly examine whether reduced DMN RSFC is an outcome of treatment-resistance or a predictor of it. We came close to this question by including treatment-resistance and duration of disease in the same model (Tables 3.3 and 3.4). Using ICA, we found that the effect of uncontrolled seizures on connectivity remains significant even after controlling for the effect of duration. Although the effect of VPA-resistance was significant on its own, it did not reach our threshold for significance ($\alpha = 0.05$) after controlling for duration. This could have been due to the small number of VPA-resistant patients in the study ($n = 13$) or to a confounding correlation between VPA-resistance and duration. VPA-resistant patients were observed to be older (by $8.1 \pm 3.5$ years, $p < 0.05$) and to have had epilepsy for a longer period of time (by $10.5 \pm 3.9$ years, $p < 0.01$) than VPA-responders. Although including duration in the model of connectivity vs. VPA response was appropriate ($F = 7.85$, $p = 0.008$), its collinearity with VPA-resistance precipitated a drop in statistical power from 0.96 to an unsatisfactory level of 0.60. A prospective study of newly-diagnosed drug-naïve IGE patients would help resolve this confound and establish whether DMN connectivity can predict treatment-resistance. At least one such study [Moeller et al. 2008b] has demonstrated deactivation of DMN regions during GSWD in a drug-naïve population.

We had not expected to observe a correlation between VPA status and age or duration, and no such correlation was observed for uncontrolled vs. controlled seizures ($p > 0.4$). As discussed in Section 1.1.3, we postulate that, due to the less favorable adverse effects profile of VPA compared to newer drugs, nowadays clinicians are postponing treatment with VPA until other treatment options have been exhausted. This bias was especially evident in women of childbearing age ($\chi^2 = 4.86$, $p = 0.053$); fortunately, we were able to recruit a similar number of men ($n = 21$) and women ($n = 20$) who had tried VPA. Response to VPA among those who had tried it was also similar between men (14/21) and women (14/20; Table 3.1).

Drugs represent another possible confound in our study, as these neuromodulatory agents affect cognition [Szaflarski & Allendorfer, 2012; Yasuda et al., 2013] and could plausibly modulate the
DMN independently from their effects on epilepsy (Section 1.3.1). Unfortunately, the large number of different AEDs combined with the high proportion of patients on AED polytherapy preclude modeling every AED’s effect separately. We instead considered the number of current AEDs (taken at the time of scanning) and the number of previously failed drugs (Table 3.1). As expected, patients who were taking more \( p = 0.023 \) or had failed more \( p < 0.001 \) medications were more likely to have uncontrolled seizures. On the other hand, number of current drugs was a poor predictor in the model of connectivity vs. uncontrolled seizures and duration \( (F = 1.51, \ p = 0.224) \). This lack of correlation is unsurprising because there is no reason to expect that all drugs would have the same effect (i.e. increasing or decreasing) on connectivity. We estimate that an additional 54 epilepsy patients would be needed to retain the statistical power of the original model and conclusively refute the possibility that observed differences in DMN connectivity were due to drug effects rather than treatment-resistance. Again, a prospective study would help to resolve these confounds.

3.4.4 Agreement Between ICA and Seed-Based Voxel Correlation

To our knowledge, this is the first study of DMN connectivity in IGE to use ICA and dual regression. We performed confirmatory analysis using the more common technique of seed-based voxel correlation. These two techniques yielded similar findings. Both showed greater changes in posterior than anterior regions. The posterior cingulate cortex (PCC) is considered the “hub” of the DMN [Greicius et al., 2003], and so may have been more severely affected by IGE. Also, posterior DMN regions are larger than anterior ones and thus may have been favored by cluster-based thresholding; ancillary ROI analysis was not affected by this factor. Seed-based voxel correlation showed decreased connectivity in the thalamus associated with uncontrolled IGE whereas ICA did not show thalamus to be part of the DMN. The interpretation of this discrepancy is unclear because thalamus is not classically considered part of the DMN [Raichle et al., 2001]. Nevertheless, thalamus is functionally and structurally connected to DMN regions in cortex [Greicius et al., 2003, 2009; Zhang et al., 2008] and has been observed to modulate DMN connectivity [Jones et al., 2011]. Reduced DMN connectivity in thalamus could arise due to GSWD produced by thalamocortical circuits [Blumenfeld, 2002; Contreras et al., 1996; Destexhe et al., 1999; Moeller et al., 2008a].
Chapter 4

Paracingulate Cortex Bridges Default Mode, Frontal, and Motor Networks

This chapter and Chapter 2 contain portions of a manuscript under review [Kay et al., 2013b].

4.1 Introduction

Seizures in IGE are characterized electrographically by generalized spike and wave discharges (GSWDs) (Section 1.2). In addition to being observed during seizures, GSWDs may also be observed interictally, i.e. between seizures [Lüders et al., 1998; Pedersen & Petersen, 1998; Smith, 2005; Yenjun et al., 2003]. However, interictal GSWD frequency is not a good predictor of seizure frequency or treatment-resistance [Staley et al., 2005]. As discussed in Section 1.1.3, approximately 20% of IGE patients are treatment-resistant and experience uncontrolled seizures despite appropriate treatment with AEDs [Holland et al., 2010; Kwan & Brodie, 2000], yet the causes of treatment-resistance are not well understood [Berg, 2009]. In this EEG/fMRI study we investigate the effects of interictal GSWD frequency on resting-state networks associated with treatment-resistance.
4.1.1 GSWDs Activate Paracingulate Cortex, Deactivate DMN

Previous EEG/fMRI studies of IGE have identified brain regions that activate or deactivate in association with interictal GSWDs (Section 1.2.2). GSWD-related activation is widespread, but it is especially pronounced in thalamus and in frontal regions [Aghakhani et al., 2004; Gotman et al., 2005; Hamandi et al., 2006; Laufs et al., 2006; Szaflarski et al., 2010, 2013]. Among frontal regions, the paracingulate cortex in particular activates more strongly with GSWD in IGE patients who are treatment-resistant [Szaflarski et al., 2013] (Figure 1.2). GSWD-related deactivation occurs specifically in regions that belong to the default mode network (DMN) [Aghakhani et al., 2004; Gotman et al., 2005; Hamandi et al., 2006; Laufs et al., 2006; Moeller et al., 2008b; Salek-Haddadi et al., 2003; Szaflarski et al., 2010, 2013]. As discussed in Section 1.4.3, the DMN is thought to be involved in consciousness, and its deactivation is thought to contribute to the semiology (Section 1.1.2) of absence seizures [Danielson et al., 2011; Vaudano et al., 2009]. As shown in Chapter 3, resting-state default mode network functional connectivity is significantly lower in IGE patients who are treatment-resistant [Kay et al., 2013a]. Thus, both paracingulate activation and DMN inhibition are associated with GSWDs and treatment-resistance.

4.1.2 Hypotheses

In this resting-state EEG/fMRI study, we investigate the relationship between the paracingulate cortex, DMN, and GSWD frequency using seed-based voxel correlation after exclusion of time points, but not subjects, associated with GSWDs. We previously (Chapters 2 and 3) identified four seed regions: two a priori seed regions in paracingulate cortex [Szaflarski et al., 2013] and PCC [McGill et al., 2012], the latter seed being situated in a DMN hub region [Greicius et al., 2003], and two a posteriori seed regions in thalamus and cerebellum. We test the hypothesis that RSFC between the paracingulate seed and DMN is correlated with interictal GSWD frequency.
4.2 Subjects & Methods

This study examined a previously described cohort of 100 IGE patients and 40 healthy control subjects [Szaflarski et al., 2013], refer to Sections 2.2 and 3.2. Subjects underwent 1-3 consecutive 20-minute EEG/fMRI scans. Some control subjects listened to self-selected music during scanning; the effect of music listening is discussed in Appendix A and was included as a covariate in analyses [Kay et al., 2012]. Eleven IGE patients were unable to complete at least one scan. Of the 89 IGE patients and 40 healthy controls who completed scanning, 15 IGE patients (23 scans) and 2 healthy controls (2 scans) were excluded due to poor quality data.

For analysis with seed-based voxel correlation, an additional 2 IGE patients (5 scans) were excluded because a high number of GSWD led to an ill-conditioned design matrix. Resting-state analysis was carried out on 72 IGE patients (152 scans) and 38 healthy controls (67 scans), see Table 2.1.

For analysis with independent component analysis (ICA), all 37 scans containing GSWD were excluded, resulting in the complete exclusion of an additional 12 IGE patients. Resting-state analysis was carried out on 189 scans from 60 IGE patients (122 scans) and 38 healthy controls (67 scans), see Table 3.1.

4.2.1 EEG/fMRI Acquisition & Processing

Methods for acquisition and processing of EEG data simultaneous with fMRI are given in Sections 2.2.1 and 2.2.2, respectively, and have been described previously [DiFrancesco et al., 2008; Espay et al., 2008; Szaflarski et al., 2013] (Section 3.2.1). Subjects were fitted with an EEG cap with electrodes arranged according to the international 10/20 system, and 64-channels of data including an ECG channel were recorded at 10 kHz concurrent with fMRI using an MRI compatible system (Compumedica U.S.A., Ltd., El Paso, TX, U.S.A.). Time marks generated by the scanner (4 T Varian Unity INOVA, Varian, Inc., Palo Alto, CA) were automatically inserted into the datastream at the onset of each volume acquisition. 400 T2*-weighted echo-planar functional volumes consisting of 30 slices each were collected during each scan with a 256 × 256 mm field of view, 64 × 64 voxel matrix, 90° flip angle, 5 mm slice thickness, and TR/TA = 3000/2000 ms.
T1-weighted MDEFT [Duewell et al., 1996; Uğurbil et al., 1993] structural images were acquired for use as an anatomical reference with an 1100 millisecond inversion delay, $256 \times 196 \times 196$ mm field of view, $256 \times 196 \times 196$ voxel matrix, 22° flip angle, and TR/TE = 13.1/6.0 ms.

EEG data were low-pass filtered at 30 Hz, gradient artifacts were reduced with an average artifact subtraction technique using the time marks generated by the scanner [Allen et al., 2000], and BCG artifacts were reduced with a linear spatial filter using pulse events identified in the ECG channel [Lagerlund et al., 1997]. All EEG data were reviewed by a board certified epilepsy specialist (JPS), and GSWD timings were marked to within 10 ms precision. To avoid possible contamination of the resting-state, 37 scans containing GSWD were excluded from independent component analysis (ICA, Section 4.2.3). These scans were not excluded from seed-based voxel correlation, but GSWD-related timepoints within scans were individually excluded from analysis (Section 4.2.2).

Functional MRI data were reconstructed and corrected for geometric distortion and Nyquist ghosting with the aid of MERS [Schmithorst et al., 2001]. Functional scans underwent slice timing correction, motion correction [Jenkinson et al., 2002], rigid-body registration to a high-resolution anatomical scan [Jenkinson & Smith, 2001], and non-linear registration [Andersson et al., 2007a,b] to an MNI152 standard using FSL [Smith et al., 2004]. Data were spatially blurred in-mask with a gaussian kernel of FWHM = 6 mm using AFNI [Cox, 1996].

