Long-Term EEG Dynamics

Following Traumatic Brain Injury

in a Rat Model of Post Traumatic Epilepsy

by

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ABSTRACT

Development of post-traumatic epilepsy (PTE) after traumatic brain injury (TBI) is a major health concern (5% - 50% of TBI cases). A significant problem in TBI management is the inability to predict which patients will develop PTE. Such prediction, followed by timely treatment, could be highly beneficial to TBI patients.

Six male Sprague-Dawley rats were subjected to a controlled cortical impact (CCI). A 6mm piston was pneumatically driven 3mm into the right parietal cortex with velocity of 5.5m/s. The rats were subsequently implanted with 6 intracranial electroencephalographic (EEG) electrodes. Long-term (14-week) continuous EEG recordings were conducted. Using linear (coherence) and non-linear (Lyapunov exponents) measures of EEG dynamics in conjunction with measures of network connectivity, we studied the evolution over time of the functional connectivity between brain sites in order to identify early precursors of development of epilepsy.

Four of the six TBI rats developed PTE 6 to 10 weeks after the initial insult to the brain. Analysis of the continuous EEG from these rats showed a gradual increase of the connectivity between critical brain sites in terms of their EEG dynamics, starting at least 2 weeks prior to their first spontaneous seizure. In contrast, for the rats that did not develop epilepsy, connectivity levels did not change, or decreased during the whole course of the experiment across pairs of brain sites. Consistent

behavior of functional connectivity changes between brain sites and the “focus” (site of impact) over time was demonstrated for coherence in three out of the four epileptic and in both non-epileptic rats, while for STLmax in all four epileptic and in both non-epileptic rats.

This study provided us with the opportunity to quantitatively investigate several aspects of epileptogenesis following traumatic brain injury. Our results strongly support a network pathology that worsens with time. It is conceivable that the observed changes in spatiotemporal dynamics after an initial brain insult, and long before the development of epilepsy, could constitute a basis for predictors of epileptogenesis in TBI patients.

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CHAPTER 1. INTRODUCTION

Overview

Records of epileptic activity date back to antiquity (Goldensohn, 1997). It was originally considered to be a condition that came about as a demonic possession and led to stigmatization of those afflicted. It was not until the times of Hippocrates that a new theory as to the cause of epileptic seizure disorders was produced. Hippocrates postulated that the observed seizures were actually a neurological disorder (Engel & Pedley, 1997). With the fairly recent advent of EEG and advances in computational power, the study of epileptic seizures has grown considerably. However, while the scientific community has indeed made great strides toward obtaining a better understanding of epilepsy, many aspects of the disorder are still shrouded in mystery.

Epilepsy is a significant global health problem affecting approximately 1% of the world population (Niedermeyer, Epileptic Seizure Disorders, 2005). Epilepsy is not a disease. It is more accurate to say that epileptic seizures are caused by a large number of diseases (Niedermeyer, Epileptic Seizure Disorders, 2005). Epileptic seizures occur when large numbers of neurons undergo abnormal paroxysmal synchronous activity. This activity may occur in relatively small brain regions (focal epilepsy) or throughout the brain (generalized epilepsy). Seizures may also occur in the cortex or deep brain structures. In order to be diagnosed as epileptic a person must have two or more unprovoked seizures. The definition of unprovoked seizures will be discussed further in subsequent chapters. Not all epileptic seizures exhibit clinical manifestations (clinical versus

subclinical seizures). Common clinical symptoms of seizures include sudden involuntary movements, falling to the floor and facial twitches.

One of the many causes of epileptic seizures is traumatic brain injury (TBI). It is estimated that as much as 50% of those that sustain a TBI will develop post traumatic epilepsy (PTE) (Agrawal, Timothy, Pandi, & Manju, 2006). Injuries sustained due to recent military conflicts overseas have sparked much interest in the field of TBI including PTE. PTE is epilepsy that develops as a result of TBI. To be diagnosed with PTE one must have a seizure greater than one week after the TBI. Seizures that occur prior to one week post injury may be early signs of the epileptogenic process but are not, themselves, indicative of epilepsy (Angeleri & al, 1999). Like the development of all forms of epilepsy, PTE is not yet predictable. This means that following TBI, there is uncertainty as to whether anti-epileptic drugs (AEDs) ought to be prescribed. A means for the clinician to accurately predict the onset of PTE prior to the first seizure would be of great use in the clinical treatment.