The quality of functional to anatomical registration was measured using the mutual information cost function [Jenkinson & Smith, 2001]. 12 functional scans with an outlying cost indicating unsatisfactory registration were excluded from the study. The quality of motion correction was measured using the normalized correlation ratio cost function [Jenkinson & Smith, 2001] of each timepoint to the reference volume. 13 additional scans with outlying costs indicating excessive motion were excluded from the study.

### 4.2.2 Seed-Based Voxel Correlation

We investigated the relationship between RSFC and GSWD frequency using four previously identified seed regions. We considered a paracingulate seed region previously associated with treatment-
resistance in the same cohort of patients [Szalffarski et al., 2013] (Figure 1.2, Section 4.2.2) with its
centroid at MNI coordinates $X = 2.0, Y = 13.6, Z = 45.9$. Study of this paracingulate seed [Kay
et al., 2013b] (Chapter 2) yielded two $a\ posteriori$ seeds in cerebellum (MNI $X = \pm 31.6, Y = -56.4,
Z = -28.8$) and thalamus (MNI $X = \pm 9.2, Y = -15.6, Z = 13.6$), which we also consider here.
Our fourth seed region comes from a previous study [Kay et al., 2013a; McGill et al., 2012] of the
DMN and is located in PCC (MNI $X = 2, Y = -58, Z = 24$), a DMN hub region [Greicius et al.,
2003].

The method of seed-based voxel correlation is further discussed in Appendix C.2. As in Sec-
tion 2.2.3, the mean timecourse of voxels within each seed region was extracted from each subject’s
fMRI data prior to spatial blurring. This timecourse was used as the regressor of interest in a
voxelwise general linear model for each subject (3dDeconvolve tool in AFNI). The 3dREMLfit tool
in AFNI was used for temporal prewhitening. Baseline drift was modeled using a first-order poly-
nomial; no global or tissue regressors were used [Fox et al., 2009; Weissenbacher et al., 2009]. We
included the six-rigid body motion [Power et al., 2012; Van Dijk et al., 2012] parameters generated
by FSL as nuisance regressors in the model. In addition, timepoints associated with high motion
measured as the normalized correlation ratio cost function [Jenkinson & Smith, 2001] to the re-
ference volume were excluded from analysis. Three timepoints were excluded: those preceeding,
including, and following each high-motion volume.

We included subjects and scans containing interictal GSWD in our analysis in order to examine
the relationship between RSFC and interictal GSWD frequency. To avoid possible contamination
of the resting-state by GSWD, timepoints associated with GSWD were excluded from analysis in a
manner analagous to the exclusion of high-motion timepoints. A total of 19 timepoints comprising
57 seconds were excluded for each GSWD: the 9 preceeding, 9 following, and 1 including the GSWD.
The exclusion of timepoints from the general linear model due to GSWD and motion resulted in
ill-conditioned design matrices for 2 IGE patients (5 scans) with very frequent GSWD who were
therefore excluded from study with seed-based voxel correlation.
4.2.3 Independent Component Analysis

We explored the spatial properties of resting-state networks associated with the paracingulate seed (Section 4.2.2) using independent component analysis (ICA), a technique discussed in Appendix C.3. As in Section 3.2.2, group ICA [Calhoun et al., 2001; Schmithorst & Holland, 2004] of scans without GSWD was carried out using GIFT [Calhoun et al., 2009]. PCA reduction was applied in two steps. Each scan was reduced to 38 temporal principal components (PCs), the number suggested by the GIFT software, prior to temporal concatenation, and the concatenated result was reduced to 25 temporal PCs prior to ICA. This process yielded 25 independent components (ICs). An IC that included motor and DMN regions was selected visually (Figure 4.2A). ICA was subsequently repeated with reduction to 45 and then 30 PCs, yielding 30 ICs, to check the robustness of the motor/DMN IC (Figure 4.2B).

4.3 Results

GSWD frequency (\# GSWD/\# scans) was significantly correlated with RSFC in IGE patients for each seed. The most widespread increases in RSFC with GSWD frequency were observed for the paracingulate seed (Figure 4.1A) and included the motor areas of precentral gyrus, insula, thalamus, and basal ganglia; the frontal regions of superior frontal, medial frontal, inferior frontal, orbitofrontal, and anterior cingulate cortex (ACC); and the posterior DMN-associated regions of posterior cingulate cortex (PCC), precuneous, lingual gyrus, and lateral occipital cortex. Increased RSFC was also observed in posterior cerebellum. RSFC with the cerebellar seed increased significantly with spike frequency for ACC, left insula, right thalamus, and posterior cerebellum (Figure 4.1B). RSFC with the thalamic seed increased significantly with spike frequency for right insula, right thalamus, and left basal ganglia (Figure 4.1C). The PCC seed designed to study the DMN exhibited significantly increased RSFC in most of the same areas as the paracingulate seed excluding orbitofrontal cortex (Figure 4.1D). The PCC seed exhibited decreased, rather than increased (as in the paracingulate seed), connectivity with the cerebellum.

ICA yielded an IC involving the paracingulate seed region with correlated and anticorrelated
Figure 4.1: Significantly ($\alpha = 0.05$, $|t| > 2.24$, $p < 0.043$, # voxels $\geq 40$) increased (red) and decreased (blue) correlations of connectivity with spike frequency (# GSWD/# scans) in epilepsy patients are overlayed on the MNI152 standard brain in radiological orientation. Seed regions are shown in green for paracingulate cortex (A), cerebellum (B), thalamus (C), and the default mode network seed in posterior cingulate cortex (D).
Anticorrelated Motor and Default Mode Resting-State Networks

Figure 4.2: Correlation (red) and anticorrelation (blue) in an independent component (IC) are overlayed on the MNI152 standard brain in radiological orientation. The same component was reproducibly observed after reduction to 25 (A) or 30 (B) principal components. The correlated (red) regions approximate the default mode network (DMN). The anticorrelated (blue) regions approximate a motor network. The IC maps are thresholded at 99% of the robust range (defined as the 2nd to 98th percentiles of voxel intensities) but are not masked or thresholded by statistical significance. A: $|t| > 9.600$. B: $|t| > 9.065$.

voxels. This IC was robust and could be observed at reductions to 25 (Figure 4.2A) and 30 (Figure 4.2B) components. The correlated (red) regions approximate the default mode network (DMN) including the PCC, cuneus, bilateral occipital/parietal cortex, ACC, medial frontal cortex, and additionally, posterior thalamus. The anticorrelated (blue) regions approximate a motor network including the juxta positional globule (which intersects paracingulate cortex), precentral gyrus, bilateral anterior insulae, and anterior thalamus. Dorsolateral prefrontal cortex (DLPFC) also appeared as an anticorrelated region bilaterally.

4.4 Conclusions

In this study we investigated the effects of GSWD on cortical-subcortical circuits and the DMN using a paracingulate seed previously shown to be associated with treatment-resistant IGE [Szaflarski
et al., 2013] and a posterior cingulate seed previously shown to be associated with the DMN [McGill et al., 2012]. We found a positive correlation in RSFC between the paracingulate seed and frontal, motor, and DMN regions with increasing GSWD frequency. Using ICA, we showed that these regions constitute anticorrelated resting-state networks. These results suggest a mechanism by which changes in cortical-subcortical connectivity facilitate inhibition of the DMN and predispose patients to GSWDs and seizures.

4.4.1 GSWD Frequency

We observed significant functional connectivity changes in IGE patients correlated with GSWD frequency despite excluding a generous amount of fMRI data (19 TRs = 57 seconds) around each GSWD detected using simultaneous EEG. We believe these results represent increases in resting-state connectivity associated with GSWD frequency. The data are shown in Figure 4.1 where paracingulate RSFC is increased in several brain regions while cerebellar and thalamic RSFC are each increased in a subset of those brain regions. Increased correlations of paracingulate RSFC with GSWD frequency (Figure 4.1A) were observed in three “networks”: a GSWD/motor network comprised of insula [Gotman et al., 2005], precentral gyrus, thalamus, and basal ganglia; a frontal network comprised of superior frontal, medial frontal, inferior frontal, orbitofrontal, and ACC; and the posterior DMN comprised of PCC, precuneous, lingual gyrus, and lateral occipital cortex. Posterior cerebellum was also involved and may be part of the GSWD/motor network.

The appearance of regions associated with the DMN in Figure 4.1A was conspicuous in that both activation in the paracingulate seed and RSFC in the DMN are associated with treatment-resistance. To test the hypothesis that DMN RSFC is correlated with GSWD frequency, we considered a seed in PCC that we had previously used to quantify DMN RSFC [Kay et al., 2013a; McGill et al., 2012; Greicius et al., 2003]. RSFC with the PCC and paracingulate seed exhibited similar patterns of correlation (Figure 4.1D). However, the involvement of frontal regions (especially orbitofrontal cortex) with the PCC seed were not as robust, suggesting that it may be more appropriate to think of the frontal and posterior DMN regions as separate network entities in this case.
4.4.2 Disruption of Anticorrelated Networks

The brain regions in Figure 4.1A are not typically active at the same time, e.g. PCC and precentral gyrus activate independently in the normal brain [Uddin et al., 2009]. In fact, the IC in Figure 4.2 shows that these motor and DMN networks are anticorrelated at rest. The absence of robust DMN or frontal involvement using alternative GSWD/motor seeds (i.e. cerebellum in Figure 4.1B and thalamus in Figure 4.1C) suggests that paracingulate cortex is a bridge, or hub region that allows the GSWD/motor network to drive resting-state activation in DMN and frontal regions. This abnormal correlation between typically anticorrelated networks in IGE patients with high GSWD frequency is a plausible mechanism by which DMN RSFC may be cumulatively disrupted [Kay et al., 2013a] and by which cognitive [Devinsky et al., 1997; Killory et al., 2011; Kim et al., 2007; Maneshi et al., 2012; Swartz et al., 1996] and psychiatric [Akanuma et al., 2008; Koepp, 2005; Trinka et al., 2006; Wandschneider et al., 2012] effects may arise.
Chapter 5

General Discussion

Epilepsy is a complex phenomenon that arises from abnormal neuronal circuits [Garcia Dominguez et al., 2005; Engel et al., 2013; Zhang et al., 2011]. Idiopathic generalized epilepsy (IGE) lacks a clearly-defined seizure onset zone and manifests several seizure types with markedly different semiologies: loss of consciousness without convulsions (absence), convulsions without loss of consciousness (myoclonic), and convulsions with loss of consciousness (generalized tonic-clonic) [Commission on Classification and Terminology of the International League Against Epilepsy, 1989; Janz, 1997; Lüders et al., 1998; Noachtar & Peters, 2009; Rudzinski & Shih, 2010]. Present on the electroencephalogram (EEG) in all three seizure types, as well as during the interictal state, the generalized spike and wave discharge (GSWD) is pathognomonic for IGE [Lüders et al., 1998; Pedersen & Petersen, 1998; Smith, 2005; Yenjun et al., 2003]. Decades of work on electrical recordings from humans [Hayne et al., 1949; Niedermeyer et al., 1969; Williams, 1953] and animal models [Avoli, 1995; Coenen & Van Luijtelaar, 2003] of IGE, and more recently, non-invasive imaging techniques [Bernasconi et al., 2003; Betting et al., 2006; Deppe et al., 2008; Pulsipher et al., 2011; Sakurai et al., 2010; Stefan et al., 2009] such as EEG/fMRI [Gotman et al., 2006], have elucidated the thalamocortical mechanism of GSWD generation [Blumenfeld, 2002; Contreras et al., 1996; Destexhe et al., 1999; Gloor, 1968; Moeller et al., 2008a] (Section 1.2). Nevertheless, there is still much to learn about the resting-state changes in the IGE brain that predispose it to GSWDs and seizures.
The topic of treatment-resistance is of particular interest to clinicians. Anti-epileptic drugs (AEDs) are the first line of therapy for IGE [Benbadis, 2005; Mantoan & Walker, 2011; Sullivan & Dlugos, 2004; Wolf & Inoue, 1984] and, unlike some focal epilepsies, surgery is contraindicated [Engel, 1993]. Some 20% of IGE patients do not achieve seizure remission despite appropriate therapy with a combination of AEDs [Faught, 2004; French, 2007; Holland et al., 2010]. Monotherapy with the archetypal AED valproate (VPA) is highly predictive of overall treatment response [Holland et al., 2010] (Section 1.1.3). However, there are no other reliable prognostic indicators for progression to treatment-resistance; neither GSWD frequency, morphology, nor presence during the interictal state predicts ultimate clinical outcome [Berg, 2009]. Therefore, the aims of this study were to characterize some of the resting-state properties of the IGE brain and investigate possible biomarkers for treatment-resistance.

5.1 Cortical-Subcortical Connectivity

A recent event-related EEG/fMRI study by us of this patient cohort found a possible GSWD-related biomarker for treatment-resistance [Szafarski et al., 2013]. In this prior study, we showed that paracingulate (i.e. medial frontal) cortex and the anterior insulae bilaterally exhibit greater GSWD-related activation in IGE patients who are resistant to VPA (i.e. treatment-resistant patients) compared to VPA-responders (Figure 1.1). This finding is consistent with the numerous frontal-lobe deficits observed in IGE patients [Devinsky et al., 1997; Killory et al., 2011; Kim et al., 2007; Koepp, 2005; Maneshi et al., 2012; Swartz et al., 1996; Wandschneider et al., 2012] (Section 1.1.5) and supports the hypothesis that certain extratralamic, cortical regions are critical to generalized seizure onset [Meeren et al., 2005; Niedermeyer, 1996; Sakurai et al., 2010]. In the present study, we used seed-based voxel correlation to investigate the resting-state functional connectivity (RSFC) of this paracingulate region with the rest of the brain. We found that RSFC between the paracingulate seed and other frontal regions, in addition to the thalamus and basal ganglia (and the default mode network, see Section 5.2), was increased in IGE patients with a greater frequency of interictal GSWDs (Section 4.3, Figure 4.1). RSFC between anterior thalamus and the paracingulate seed was increased in IGE patients vs. healthy controls (Section 2.3.2, Figure 2.2). These results are
consistent with the notion that a specific cortical region (i.e. paracingulate cortex) drives ictogenesis and cognitive deficits.

RSFC was significantly increased between paracingulate cortex and anterior, but not posterior, thalamus. In fact, it would appear that these thalamic regions exhibit opposite connectivity with the paracingulate seed (Figure 2.3). Although the thalamus has typically been thought of as a monolithic entity with respect to its role in IGE, this and other recent studies [Betting et al., 2006; O’Muircheartaigh et al., 2012; Tyvaert et al., 2009] provide evidence that the thalamus may have functional subdivisions important to its role in ictogenesis. This may explain why some similar studies were unable to show conclusively an IGE-related difference in “thalamic” connectivity [Moeller et al., 2011]. These findings recommend the use of techniques capable of resolving adjacent thalamic nuclei, such as 7T fMRI, when designing future studies.

With respect to treatment-resistance, we had perhaps expected to see a difference in thalamocortical RSFC between patients with controlled and uncontrolled seizures, consistent with the thalamocortical hypothesis of GSWDs. Instead, we observed reduced RSFC between the paracingulate seed and cerebellum, a subcortical structure that has not typically been investigated in the context of IGE. We also observed a widespread reduction in cerebellar RSFC with thalamus, basal ganglia, and cortex (Section 2.3.1, Figure 2.1). Whereas our other results clearly support the hypothesis of thalamocortical circuits giving rise to epilepsy, these results suggest that cerebellar influence may be particularly crucial within epilepsy patients when it comes to treatment-resistance. Since our fMRI techniques were not well-tuned to detection of cerebellar signal, we believe additional investigation is required to establish reduced cerebellar RSFC as a biomarker for treatment-resistance. It is notable that the present armamentarium of AEDs does not include drugs that specifically target cerebellar circuits with basal ganglia or cortex, save for experimental use of amantadine [Shahar & Brand, 1992].

5.2 Default Mode Network Connectivity

The default mode network (DMN) is a resting-state network thought to support consciousness [Fox et al., 2005; Morgan et al., 2008; Raichle et al., 2001; Vanhaudenhuyse et al., 2010]. GSWDs produce
specific deactivations in DMN regions [Aghakhani et al., 2004; Gotman et al., 2005; Hamandi et al., 2006; Laufs et al., 2006; Moeller et al., 2008b; Salek-Haddadi et al., 2003; Szaflarski et al., 2010, 2013], and inhibition of the DMN during absence seizures is thought to contribute to their loss-of-consciousness semiology [Danielson et al., 2011; Vaudano et al., 2009] (Section 1.4.3). Recent studies have observed reduced DMN connectivity in IGE patients compared to healthy controls [McGill et al., 2012; Luo et al., 2011; Wang et al., 2011]. Our results confirm this relationship and show that DMN RSFC is even further reduced in treatment-resistant IGE patients compared to healthy responders (Section 3.3.2, Figure 3.3). These findings establish DMN RSFC as a novel biomarker for treatment-resistant IGE, albeit not a very useful one in isolation given the considerable overlap in RSFC between treatment-resistant and responsive patients. Nevertheless, the observation that DMN RSFC is associated with uncontrolled seizures may lead to mechanistic insights into this relatively unknown condition.

Although GSWDs are strongly associated with seizures, they may also occur interictally without causing any clinical signs of a seizure [Lüders et al., 1998; Pedersen & Petersen, 1998; Smith, 2005; Yenjun et al., 2003], and interictal GSWD frequency does not reliably predict treatment-response [Berg, 2009]. Insofar as seizures do occur, they are most common in a patient who is drowsy or sleeping [Janz, 1997; Noachtar & Peters, 2009; Rudzinski & Shih, 2010]. We speculate that DMN connectivity may partly explain these observations. Since DMN regions are deactivated by GSWDs, and since inhibition of the DMN is thought to contribute to seizure semiology, we speculate that high DMN connectivity may be protective against progression of interictal discharges to absence seizures. Supposing that the preceding hypothesis is true, we would then expect patients with lower DMN RSFC to be more likely to experience seizures instead of interictal GSWDs. DMN connectivity is lowest in patients who are drowsy or sleeping [De Havas et al., 2012; Gujar et al., 2010; Horovitz et al., 2009; Sâmann et al., 2011], and this is precisely when seizures are most common.

DMN RSFC is decreased in epilepsy, and duration of epilepsy is correlated with loss of DMN RSFC in treatment-resistant patients (Section 3.3.2, Figure 3.4). One possible mechanism for loss of DMN connectivity is dynamic synaptic remodelling, perhaps as a result of DMN deactivation.
during GSWDs. We found that RSFC between the DMN and GSWD/motor networks is increased in patients with high GSWD-frequency and speculate that GSWD/motor networks drive DMN regions to activate excessively during the resting-state (Section 4.3, Figure 4.1). This may represent another source of dynamic remodelling leading to reduced DMN connectivity, but it may also reduce DMN connectivity via excitotoxic cell death as a result of excessive activation in DMN regions. The correlation between reduced DMN RSFC and disease duration may reflect the cumulative effects of neuronal impairment in patients susceptible to excitotoxic injury. It is important to distinguish which of these potential mechanisms is at work, because excitotoxic injury to the DMN is preventable with existing NMDA antagonists such as memantine and adamantine [Lorenzi et al., 2011]. Future investigations using voxel based morphometry (VBM) to quantify cortical atrophy, magnetic resonance spectroscopy (MRS) to quantify NAA/Cr ratios (a biomarker for metabolic injury), or positron emission tomography (PET) to quantify impaired glucose metabolism would be helpful in answering this clinically important question.

5.3 Limitations

This resting-state EEG/fMRI study investigated a large cohort of 60 IGE patients (72 including runs with GSWD) and 38 healthy controls, after excluding subjects with poor-quality or unusable data. In this section we acknowledge limitations in our study design so as to aid in interpretation of our results and the design of future studies.

5.3.1 AED Effects

Epilepsy likely arises from complex interactions between neuronal circuits [Garcia Dominguez et al., 2005; Zhang et al., 2011]. Treating patients with multiple neurologically active pharmaceutical agents does not simplify interpretation of experimental results. In addition to the many studies investigating IGE-related changes in the brain, some studies are now beginning to investigate AED-related changes in the brain [Szaflarski & Allendorfer, 2012; Yasuda et al., 2013]. We discuss the statistical correlates of treatment with multiple AEDs in Section 3.4.3, but our study size does not permit modelling the effect of every AED and combination of AEDs on RSFC. We may argue ad
*ignorantium* that, since the effects of every AED are not known, that they cancel each other out in the average and do not influence our results. However, many AEDs have similar mechanisms of action, and it is possible that even in a study with physician-directed treatment such as ours, a consistent and significant effect of AEDs may be missed.

The most ideal study design would include a very large number of patients such that the effects of each AED could be modelled and controlled for. Such a study design would have the added benefit of elucidating the statistical effects of each AED. Unfortunately, given the cost of scanning and the availability of suitable subjects, such a study would be prohibitively expensive and require coordination across multiple sites. A more reasonable approach might seek to control experimentally which AEDs are administered to study participants. This could be done prospectively to investigate an AED of interest or retrospectively to control for the effects of frequently-used AEDs.

### 5.3.2 Prospective Study Design

It is unclear from the present study whether altered RSFC is an antecedent cause of IGE or a subsequent effect of it. For example, we speculate that reduced DMN RSFC predisposes to (i.e. causes) treatment-resistance, but we cannot say with certainty that it is not an incidental effect (that may nevertheless be useful as a biomarker). In order to answer this question definitively, we would need to scan patients at multiple timepoints including time of first seizure onset. Such a prospective study design would also be helpful in obtaining data from drug-naïve patients without limiting their treatment options [Moeller et al., 2008b]. A well-funded study might even seek to obtain data from patients at high-risk of developing IGE (e.g. siblings of IGE patients) to investigate whether changes in RSFC precede disease onset and if disease may be prevented in patients who are genetically at-risk.

### 5.3.3 Temporal Resolution

The chief purpose of simultaneous EEG/fMRI is to circumvent the hemodynamic response and temporal constraints of fMRI alone. Although the EEG data is very useful in supplementing the fMRI data, i.e. with GSWD timing, it does not account for aliasing of physiological signal into the
fMRI data. Physiological regressors such as heartbeat, breathing, and movement have been shown to affect the quality of resting-state data [Fox et al., 2009; Starck et al., 2010]. Unfortunately, these regressors were not recorded in our study.