Research Objectives

The development of post traumatic epilepsy is of major concern to those who have suffered from TBI. Currently, patients must decide prior to their first epileptic seizure if they would like to begin treatment with AEDs and to risk been afflicted with potential AED side effects or forgo treatment and possibly suffer full-blown epilepsy later on. This is clearly not an easy decision.

The main aim of this research was to provide a more in-depth understanding of epileptogenesis following TBI. This was accomplished through time series analysis of intracranial EEG recorded from rats subjected to controlled cortical impact (CCI). In addition, we expected to discover indicators for prediction of the level of seizure susceptibility and upcoming epileptic seizures. It is plausible to assume that those who will eventually develop epileptic seizures will have different processes occurring in their brain than those who will not develop seizures.

During epileptic seizures the brain exhibits high levels of functional connectivity. Our research investigated whether there is a similar increase in connectivity during epileptogenesis, as well as the critical brain sites between which this functional connectivity is mostly exhibited. It is thought that the focus of epileptic activity drives the other brain sites during seizures (Meeren, Pijn, Van Luijtelaar, Coenen, & Lopes da Silva, 2002). Assuming that the site of impact in CCI would become the epileptogenic focus in those rats that would eventually develop epilepsy, this research attempted to detect a trend in the level of functional connectivity between the site of impact (the potential driver) and the other brain sites we were recording EEG from. To establish sensitivity and specificity of such a theory, it is crucial to also check that such a trend occurs in the rats that will become epileptic and not the ones that will not.

Thesis Organization

This thesis is organized as follows. Chapter 2 provides a background on physiology and epilepsy. The scientific progress made toward understanding epilepsy is tracked from ancient times up through the early 1900's (the beginning of modern neuroscience). Such progress included the realization that epilepsy was a neurological condition as opposed to a curse from the gods, the discovery of electrical activity within the human body and specifically the brain, and the ability to record this activity. Clarification and brief description of the definition and nature of epilepsy is provided in this chapter as well as an overview of prevalence, common causes and treatment options. The discovery of AEDs is also presented in this chapter. Traumatic brain injury and its possible repercussions will then be discussed together with epidemiology of TBI as well as treatment options and their side effects. Following the section on TBI is an overview of PTE. The significance, prevalence and implications of this disorder will be presented. Methods and results of studies conducted to date on epileptogenesis after TBI will also be discussed.

Chapter 3 presents the experimental methods that we employed in this study. The means by which the CCI was administered, the specifications of the EEG recording, and care of the animals are discussed in this chapter. The methods and measures of data analysis used are also presented in this chapter. In particular, the theory and application to EEG of coherence and Lyapunov exponent measures of dynamics are included. Chapter 4 presents the results from this study. It was found that those rats that eventually developed epilepsy showed increased levels

of functional connectivity throughout the study while those that did not develop epilepsy showed no such trend. Chapter 5 offers concluding remarks and suggestions for future research.

CHAPTER 2. BACKGROUND

Epilepsy

History of epilepsy

It is probable that epilepsy has been present in the human population since the dawn of man. In fact, there is record of generalized seizures taking place 3,000 years ago. (Goldensohn, 1997) The term, "epilepsy" comes from the Greek word, "epilamvanein" meaning, "to be seized" or "to be attacked" (Engel & Pedley, 1997). The word, "epilepsy" comes from the idea that seizures are a result of being attacked by the gods. This was the only publicly professed cause of epilepsy until, in approximately 460 B.C.. Hippocrates postulated that epilepsy was a disease of the brain. Furthermore, he proposed that the treatment of epilepsy should consist of a change in diet and drugs rather than the religious methods that were widely practiced at the time (Engel & Pedley, 1997). Unfortunately, this profound insight into the source of seizures and what treatment methods may be effective in reducing their number and severity was lost on the world until centuries later (Goldensohn, 1997).

Approximately 2,200 years after Hippocrates hypothesized that epilepsy was a disorder of the brain an Italian scientist by the name of Luigi Galvani founded the field of electrophysiology (Goldensohn, 1997). Galvani, through over a decade of experiments, showed that electrical activity was present in animals. One experiment that worked to show this was one in which Galvani administered electrical stimulation to the crual nerve and observed muscle contraction in distal

leg muscles. However, like Hippocrates, Galvani's work was not be widely accepted for many (about 30) years after his death (Goldensohn, 1997).

In 1849 a book, entitled "Investigations on Animal Electricity", was written by Emil Du Bois-Reymond. In this two-volume book, a procedure for recording electric potentials from muscles was detailed (Goldensohn, 1997). With the foundation for electromyography firmly in place, a number of other scientists began to investigate the area and, approximately twenty-five years later, Richard Caton published a short paper describing his observation of "continuous spontaneous electrical activity of the brain" (Goldensohn, 1997). However, the technology at the time did not allow Caton to record the signals he observed. Unfortunately, such type of records would not be generated for another fifty years (Goldensohn, 1997).

The turn of the twentieth century saw an emergence of renowned clinicians that worked in the area of epilepsy. Dr. J. Houghlings Jackson, William Growers and Wilhelm Somner, among others worked to produce a plethora of important discoveries (Goldensohn, 1997). Arguably, the most significant of these discoveries was published in 1880 by Wilhelm Somner. He found that lesions in the hippocampus were the cause of some seizures. Additional credence was given to this result in 1889 when E. Bratz characterized hippocampal sclerosis. (Goldensohn, 1997) This was also the period of time that Camillo Golgi and Santiago Ramon y Cajal were performing their famous work on the structure of

the nervous system. This work led to the creation of the "Neuron Theory" and the 1906 Nobel Prize for Physiology and Medicine (Fishman, 2007).

Along with work done to determine the cause of epilepsy, there was also much done around this period to determine effective therapies. Potassium bromide was suggested as a therapeutic agent in 1857 by Sir Charles Locock. In his study, 14 of the 15 patients saw a reduction in seizures (Goldensohn, 1997). This was a significant breakthrough in the field of epilepsy research because up until this time there were no effective remedies available to those suffering from this disease. By 1861 bromide was widely used to this end. This lasted for approximately 60 years and was said to provide complete control in about 25% of patients and a 50% seizure reduction in 25% of patients. (Goldensohn, 1997) The drug that effectively took the place of bromide was phenobarbital as it was shown to have markedly reduced side effects. Then, in 1937, Tracy Putnam and H. Houston Merrit set out to find a more effective AED. This active search for an AED was the first of its kind and was based on three hypotheses:

1. "Effective anticonvulsants need not be soporific and that such compounds exist" (Goldensohn, 1997)
2. "Anticonvulsants will protect against experimentally induced seizures and indicate their clinical value." (Goldensohn, 1997)
3. Compounds with phenyl groups are good candidates because phenobarbital, with its phenyl ring, is the only barbiturate that is an effective anticonvulsant." (Goldensohn, 1997)

Armed with this set of ideas, Putnam and Merrit devised an experiment in which they induced seizures in cats and individually tested the efficacy of 19 phenyl group compounds. The results of the experiment seemed to indicate that diphenylhydantoinate would be of use in a clinical setting. It turns out that this drug does indeed work well to treat epilepsy (Goldensohn, 1997). In fact, it is still in use today under the commercial name Dilantin. More than just finding an effective AED, this study and its subsequent publications helped to confirm each of the three above stated hypotheses (Goldensohn, 1997).

While the aforementioned men were hard at work developing and testing effective AEDs there were many other scientists working to gain a deeper understanding of epilepsy through the investigation of the electrical activity of the brain. These investigations included an 1870 study by G. Fritsch and Julius Eduard Hitzig which showed that electrical stimulation of the brain could affect the state of one's body. Danilevsky, Marxow and Beck each independently continued this line of work and made such discoveries as alpha blocking, and continuous observation of rhythmic activity in the brain (Niedermeyer, Historical Aspects, 2005). This type of work was hamstringed by the fact that there was not yet a way to record the aforementioned electrical activity. This changed when in 1904 Napoleon Cybulski presented studies in which he examined EEG in graphical form. These studies also were the first in which EEG was recorded during a seizure (induced by electrical stimulation) (Niedermeyer, Historical Aspects, 2005). Approximately 16 years later, Hans Berger became the first person to

record EEG from humans and thus helped to usher in the era of modern neurology (Niedermeyer, Historical Aspects, 2005).