Although the temporal resolution of EEG is superior to fMRI, it is ultimately limited by the electrical properties of the skull and is not ideal for detection of high frequency oscillations (HFOs) [Arroyo & Uematsu, 1992]. Furthermore, removal of fMRI-related noise from the EEG recording necessitates aggressive low-pass filtering, which further attenuates high-frequency information (Appendix C.1). There does not seem to be any way around these limitations, at least so far as an EEG/fMRI study is concerned. Supplemental MEG recordings might be useful to a study in which HFOs are important.

5.3.4 Anatomical Segmentation

In this study we used functional data to identify regions of interest and used high-resolution anatomical data in a supporting role to identify the locations of functional findings. It may also be useful to define a priori anatomical regions in which to investigate functional (e.g. RSFC) or anatomical (e.g. cortical thickness) changes. We found that susceptibility artifacts related to the presence of the EEG cap and cabling made it very difficult for automated algorithms to effectively segment the brain into anatomical regions. Future studies might wish to obtain a high-resolution anatomical scan without the EEG cap or else employ rigorous shimming techniques to minimize the influence of susceptibility artifacts on the recorded data.

5.4 Future Directions

5.4.1 Whole-Brain Connectivity

This study used seed-based voxel correlation and independent component analysis (ICA) to investigate resting-state networks in IGE patients. Graph theory is another mathematical technique that can be used to investigate network organization in the whole brain using functional (i.e. fMRI) or structural (i.e. DTI) data [Achard et al., 2006; Guye et al., 2010]. The brain is divided into nodes,
typically predefined anatomical regions, and connectivity between nodes is used to construct an adjacency matrix describing the edges in the network [Bullmore & Sporns, 2009; Salvador et al., 2005]. Graph theory provides a mathematical framework for summarizing the adjacency matrix into a scalar statistic, such as small-worldness, which may then be compared between experimental groups [Achard et al., 2006]. This technique is sensitive to pathological changes in brain state in general [Guye et al., 2010] and also in IGE specifically, where small-worldness is reduced in IGE patients compared to healthy controls [Zhang et al., 2011].

We attempted to apply graph theoretical analysis to our data, but we encountered difficulties with automated segmentation due to data-quality issues discussed in Section 5.3.4. Our preliminary results, shown in Appendix B, were not statistically significant, nor did they produce reasonable values for small-worldness. A future study using labor-intensive manual segmentation of these data or different, higher quality data could yield interesting results with respect to whole-brain connectivity in treatment-resistance IGE.

5.4.2 Relationship with Cognitive and Psychiatric Symptoms

IGE, and JME in particular, is associated with cognitive [Killory et al., 2011; Kim et al., 2007; Koepp, 2005; Devinsky et al., 1997; Maneshi et al., 2012; Swartz et al., 1996; Wandschneider et al., 2012] and psychiatric [Akanuma et al., 2008; Trinka et al., 2006] symptoms in addition to seizures (Section 1.1.5). These may remain a significant source of morbidity even for patients in whom seizures are well-controlled [Akanuma et al., 2008]. It is therefore clinically important to understand the association between IGE and psychiatric disease so that both conditions may be better managed. Our findings demonstrated significant changes in cognitive and emotive networks. We speculated that increased RSFC between GSWD/motor networks and the DMN correlated with GSWD frequency may result in disruption of the DMN (Section 4.4.2). We also observed such a correlation with frontal regions and speculate that disruption of cognitive networks in the frontal lobes may contribute to the supratentorial symptoms observed in IGE.

Our study design did not record measures of cognitive performance or psychiatric state. IGE is, by definition [Commission on Classification and Terminology of the International League Against
Epilepsy, 1989], associated with normal overall intelligence, and the effect of specific cognitive impairments associated with IGE had not been reported until recently [Killory et al., 2011; Swartz et al., 1996; Vulliemoz et al., 2011]. Future studies of IGE should make an effort to obtain data on cognitive and psychiatric variables so that the relationship between RSFC and these clinically important symptoms may be investigated. A new study protocol might retrospectively obtain psychometric data on the existing cohort of IGE patients or prospectively recruit a new patient cohort with specific cognitive or psychiatric conditions.
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Appendix A

Effect of Music Listening

This appendix contains portions of a published paper [Kay et al., 2012].

A.1 Rationale

Resting-state EEG/fMRI presents a challenge in terms of subject compliance. Participants are required to wear an uncomfortable EEG cap and lie absolutely still in a scanner with eyes closed for, in our study, up to 90 minutes. We allowed subjects to listen to self-selected music (i.e. by bringing in a music player or selecting from a library of compact discs) in an effort to facilitate relaxation and thereby increase subject compliance. However, music-listening is not a typical resting-state condition, and few studies have systematically investigated its effects on the resting-state. In this study we asked whether subjects who listen to music are truly “resting” and whether music-listening is a valid condition under which to obtain resting-state data for the purpose of our investigations of epilepsy.

We were particularly interested in the effects of music on the default mode network (DMN), a robust resting-state network that is sensitive to variation between subjects and groups [Buckner et al., 2008; Calhoun et al., 2008; Damoiseaux et al., 2006; Morgan et al., 2008; van de Ven et al., 2004] (Section 1.4.3). Although robust, the DMN is modulated by task performance [Calhoun et al., 2008; Fair et al., 2007; Gordon et al., 2012; Greicius & Menon, 2004]. Even an activity as simple as looking at a fixation cross produces measurable changes in the DMN – although these slight
changes do not appear to represent fundamental alterations in the structure of the DMN [Yan et al., 2009]. Music is a phenomenon universal to human cultures that is recognized even by infants [Andrade & Bhattacharya, 2003]. Music has many parts, some of which, such as speech and rhythm, are left-lateralized, while others, such as timbre and tone, are right lateralized [Andrade & Bhattacharya, 2003]. Music-processing as a whole is thought to have a right-sided bias [Klostermann et al., 2009]. Processing of music is distributed across many brain regions including brainstem, thalamus, hippocampus, insula, auditory cortex, limbic system, and multimodal cortical association areas [Andrade & Bhattacharya, 2003; Bamiou et al., 2003; Klostermann et al., 2009; Watanabe et al., 2008]. Some of these regions are known to participate in RSNs with hippocampus, cingulate cortex, and multimodal cortex – parts of the DMN. It is, therefore, not unreasonable to expect that music has modulating effects on RSNs and that it may affect the DMN.

The hypothesis guiding this work was that while we did not expect to observe fundamental changes in the DMN related to music-listening vs. the no-music condition, we anticipated that music would have moderating effects on RSNs including the thalami and the superior temporal gyri (Heschl’s gyri). We used independent component analysis (ICA), the same method employed in our epilepsy studies (Sections 3.2.2 and 4.2.3), to qualitatively and quantitatively characterize changes in resting-state networks and the DMN associated with music-listening in our cohort of control subjects (see Tables 2.1 and 3.1).

A.2 Findings

A.2.1 Alpha Rhythm

Methods

EEG processing and acquisition were performed as in Sections 2.2.1, 3.2, and 4.2. The processed EEG data were then divided into 400, TR = 3000 ms epochs starting at the onset of each volume acquisition. These epochs were reviewed by the authors for remaining artifact contamination. Contaminated epochs were excluded from the study. Each surviving epoch was detrended, demeaned, and scaled via division by its standard deviation. To obtain an estimate of power spectral density
(PSD), a smoothed periodogram of each epoch was computed for each of four bipolar occipital channels according to previously described methods [Brillinger, 2001; Brockwell & Davis, 2009]. Smoothing was achieved using a gaussian kernel with a bandwidth of two standard deviations resulting in a smoothing window of width equal to that of the 8–13 Hz alpha frequency band. Finally, the average PSD over the alpha band, $y$, was computed from the smoothed periodogram $p$ according to Equation A.1 where $\omega_1$ is the first of $N$ frequency components in the alpha band [DiFrancesco et al., 2008]. This process was repeated for the 13 – 30 Hz beta frequency band.

$$y = \frac{1}{N} \sum_{i=1}^{N} p(\omega_i)$$ \hspace{1cm} (A.1)

Each $l^{th}$ observation on average PSD for the $k^{th}$ channel and the $j^{th}$ subject in the $i^{th}$ group (music vs. control), $y_{ijkl}$, was fit to a linear model with mean $\mu$, group effect $\gamma$, subject effect $\eta$, channel effect $\nu$, and subject-channel interaction $\tau$. The heteroscedastic, fixed-effects model shown in Equation A.2 was chosen to best account for natural inter-subject variability and for the interaction between subject and channel arising from slight, unavoidable variation in electrode placement. This model was used to test the null hypothesis of no difference in group effect, $H_0$: $\gamma_1 = \gamma_2$, against its alternative, $H_0$: $\gamma_1 \neq \gamma_2$, at a significance threshold of $\alpha = 0.05$.

$$y_{ijkl} = \mu + \gamma_i + \eta_j + \nu_k + \tau_{jk} + \varepsilon_{ijkl}, \varepsilon_{ijkl} \sim \mathcal{N}(0, \sigma_{jk}^2)$$ \hspace{1cm} (A.2)

**Results**

Data from one subject in each of the music and control groups was discarded due to difficulties in fMRI artifact removal. EEG data were successfully processed for the other 38 subjects. Four bipolar occipital channels, P3-O1, P4-O2, P7-O1, and P8-O2, were used for estimation of average power spectral density (PSD) in 29 subjects. Due to difficulty recording from the O1 or O2 leads (poor electrode impedance), adjacent channels were substituted in nine subjects. Of the 29,028 TR = 3000 ms epochs recorded, 25,759 (88.7%) survived processing and review for artifact contamination. This yielded 103,036 estimates of average PSD in the alpha band: 50,224 from the music group and 52,812 from the control group (difference $< 2.5\%$ at $\alpha = 0.05$ significance level; this difference
is statistically significant but is unlikely to bear any clinical importance). Average power spectral density in the alpha band was found to be significantly higher in the music group than in the control group with $p < 0.001$. Average power spectral density in the beta band was found to be significantly lower in the music group than in the control group with $p < 0.001$.

**Discussion**

Behaviorally, increased alpha rhythm amplitude is associated with increased relaxation and introspection [Niedermeyer, 1999; Plotkin, 1976]. Thus, the finding of increased alpha rhythm amplitude in the music group suggests that music-listening enhances these features. In contrast to a previous EEG/PET study [Nakamura et al., 1999], we observed reduced beta rhythm amplitude in the music group. Beta rhythm is chiefly associated with activation in sensorimotor cortex [Baker, 2007], so its interpretation in the context of music-listening is unclear. A possible explanation for the discrepancy between our finding and that of the previous study is the use familiar, self-selected music in our study versus traditional Gamelan music, to which a non-audible high-frequency component $> 22$ kHz was added, in the previous study. The loud noises generated by the 4T MRI scanner in our study could also have been a contributing factor.

A.2.2 Independent Component Analysis

**Results**

Imaging data were analyzed using three complementary approaches. For the first approach, ICA was applied separately to the music and control groups using CCHIPS software with methods similar to Sections 3.2.2 and 4.2.3. This yielded 52 independent components (ICs) for the music group and a distinct 55 ICs for the control group. Two symmetric, multi-regional resting-state networks (RSNs) were identified from the 55 control ICs. A visual search of the 52 music ICs identified the two most visually similar RSNs from the music group. Spatial maps of the ICs for these two RSNs are shown in Figure A.1.

On visual inspection, one pair of ICs comprised of Brodmann Areas (BAs) 9, 10, 23, and 31 was highly similar between groups. In this study, we call it IC1. The other pair of ICs, IC2, was also
Figure A.1: Similar spatial maps for resting-state independent components (ICs) in the music (top, \( n = 19 \) subjects) and control (bottom, \( n = 19 \)) groups. Background is the averaged (\( n = 38 \)) anatomical brain. Slices are in radiological orientation (left = right). Left component (IC1): Brodmann areas (BAs) 9, 10, 23, and 31. Right component (IC2): BAs 8, 9, 23, 30, 31, and 32 (both) + 35 and 36 (music) and 10 (control). Clusters of > 50 voxels with z-values > 2.58 are shown. Talairach Z coordinates of first and last slices of each IC are indicated. Slices are 5 mm apart.
similar between groups. IC2 comprised BAs 8, 9, 23, 30, 31, and 32 in both groups but included BA 10, medial frontal gyrus (MFG), only in the control group and BAs 35 and 36, hippocampus, only in the music group. However, the involvement of hippocampus/precuneus was observed in isolation in ICs from both groups comprising BAs 17, 18, 30, and 36 (not shown).

**Discussion**

Differences in IC2 merit further discussion. Although IC2 shows greater hippocampal involvement in the music group, an IC for hippocampus is evident in both groups (not shown). If a difference between groups exists, it is not that hippocampus is absent from the RSN in the control group, but rather that hippocampus is more strongly associated with this RSN in the music group. This interpretation would be consistent with recent findings that hippocampus is associated with the DMN during memory recall but not during memory encoding [Huijbers et al., 2011]. Greater association of hippocampus with regions involved in the DMN in the music group could be an indicator of increased memory recall during music-listening, for example, the recall of song lyrics or musical score.

IC2 also shows greater involvement of medial frontal gyrus (MFG) in the RSN of the control group. The precise role of this region within BA 10 is not well understood, however activation in this region has been associated with attention to environmental (but not internally generated) stimuli [Burgess et al., 2007; Gilbert et al., 2005]. Greater involvement of MFG in the control group could imply greater attentiveness to external stimuli (i.e. scanner noises, physical discomfort, etc.), with lesser involvement of MFG in the music group implying greater introspection, or lack of attention to environmental stimuli, during music-listening. Nevertheless in IC1, where MFG is also involved in an RSN, there is no discernible difference between groups on visual inspection. The finding of different MFG involvement in IC2 is therefore equivocal, with MFG possibly playing different roles in the RSNs described by IC1 and IC2.
A.2.3 Dual Regression

In our second approach, we repeated registration and group ICA as using the MELODIC routine in FSL [Smith et al., 2004], which includes dual regression (spatio-temporal regression, STR) function, with methods similar to Sections 3.2.2 and 4.2.3. We obtained 52 ICs jointly describing the music and control groups. Of these ICs, the one most characteristic of the DMN [Greicius et al., 2004] is shown in Figure A.2A (top). Regions represented in this IC were hippocampus (BAs 27, 34, 36, 28, and 35), inferior frontal lobe (BA 47), lingual gyrus (BAs 18 and 19), PCC (BAs 23, 29, 30, and 31), precuneus (BA 7), DLPFC (BA 8), and biparietal involvement of BAs 19, 22, 29, and 39. Significant ($\alpha = 0.05$) group-level differences in this IC found using dual regression and corrected for multiple comparisons using the Randomise routine in FSL are shown in Figure A.2B (bottom). Overall, there was slightly increased co-activation in right parietal cortex in the music group (BA 19) and left parietal cortex in the control group (BAs 19 and 39). We also observed a right-sided cluster of increased co-activation overlapping retrosplenial regions (BA 29) in the control group, however much of this cluster occupied white matter.
The DMN is observed to be grossly unchanged by music-listening. The laterality observed in posterior parietal cortex may reflect a right-sided preference for music processing [Klostermann et al., 2009].

A.2.4 Graph Connectivity

Methods

In our third approach we compared resting-state connectivity between groups using undirected graphical analysis [Salvador et al., 2005]. This technique represents suprathreshold correlations between regions, or “nodes”, as connections, or “edges” in a graph. In an undirected graph, the direction of the causal relationships (i.e. information flow) between nodes is not established [Brillinger, 1996]. To obtain nodes we again performed group ICA an all subjects using CCHIPS with methods similar to Sections 3.2.2 and 4.2.3. This yielded a single, shared set of ICs common to both music and control groups. We searched for ICs describing either a single cluster of contiguous voxels or else a pair of clusters of contiguous voxels separated by and symmetric about the midline – a single region. Although ICs describing more than one such region are also valid RSNs, graphical analysis is typically limited to nodes comprising single regions [Bullmore & Sporns, 2009] as the interpretation of connectivity between nodes comprised of multiple, possibly overlapping regions is unclear. In cases where two or more ICs described the same region, the IC with the fewest voxels outside (not contiguous with) the region was used. These “extra” overlapping ICs were excluded because they would exhibit high temporal correlation with each other and would therefore introduce problems of collinearity into the graphical analysis [Fiecas et al., 2010]. We identified ICs for 11 regions, which we proceeded to use as nodes (Figure A.3).

We obtained the spectral coherence (SC) and partial spectral coherence (PSC) [Brillinger, 1996] between each of 55 pairings of the 11 nodes over the 0 – 0.1 Hz frequency band [Achard et al., 2006; Biswal et al., 1995; Cordes et al., 2001] using the back-projected IC time courses for these nodes in each subject. SC and PSC are related but distinct measures [Brillinger, 1996]. SC describes the relationship between two nodes independent of other nodes, or total link strength, and is thus suitable for pairwise comparisons between nodes. PSC describes the relationship between two nodes
conditional upon other nodes, or direct link strength, and is thus suitable for determining edges in a network graph. SC and PSC are analogous to the time-domain measures of correlation and partial correlation, respectively.

We computed SC and PSC according to previously described methods [Fiecas et al., 2010; Salvador et al., 2005]. Briefly, the SC between two nodes $i$ and $j$ at frequency $\lambda$ is given by $f_{i,j}(\lambda)$ below where $Y_i(t)$ is the value of node $i$ at time $t$:

$$f_{i,j}(\lambda) = \frac{1}{2\pi} \sum_{u=-\infty}^{\infty} \text{Cov}[Y_i(t+u), Y_j(t)] e^{-i\lambda u}$$ (A.3)

The PSC between two nodes $i$ and $j$, $f_{i,j}^p(\lambda)$, was computed from the matrix of SC values, $[f(\lambda)]$, as follows:

$$f_{i,j}^p(\lambda) = \frac{\{|[f(\lambda)]^{-1}\}_{i,j} - |([f(\lambda)]^{-1})_{i,i} - ([f(\lambda)]^{-1})_{j,j}|^2}{(|[f(\lambda)]^{-1}|)_{i,j}}$$ (A.4)

We averaged the within-subject SC and PSC values across each group to obtain a total of 110 averaged SC values and 110 averaged PSC values. The PSC values were ranked from lowest (weakest link) to highest (strongest link) and plotted versus PSC rank. The inflection point of this plot was identified and used as the PSC cutoff threshold to select the edges in the undirected connectivity graph. We created two undirected network graphs with identical nodes, one for each group, by drawing an edge between pairs of nodes for which the PSC within the given group was greater than or equal to the PSC threshold [Salvador et al., 2005] (Figure A.4).

**Results**

Of 53 ICs, 33 were excluded as noise, 6 were excluded because they described more than one region, and 3 because they described the same region as another IC. This left 11 symmetric, single-region ICs for use as network nodes in graphical analysis. One of these ICs contained three clusters of contiguous voxels, but we included it in our analysis because it was symmetric about the midline and all voxels resided within motor cortex. These 11 ICs constitute the basis for the 11 network nodes shown in Figure A.3. They were periaqueductal grey (PAG), thalamus (TH), basal ganglia (BG), insula as BA 13, cuneus as BAs 17, 18, and 30, auditory cortex as BAs 40, 41, and 42,
Figure A.3: Spatial maps of independent components (ICs) used as nodes and their locations or Brodmann areas (BAs). Background is the averaged \((n = 38)\) anatomical brain. Slices are in radiological orientation (left = right). Left to right, top to bottom, ICs are: periaqueductal grey (PAG), thalamus (TH), basal ganglia (BG), insula, cuneus, auditory, posterior cingulate (PCC), anterior cingulate cortex (ACC), frontopolar, and motor cortices. Clusters of > 50 voxels with \(z\)-values > 2.58 are shown. Talairach coordinates of slices are indicated.

posterior cingulate cortex (PCC) as BAs 23 and 31, anterior cingulate cortex (ACC) as BAs 24, 32, 33, and 46, frontopolar cortex as BAs 9 and 10, dorsolateral prefrontal cortex (DLPFC) as BAs 9 and 46, and motor cortex as BAs 4 and 6.

After identifying these 11 network nodes, we assessed the average spectral coherence (SC) between them. SC is a measure of total link strength and the frequency-domain equivalent of correlation coefficient [Brillinger, 1996]. The differences in total link strength between the music and control groups were minimal. After correcting for multiple comparisons using false discovery rate (FDR, \(n = 55\)), only one SC value was significantly different between groups. This SC value corresponded to the total link strength between PAG and thalamus, which was significantly higher in the music group (0.669) than the control group (0.485) with uncorrected \(p < 0.0001\).
We had expected the total link strength between thalamus and auditory cortex to be higher in the music group than the control group. While this was the case with SC values of 0.693 and 0.597, respectively, the associated uncorrected \( p = 0.006 \) did not survive correction for multiple comparisons. At this \( p \)-value threshold, the total link strength between thalamus and insula would also have been significantly higher in the music group (0.629) than the control group (0.502) with uncorrected \( p = 0.004 \).

This analysis was followed by calculating average partial spectral coherence (PSC), which is a measure of direct link strength and the frequency-domain equivalent of partial correlation coefficient [Brillinger, 1996]. We constructed two undirected graphs with identical nodes, one graph for each group, by drawing an edge between nodes whose PSC met or exceeded a threshold PSC of 0.3261 determined using methods previously described [Salvador et al., 2005]. These graphs are shown in Figure A.4. We considered five nodes, cuneus, PCC, ACC, DLPFC, and frontopolar cortex, to be members of the default mode network (DMN). We examined the DMN by considering edges between nodes that belong to the DMN (within-DMN edges) and between nodes that belong to the DMN and those that do not (without-DMN edges). There was one more within-DMN edge in the music group between DLPFC and frontopolar cortex. In total, there were two more without-DMN edges in the control group involving ACC. The total number of edges in the music group was one higher than the total number of edges in the control group.

**Discussion**

Using PSC, we observed increased connectivity between nodes associated with the DMN (within-DMN connectivity) and decreased connectivity between these DMN nodes and those nodes not associated with the DMN (without-DMN connectivity) in the music group. In other words, DMN activity was more strongly correlated with regions within the DMN and less strongly correlated with regions that represent the external environment. The DMN is thought to serve dual roles of introspection, i.e. monitoring of internal stimuli, and monitoring of the external environment [Buckner et al., 2008]. The observed pattern of connectivity suggests that the role of introspection predominates during music-listening. However, due to the small number of nodes used in our analysis, this
Figure A.4: Undirected graph of partial spectral coherences (PSC) between nodes from Figure A.3. Midline and bilateral nodes are shown over the left hemispheric surface. Edges are drawn between nodes with PSC > 0.3261 inflection point. Locations or Brodmann areas (BA) of nodes are indicated. PAG = periaqueductal grey, TH = thalamus, BG = basal ganglia. Nodes identified as part of the canonical default mode network (DMN) are colored gold. All other nodes are colored green. Edges colored red are present only in the music group, edges colored blue are present only in the control group, and edges colored purple are present in both groups. [Background image from Wikimedia Commons: Figure 728 from 1918 Gray’s Anatomy vectorized by user Mysid.]
finding is not statistically significant.

Differing patterns of connectivity were also observed outside of the DMN. Using SC, we observed trends of higher total link strength between thalamus and auditory cortex and between thalamus and insula in the music group accompanied by an edge between insula and auditory cortex present only during music-listening. These findings should not come as surprise since both auditory cortex and insula are involved in the processing of sounds. Insula plays an important role in language processing, whether in verb generation [Karunanayaka et al., 2010], story processing [Karunanayaka et al., 2007], semantic processing [Kim et al., 2011], or auditory processing [Bamiou et al., 2003]. We would expect insula to be recruited in the processing of lyrics and perhaps even nonverbal elements of music. Another explanation for this between-group difference could be insula’s connections with limbic and autonomic systems. As insula exerts considerable control over the autonomic nervous system [Critchley et al., 2000; Tokgözoglu et al., 1999], it could plausibly be the mechanistic actor through which music promotes relaxation.

A.3 Conclusion

Music-listening produces relaxation and a more introspective pattern of connectivity in the DMN. Although the resting-state networks, including the DMN, are modulated by music-listening, the fundamental structure of the DMN is unaltered. Therefore, music-listening is a valid condition under which to record resting-state data. Our findings of altered connectivity in auditory cortex, thalamus, and insula recommend inclusion of music-listening as a covariate in resting-state analyses comparing subjects who were and were not listening to music.
Appendix B

Whole-Brain Connectivity

This study used seed-based voxel correlation and independent component analysis (ICA) to investigate resting-state networks in IGE patients. Graph theory is another mathematical technique that can be used to investigate network organization in the whole brain using functional (i.e. fMRI) or structural (i.e. DTI) data [Achard et al., 2006; Guye et al., 2010]. The brain is divided into nodes, which may be anatomical regions, and connectivity between nodes is used to construct an adjacency matrix describing the edges in the network [Bullmore & Sporns, 2009; Salvador et al., 2005]. Graph theory provides a mathematical framework for summarizing the adjacency matrix into a scalar statistic, such as small-worldness, which may then be compared between experimental groups [Achard et al., 2006]. This technique is sensitive to pathological changes in brain state in general [Guye et al., 2010] and also in IGE specifically, where small-worldness is reduced in IGE patients compared to healthy controls [Zhang et al., 2011].

B.1 Summary of Methods

Graph theory is an approach to resting-state functional (also structural) connectivity that is sensitive to pathological changes in brain state [Guye et al., 2010] and has been used to study IGE [Zhang et al., 2011]. A graph is a collection of nodes connected by edges. In an undirected graph, the edges are scalars that do not provide information about the causal relationship between nodes [Brillinger, 1996]. Such an undirected graph may be completely described by an adjacency matrix, such as in
Figure B.1. The sets of rows and columns in an adjacency matrix each correspond to the complete set of nodes such that each cell in the matrix is the edge weight between the two nodes in the corresponding row and column. Therefore, an adjacency matrix is symmetric. Edges may be binary (i.e. either 0 or 1) or weighted (i.e. between 0 and 1). In an undirected acyclic graph, nodes are not connected to themselves so the diagonal of the adjacency matrix is all zeros. An undirected acyclic graph is ideal for representing resting-state functional connectivity (RSFC) between brain regions.

There are many ways to select nodes, but selection based on anatomical regions of interest is typical. The Freesurfer software may be used to segment a subject’s high-resolution anatomical image, and it has the advantage of being able to track the precise cortical surface, distinguishing between gyri, sulci, subcortical tissue, white matter, cerebrospinal fluid, and skull. Unfortunately, artifacts in our anatomical data prevented the automated use of Freesurfer in our study. Instead, we used the automated anatomical labelling (AAL) atlas [Tzourio-Mazoyer et al., 2002], which divides the brain into 90 cortical and subcortical regions of interest in stereotaxic space, to define the 90 nodes in our study. Therefore, the dimensions of our adjacency matrices were $90 \times 90$.

During seed-based voxel correlation and ICA, we motion corrected the functional data to a reference frame using a rigid-body linear transformation [Jenkinson et al., 2002]. We then registered the reference frame to the anatomical volume using a 7 degrees of freedom linear transformation (3 translations, 3 rotations, 1 global scale) [Jenkinson & Smith, 2001]. Finally, the anatomical volume was transformed to MNI152 stereotaxic space using a non-linear transformation [Andersson et al., 2007a,b]. In order to generate edges between the AAL nodes, we performed these transformations in reverse. The AAL atlas was transformed from stereotaxic space to each subject’s anatomical space, then to the functional reference frame, and finally to each functional volume. After slice-timing correction, the average functional timecourse inside each AAL node was extracted. Unlike as with Freesurfer, this technique did not clearly delineate the cortical surface, and so signal from white matter and cerebrospinal fluid was unavoidably included in the average timecourses.

Let the average timecourse for node $i$ in a given subject be $t_i$ where $1 < i < N = 90$. We used two measures of connectivity [Brockwell & Davis, 2009] to define the edge weights between between
a pair of nodes \( \{i,j\} \). One measure was absolute pairwise correlation \( r_{i,j} \), given in Equation B.1, which describes the total link strength between two nodes irrespective of the influence of other nodes.

\[
r_{i,j} = \left| \frac{\text{Cov}(t_i, t_j)}{\sqrt{\text{Var}(t_i)\text{Var}(t_j)}} \right|
\]

Consider the symmetric (i.e. \( r_{i,j} = r_{j,i} \)) variance-covariance matrix \( \mathbf{R} \) given in Equation B.2.

\[
\mathbf{R}_{N \times N} = \begin{bmatrix}
1 & r_{1,2} & \cdots & r_{1,N} \\
r_{2,1} & 1 & \cdots & r_{2,N} \\
\vdots & \vdots & \ddots & \vdots \\
r_{N,1} & r_{N,2} & \cdots & 1
\end{bmatrix}
\]

(B.2)

By inverting \( \mathbf{R} \) we obtain the second, complementary measure: partial correlation \( r_{i,j}^p \), given in Equation B.3, which describes the direct link strength between two nodes conditional on all other nodes.

\[
r_{i,j}^p = \left| -\frac{\{[\mathbf{R}]^{-1}\}_{i,j}}{\sqrt{\{[\mathbf{R}]^{-1}\}_{i,i}\{[\mathbf{R}]^{-1}\}_{j,j}}} \right|
\]

(B.3)

The adjacency, or weight matrix \( \mathbf{W} \) for a given subject is then constructed from pairwise or partial correlation according to Equation B.4.

\[
\text{pairwise } \mathbf{W}_{N \times N} = \begin{bmatrix}
0 & r_{1,2} & \cdots & r_{1,N} \\
r_{2,1} & 0 & \cdots & r_{2,N} \\
\vdots & \vdots & \ddots & \vdots \\
r_{N,1} & r_{N,2} & \cdots & 0
\end{bmatrix}, \quad \text{partial } \mathbf{W}_{N \times N} = \begin{bmatrix}
0 & r_{1,2}^p & \cdots & r_{1,N}^p \\
r_{2,1}^p & 0 & \cdots & r_{2,N}^p \\
\vdots & \vdots & \ddots & \vdots \\
r_{N,1}^p & r_{N,2}^p & \cdots & 0
\end{bmatrix}
\]

(B.4)

The usefulness of graph theory is to take the adjacency matrix and summarize it as a scalar statistic which may then be compared between groups. A more conventional analysis might pick several nodes of interest (e.g. thalamus and medial frontal cortex) and compare their pairwise or partial correlation between groups. Although valid, this strategy suffers from problems of collinearity and multiple comparisons as \( N \) grows large. With graph theory, we do not concern ourselves
with specific nodes and instead consider whole-brain connectivity and organization. In this way, we can make sense of a large number of nodes using summary statistics with a relatively straightforward interpretation.

In our preliminary analysis, we considered the measure of small-worldness, $\sigma$. Small-worldness describes the “cliquishness” of a graph, i.e. the propensity of separate groups of nodes to be connected through influential hub nodes [Achard et al., 2006]. IGE is associated with a loss of small-worldness [Zhang et al., 2011], which may be due to the presence of spurious connections that bypass inhibitory or regulatory hubs.

Computation of small-worldness requires the computation of several additional graph measures. The following definitions are reproduced from the literature [Bullmore & Sporns, 2009; Salvador et al., 2005]. Let the degree $k_i$ of a node be the number of edges connected to that node. Let $\{W\}_{i,j} = w_{i,j}$. For a weighted graph, $k_i$ is given by Equation B.5.

$$k_i = \sum_j w_{i,j} \quad \text{(B.5)}$$

From degree we can obtain the clustering coefficient $c_i$ of a node given in Equation B.6. It describes the likelihood that nodes a node is connected to (i.e. its neighbors) are themselves connected to each other.

$$c_i = \frac{\sum_{\{j,k\}} (w_{i,j} w_{i,k} w_{j,k})^{\frac{1}{2}}}{k_i (k_i - 1)} \quad \text{(B.6)}$$

Degree also yields the shortest path length (or distance) $d_{i,j}$ between two nodes for which the weighted version is given by Equation B.7 as the sum of the reciprocal edge weights on all paths $\varsigma(i,j)$ between $i$ and $j$.

$$d_{i,j} = \sum_{m,n \in \varsigma(i,j)} \frac{1}{w_{m,n}} \quad \text{(B.7)}$$

Clustering coefficient and shortest path length can be summarized over all nodes to yield the overall clustering coefficient $C$ and overall path length $D$ of the graph. $C$ is given in Equation B.8 as the arithmetic mean clustering coefficient, and $D$ is given in Equation B.9 as the harmonic mean.
shortest path length.

\[ C = \frac{1}{N} \sum_{i=1}^{N} c_i \]  
(B.8)

\[ D = \left[ \frac{1}{N(N-1)} \sum_{i=1}^{N} \sum_{i \neq j} \frac{1}{d_{i,j}} \right]^{-1} \]  
(B.9)

Each of \( C \) and \( D \) is partly a function of the overall degree \( K \) (i.e. number of edges) in a graph. Therefore, it is necessary to normalize these measures using a null model. The null model is a random graph with the same degree as the graph of interest. It may be obtained empirically by randomly “rewiring” edges in the original graph. Let \( C_0 \) and \( D_0 \) be the overall clustering coefficient and shortest path length of a null graph. Then normalized overall clustering coefficient \( \gamma \) and normalized shortest path length \( \lambda \) are given by Equation B.10.

\[ \gamma = \frac{C}{C_0}, \lambda = \frac{D}{D_0} \]  
(B.10)

Finally, small worldness \( \sigma \) is defined as the ratio in Equation B.11.

\[ \sigma = \frac{\gamma}{\lambda} \]  
(B.11)

### B.2 Preliminary Data

Samples of adjacency matrices are shown for a representative subject (BPK) in Figure B.1. Below each adjacency matrix is a histogram for visualizing the distribution of edge weights. Note how the adjacency matrix using partial correlation (right) appears random and how the histogram (blue) of partial correlation edge weights does not differ much from the least-squares fit Beta distribution (in red). These figures indicate a graph with low signal to noise ratio, possibly as a result of poor quality data or inappropriate segmentation (i.e. AAL vs. Freesurfer).

The small world coefficient \( \sigma \) was calculated in each subject over a range of degrees by thresholding the adjacency matrix at different cutoff weights to generate graphs with different costs (cost = degree/\text{max}[degree]). \( \sigma \) was then averaged across experimental groups as in a previ-
Figure B.1: Edge weights from a representative subject (BPK). Top: adjacency matrices are displayed as a heatmap with black = low connectivity and yellow = high connectivity. Bottom: histogram of normalized (between 0 and 1) edge weights. A least-squares fit beta distribution is displayed over the histogram in red. Left: edge weights are calculated as pairwise correlation. Right: edge weights are calculated as partial correlation.
ous study of IGE [Zhang et al., 2011]. Results for IGE patients vs. healthy controls and IGE patients with uncontrolled vs. controlled seizures are shown in Figure B.2. There was no statistically significant difference between groups. Moreover, the range of $\sigma$ was unusually low (typically $\sigma \gg 1$ [Achard et al., 2006; Salvador et al., 2005]), and group trends were sometimes opposite of previously published findings [Zhang et al., 2011]. We concluded that graph theoretical analysis might not be possible on these data, and that it would minimally require time-consuming manual segmentation to generate higher-quality nodes.
Figure B.2: Average small world coefficient (σ) within a group of subjects is shown for weighted graphs over a range of network wiring costs. Edge weights were calculated as pairwise correlation (top: A,B) or partial correlation (bottom: C,D). A,C: all IGE patients vs. healthy controls. B,D: IGE patients with uncontrolled (Seizures-) vs. controlled (Seizures+) seizures.
Appendix C

Review of Methods

C.1 Attenuation of fMRI-Related Artifact in EEG

Simultaneous use of EEG and fMRI is considered superior to the use of either modality alone because it provides the temporal resolution of EEG combined with the spatial localizing power of fMRI. Nevertheless, attempting to record electrical potentials on the order of microvolts from inside the center of a superconducting magnet results in artifacts that are not present in a conventional EEG recording. We discuss methods for removal of two such artifacts, the gradient and ballistocardiographic (BCG) artifacts, below. Samples of these artifacts may be seen in Figure C.1 of an IGE patient exhibiting GSWD and in Figure C.2 of a healthy control subject exhibiting normal alpha rhythm.

C.1.1 Gradient Artifact

An MRI scanner contains electromagnetic “gradient” coils that are used to vary the intensity of the magnetic field over space and time (i.e. create a magnetic field gradient) as part of the normal process of image acquisition. The gradient coils are capable of producing large changes in the magnetic field very quickly – in our study, at 15 Hz. Physics tells us that a conducting loop (such as an EEG electrode) placed in a varying magnetic field will experience an induced current. In the case of a resistive conductor such as an EEG electrode, this induced current will produce an
post-BCG reduction

alpha rhythm (≈ 11 Hz)

gradient artifact (2 seconds)

BCG artifact (≈ 1.3 Hz)

GSWDs (3 Hz)

post-gradient reduction

original data

post-BCG reduction

Figure C.1: See caption on next page.
Figure C.1: Ten seconds of EEG data were recorded from an IGE patient simultaneous with fMRI and are shown in a bipolar, double-banana montage. Normal 11 Hz alpha background rhythm (left) is followed by generalized spike and wave discharges (GSWDs) at 3 Hz (right). Top: raw data. Examples of ballistocardiographic (BCG) and gradient artifacts are marked. Middle: data after low-pass filtering and gradient artifact reduction. Note GSWDs clearly visible at right and ambiguous waveforms at left. Bottom: data after BCG artifact reduction. Note the clear distinction between the background rhythm at left and GSWDs at right.

electrical potential at the amplifier that is many orders of magnitude larger than neuronal signal of interest. These potentials may be seen in Figures C.1 and C.2 (top) whenever the gradient coils are activated.

Fortunately, reduction of the gradient artifact is simple provided the correct conditions are met. The gradient waveform is very precise, and so the artifact waveform is highly similar between TR intervals and, in a continuous acquisition, even from slice to slice. By averaging several consecutive gradient artifacts, it is possible to create a template of the gradient artifact from which the neuronal signal of interest has been averaged out. Subtracting this average template from the original signal produces the desired reduction of the gradient artifact [Allen et al., 2000]. Subsequent application of a low-pass filter with a sharp roll-off starting at between 30 and 40 Hz greatly attenuates any residual gradient artifact.

Quality of the template is affected by a number of factors such as movement, aliasing, and clipping of the recorded signal. It is therefore desirable to record data from a stationary subject at a high sampling rate and high dynamic range, and to ensure that the amplifier is shielded from very high frequency signals such as the MRI radiofrequency (RF) pulse. Using a running average (as opposed to averaging over all artifacts in the recording) helps to confine problems related to motion to the time in the recording at which the motion occurred. We have found that a running average of 3–7 gradient waveforms produces good results.

Accurate timing information is also essential to producing a high-quality average artifact template. Without knowing when the artifact waveform begins and ends, it is not possible to obtain an average artifact template or to subtract it from the appropriate portion of the EEG data. It is obvious when each TR begins from cursory examination of Figures C.1 and C.2 (top), and using some sort of voltage thresholding algorithm to detect the time of gradient onset may provide
Figure C.2: See caption on next page.
acceptable results. A more ideal setup inserts time marks generated by the scanner at the onset of each TR or slice into the EEG recording, thus obviating the need to tune a gradient detection algorithm. However, without specialized equipment, the time marks generated by the scanner may be variably offset by a few samples from the true onset of the gradient artifact. This situation may be improved by maximizing the cross-correlation between the time mark and the recorded signal over a limited number of samples. We have found that cross-correlation over 25 samples as 10 kHz produces good results.

C.1.2 Ballistocardiographic Artifact

The gradient artifact results from induction of current in a stationary electrode placed in a time-varying magnetic field. Current may also be induced in a moving electrode placed in a space-varying magnetic field or a rotating electrode placed in a homogenous magnetic field. Both of these latter conditions are satisfied by gross subject movement, which produces difficult-to-remove artifacts. However, the magnetic field in the MRI scanner is so strong that motion-related artifact will be observed even for a stationary subject due the pulsatile movement of electrodes placed on top of blood vessels. This artifact is called the ballistocardiographic (BCG) artifact (Figures C.1 and C.2, middle).

Unlike the gradient waveform, the subject’s heartbeat is not very precise – it varies considerably over time. Therefore, the average artifact subtraction approach used for gradient artifact reduction does not produce good results with the BCG artifact. Instead, we use a more general, linear spatial filtering approach that takes advantage of how the artifact appears across multiple channels. The spatial filtering strategy below is reproduced from the literature [Lagerlund et al., 1997].

Let $E$ be an $n \times m$ matrix where $n$ is the number of temporal samples in the EEG recording and $m$ is the number of channels (i.e. electrodes) such that $\bar{e}(t)$ is the set of voltages recorded at
all channels at time $t$. We can use singular value decomposition (SVD) to express $E$ in terms of an $n \times m$ matrix of principal components $P$ as per Equation C.1 where $A$ is diagonal such that each $a$ is the magnitude of the corresponding principal component $p$.

$$E = P A M^T, \quad M^T M = M M^T = 1$$ (C.1)

If we know that a certain principal components $p$ is related to the BCG artifact, then we can set the corresponding column of $M$ to $0$ to obtain $M'$. This may be done for multiple columns in the case that multiple principal components are artifact-related. We may then generate a spatial filter $S$ as in Equation C.2 and use it to obtain the artifact-free signal $E'$ as $\vec{e}'(t) = S \vec{e}(t)$.

$$S = M' M^T$$ (C.2)

The question now becomes: how do we know which principal components are related to the BCG artifact and which are related to the neuronal signal of interest? To solve this, we record an extra EKG channel and use it to identify heartbeat timings. We then epoch non-overlapping time blocks of the EEG around each heartbeat and obtain an average. This process will average out the neuronal signal and produce an average template of the BCG artifact, analogous to the average template of the gradient artifact. Applying SVD to this data will produce principal components that are mostly related to the BCG artifact. We set the first $q < m$ principal components accounting for most of the observed variance to zero in order to generate our spatial filter. When the average template is of good quality, we find that zeroing out 99% of the variance produces good results.

### C.2 Seed-Based Voxel Correlation

Seed-based voxel correlation is similar to conventional, event based fMRI analysis. In an event-based analysis, we know *a priori* the timing and magnitude of some external event (e.g. a button press). The event timing is convolved with a hemodynamic response function (HRF) to produce a vector $\vec{r}$ describing the expected fMRI signal. Let $\vec{y}$ be the observed signal. A simple event-related analysis
solves the least-squares optimization problem in Equation C.3 where \( n \) is the number of subjects. The resultant \( \beta \) and \( t \)-values describe the statistical correlation between the event and activation at a particular voxel.

\[
\tilde{y} = \tilde{\tau}\beta + \varepsilon, \quad \varepsilon \overset{iid}{\sim} \mathcal{N}(0, \sigma^2), \quad t = \frac{\beta}{\sqrt{\frac{\sigma^2}{n}}} \sim \mathcal{T}_{n-1}
\]  

(C.3)

It is common (and highly advisable) to include covariates that model such factors as the mean, baseline drift, and motion. A more realistic event-related design is shown in Equation C.4 where \( \tilde{m} \) is a vector of motion regressors and \( \tilde{\eta} \) is some polynomial baseline function. \( X \) is referred to as the design matrix.

\[
\tilde{y} = XB + \varepsilon, \quad \varepsilon \overset{iid}{\sim} \mathcal{N}(0, \sigma^2), \quad X = \begin{bmatrix}
1 & p_1 & \tau_1 & m_1 \\
1 & p_2 & \tau_2 & m_2 \\
\vdots & \vdots & \vdots & \vdots
\end{bmatrix}, \quad B = \begin{bmatrix}
\mu & \phi & \beta & \omega
\end{bmatrix}
\]  

(C.4)

In resting-state analysis, there is no external timecourse we may use to construct \( \tilde{\tau} \). Instead, we select a voxel of interest (i.e. a voxel located in an anatomical structure relevant to our \textit{a priori} hypothesis) and use the timecourse of that voxel as \( \tilde{\tau} \). The average timecourse of a small region of interest may also be used. There is no need to convolve this timecourse with an HRF because it is already in the domain of the fMRI signal. Equation C.3 now yields the statistical correlation of each voxel in the brain with our “seed” voxel. It is common to measure this correlation using the sum squared error \( r^2 \), which is given by Equation C.5 in terms of Equation C.4. Since \(-1 \leq r \leq 1\), it is common to transform \( r \) to a \( z \)-statistic using the Fisher transform \( z = \text{atanh}(r) \) for the purpose of higher-level statistics.

\[
r^2 = \tilde{y}^T \left[ I - X (X^T X)^{-1} X^T \right] \tilde{y}
\]  

(C.5)

To quote Dr. Peter Bandettini, “Everything in the brain is correlated.” The effect of this observation is to make all of our \( z \)-values very high, such that every voxel appears to be “significantly” correlated with every other voxel. Movement and physiological artifacts account for some of this surplus correlation [Power et al., 2012; Van Dijk et al., 2012]. Much of this surplus correlation is cancelled out in group-level contrasts such that results may be reasonably easy to interpret.
Several strategies exist for dealing with greater-than-desired correlation at the individual level by adding columns to the design matrix $X$. Making more realistic assumptions about \( \varepsilon \) to account for temporal autocorrelation (i.e. with temporal prewhitening or an autoregressive model) also helps to reduce observed correlation.

Since movement and physiological artifacts contribute to observed correlation, the inclusion of motion parameters or physiological regressors in the design matrix is advantageous. In scenarios where these data are not available or do not produce the desired result, it is tempting to add additional covariates to the design matrix. In the past, the global mean timecourse or the global timecourse from non-grey matter tissue classes (i.e. white matter and cerebrospinal fluid) have been included in the design matrix. However, it is has been shown that these additional regressors introduce their own artifactual biases to the analysis and should be avoided [Fox et al., 2009; Weissenbacher et al., 2009].

### C.3 Independent Component Analysis

Independent component analysis (ICA) is an alternative approach to resting-state analysis that identifies correlated sets of brain regions, or resting-state networks, conditional on all other resting-state networks. Although the interpretation of ICA results is similar to seed-based voxel correlation (Section C.2), the methodology is very different and the amount of surplus correlation less, making ICA only slightly susceptible to physiologic regressors [Starck et al., 2010] and more robust than seed-based voxel correlation against structured noise [Damoiseaux et al., 2006; Ma et al., 2007]. The methods described here are reproduced from the literature [Calhoun et al., 2001; Erhardt et al., 2011].

Consider a set of fMRI scans from \( m \) subjects where each scan is comprised of \( T \) timepoints from \( V \) voxels such that the demeaned data from subject \( i \) is given by the matrix \( Y_i \) which has \( T \times V \) rows and \( V \) columns. Independent component analysis assumes that the observed signal \( Y_i \) is the linear product of some mixing matrix \( A_i \) and the “true” source signal \( S_i \) such that:

\[
Y_i = A_i \, S_i
\]  

(C.6)
Figure C.3: The spatial distribution of this group \((n = 98)\) independent component (IC) favors white matter, specifically the corona radiata. Voxels above 99\% of the robust range \((t > 15.527)\) are shown in red over the MNI152 standard brain in radiological orientation.

Where \(S_i^T\) is selected such that its columns are maximally independent (hence the name independent component analysis) or, equivalently, such that mutual information between its columns is minimized. Although independence implies orthogonality, note that independence between columns is maximized, not guaranteed. Thus, the columns of \(S_i^T\) are not necessarily orthogonal except in the case of ICA algorithms that explicitly enforce an orthogonality constraint. Note also that no error term is included in Equation C.6, consistent with a “noise-free” ICA approach. MELODIC, a part of FSL [Smith et al., 2004], is the only neuroimaging ICA tool that models noise.

From this description, it is clear why ICA offers superior separation of structured noise. Structured noise signals are sequestered in their own components, and components modelling signals of interest are selected so as to be maximally independent from the structured noise components. See Figure C.3 for an example of a structured noise component. Note, also, that no seed voxel timecourse is included in Equation C.6. This is in some ways advantageous in the sense that ICA is purely data driven and not influenced very much by the a priori assumptions of the investigator. On the other hand, the investigator is not free to select a voxel or region of interest and is thus “out of luck” if a suitable component containing the interesting region is not generated.

### C.3.1 Data Reduction

From Equation C.6, we can see that the number of components generated is equal to the number of fMRI timepoints \(T\). This becomes unwieldy as the typical number of fMRI timepoints is large
(> 100) and, while objective means of selecting the optimal number of components exist [Li et al.,
2007], this number is typically < 50. We need to reduce the number of ICs generated in Equation C.6
from \( T \) to some number \( T_1 < T \). This will allow us to generate a more useful number of components,
and it will have the added benefit of making the solution of Equation C.6 more computationally
tractable.

For each subject \( i \), consider a singular value decomposition (SVD) of the covariance matrix
of \( Y_i \), \( \Sigma_i \), into a unitary and diagonal matrix \( D \):

\[
\Sigma_i = \begin{bmatrix} F_i & F_{i2} \end{bmatrix} \begin{bmatrix} D_i & 0 \\ 0 & D_{i2} \end{bmatrix} \begin{bmatrix} F^T_i \\ F^T_{i2} \end{bmatrix}
\]  

(C.7)

Where \( F \) and \( D \) are the first \( T_1 < T \) ordered eigenvectors and eigenvalues, respectively. Note
that if we take the partition \( F \) to be unitary then:

\[
F^\perp = \left( F^T F \right) F^T = F^T_{T_1 \times T}
\]  

(C.8)

We can reduce or “compress” the data \( Y_i \) to obtain \( Y_i^* \):

\[
Y_i^* = F_{i1 \times T}^\perp Y_{1 \times T}^i
\]  

(C.9)

And we can “uncompress” \( Y_i^* \) to obtain a prediction of the original data:

\[
\hat{Y}_i = F_{T \times T_1} Y_{i1 \times T_1 \times V}^*
\]  

(C.10)

From here we could substitute \( Y_i^* \) for \( Y_i \) in Equation C.6 to obtain a more useful number of
ICs, \( T_1 \).

C.3.2 Concatenation

Thus far we have concerned ourselves with ICA on a single subject. In practice, we will typically
be interested in ICA results across a group of multiple subjects. Unlike anatomically constrained
methods such as seed-based voxel correlation, there is no guarantee that any two subjects will have the same set of independent components. Therefore, we need a way to constrain our analysis such that we generate a single set of components for a group of subjects [Calhoun et al., 2001]. This is achieved with temporal concatenation, a method found to be superior to spatial concatenation [Schmithorst & Holland, 2004].

Beginning with $Y_i^*$ from Equation C.9 we concatenate along the temporal dimension to obtain:

$$ Y^*_M \times V = \begin{bmatrix} Y_1^* \\ Y_2^* \\ \vdots \\ Y_m^* \end{bmatrix} \quad \text{(C.11)} $$

As before, we need to perform data reduction in order to limit the number of ICs generated. We can apply SVD as in Equation C.7 to obtain a generalized reducing matrix $G^-$ and a reduced concatenated dataset $X$:

$$ X_{T_2 \times V} = G^- \times V_{T_2 \times M \times T_1 \times V} \quad \text{(C.12)} $$

Where:

$$ G^-_{T_2 \times M \times T_1} = \begin{bmatrix} G^T_{1 \times T_2 \times T_1} & G^T_{2 \times T_2 \times T_1} & \ldots & G^T_{M \times T_2 \times T_1} \end{bmatrix} \quad \text{(C.13)} $$

And:

$$ Y^*_{M \times T_1 \times V} = G \times X_{M \times T_1 \times T_2 \times V} \rightarrow \tilde{Y}_i_{T \times V} = F_i \times Y^*_i_{T \times T_1 \times V} = F_i \times G_i \times X_{T \times T_2 \times V} \quad \text{(C.14)} $$

Note that, since we now control the number of ICs generated by selecting the $T_2$ dimension of $G^-$, it is no longer strictly necessary to perform data reduction on each individual subject prior to temporal concatenation. (This step, however, helps to make the process of finding $G^-$ using SVD computationally tractable for large numbers of subjects $M$.) If we choose to omit the first level of data reduction, as is done by MELODIC, then we can simply take $F_1 = I_{T \times T^*}$.

Now we may substitute $X$ from Equation C.12 for $Y_1$ in Equation C.6 to obtain:

$$ X_{T_2 \times V} = A \times S \quad \text{(C.15)} $$
As before, each column in $A$ and row in $S$ is the timecourse and spatial map, respectively, of one of $T_2$ ICs where $T_2$ is a number chosen by the investigator (i.e. using MDL criteria [Li et al., 2007]) to obtain a desired number of ICs such that $T_2 < T_1 < T$. The results are generalizable in that each timecourse from $A$ and spatial map from $S$ is an aggregate for the set of all subjects included in analysis.

C.3.3 Backprojection

Having obtained a single set of components for a group of subjects, we now need to find the timecourses $R_i$ and spatial maps $S_i$ of all the ICs in Equation C.15 for each subject $i$ before we can proceed with high-level statistical comparisons between subjects and groups. The process of projecting the group maps back onto individual subjects is called backprojection. We begin by defining the model in Equation C.16. Note from this Equation that if we know one of $R_i$ or $S_i$ then we can readily obtain the other.

$$ Y_i \in \mathbb{R}^{T \times V} = R_i \in \mathbb{R}^{T \times T_2} S_i \in \mathbb{R}^{T_2 \times V} + \varepsilon \overset{iid}{\sim} \mathcal{N}(0, \sigma^2) \tag{C.16} $$

Several backprojection strategies exist. In our studies we favor the dual regression (also known as spatiotemporal regression, STR) approach [Filippini et al., 2009], but the GICA3 algorithm [Erhardt et al., 2011] has many advantages to recommend its use, especially when the backprojected timecourse $R_i$ is of primary interest. In the case of dual regression, we assume that $S_i = S$ to obtain $R_i$ from Equation C.16, with the addition of a ones column to model a grand mean:

$$ Y_i^T \in \mathbb{R}^{V \times T} = \begin{bmatrix} 1 & S_i^T \end{bmatrix} \begin{bmatrix} 1^T \\ R_i^T \end{bmatrix} + \varepsilon^T \longrightarrow \begin{bmatrix} 1 & R_i \end{bmatrix} \begin{bmatrix} Y_i^T \\ S \end{bmatrix} = Y_i^T \begin{bmatrix} 1^T \\ S \end{bmatrix} \tag{C.17} $$

Note that this first of two (dual) regressions takes observations over space (spatio). The next regression step will take observations over time (temporal). The assumption of $S_i = S$ would result in all subjects sharing the same backprojected spatial map, $S_i = S_j \forall (i, j)$. To obtain a subject-specific backprojected spatial map, $S_i$ is re-estimated using the estimate of $R_i$ from Equation C.17.
via Equation C.16:

\[
\textbf{Y}_i = \begin{bmatrix} 1 & \textbf{R}_i \\ \textbf{S}_i \end{bmatrix}_{T \times T_2} \begin{bmatrix} 1^T \\ \textbf{S}_i \end{bmatrix}_{T_2 \times V} + \varepsilon \rightarrow \begin{bmatrix} 1^T \\ \textbf{S}_i \end{bmatrix}_{T_2 \times V} = \begin{bmatrix} 1 & \textbf{R}_i \\ \textbf{S}_i \end{bmatrix}_{T \times T_2}^{-1} \textbf{Y}_i
\]  

(C.18)