Current Clinical Understanding/Treatment Options

Epilepsy is one of the most common neurological disorders and affects approximately one percent of the world population. Some studies indicate that the prevalence of epilepsy may be as low as 0.5% while others state that the percentage could be as high as 5% (Niedermeyer, Epileptic Seizure Disorders, 2005). Temporal lobe epilepsy (by far the most common form of epilepsy) most frequently affects those in early/mid adulthood. While it is possible for epilepsy to affect those of all ages, it seems that "infancy and senium dilute the clinical and EEG semiology". (Niedermeyer, Epileptic Seizure Disorders, 2005) It should also be noted that epilepsy is equally common in all races and both sexes.

Epilepsy is not a disease but rather a condition and epileptic seizures can be caused by a wide range of diseases. Epileptic seizures are electrical disturbances in the brain and have a variety of clinical manifestations. Because the electrical disturbance can take place in the entire brain or just parts, there are various classifications of seizures (Niedermeyer, Epileptic Seizure Disorders, 2005). Seizures can be broadly classified into two categories: clinical and subclinical. (Westbrook, 2000) A clinical seizure is one that has a clinical manifestation, as opposed to a subclinical seizure which can only be detected through observation of the EEG. Seizures can be further classified by the extent of the brain that takes part in the seizure. Generalized seizures are those that affect large areas of the

brain, often affecting both hemispheres. This type of seizure typically occurs without a preceding aura and generally with no identifiable focus. Partial seizures are those that typically originate from a small area of the brain (focus). (Westbrook, 2000) In partial seizures, because only a small area of the brain is significantly affected, the location of the focus plays a large role in determining what the clinical manifestations will be. (Niedermeyer, Epileptic Seizure Disorders, 2005) Note that the aforementioned seizure classifications, while accurate, are not exhaustive.

As discussed earlier, in ancient times it was thought that epilepsy was a result of one being possessed by evil spirits. While we now know that this is not the case, many epileptic seizure disorders are idiopathic (without known cause). Before beginning discussing the causes of epilepsy it is crucial to point out that a single seizure does not warrant a diagnosis of epilepsy (Niedermeyer, Epileptic Seizure Disorders, 2005). Typically, one must have two or more independent seizure episodes before he/she is deemed, "epileptic". Despite the mystery that shrouds many such disorders there are many known causes. During the first two years of life the following are some of the most common causes of epileptic seizures: perinatal asphyxia, early central nervous system (CNS) infection, cerebral malformations, trauma and inborn errors of metabolism (Niedermeyer, Epileptic Seizure Disorders, 2005). Some of these causes also afflict those in the 3-20 age range (Niedermeyer, Epileptic Seizure Disorders, 2005). For those over age 21 the most common causes of epilepsy are: trauma, brain tumor, chronic

alcoholism, cerebral arteriosclerosis, and residual epilepsy due to an early CNS infection (e.g. cerebral palsy). (Niedermeyer, Epileptic Seizure Disorders, 2005). There are many causes of epileptic seizures with most not well understood; there are however, a few things that happen before/during seizures that are well established.

Immediately preceding an epileptic seizure a paroxysmal depolarizing shift (PDS) occurs. This shift dramatically changes the membrane potential of cortical as well as deep brain structures. In fact, this type of shift can result in a membrane potential changing from -85mV (a fairly standard resting potential) to +30mV (approximately 80mV higher than a typical threshold potential) (Niedermeyer, Epileptic Seizure Disorders, 2005). This drastic potential shift results in rapid bursts of neuronal spiking (the term, "spiking" here refers to the generation of an action potential of a single neuron as opposed to the spikes one may see on an EEG which are discussed below) (Niedermeyer, Epileptic Seizure Disorders, 2005). The cause of a PDS is still heavily debated. It is believed, however, that there may be a problem with the ion channels responsible for carrying out the electrical functions of the cell and maintaining a constant resting membrane potential. It is also thought that γ-Aminobutyric acid (GABA) may play a major role in causing a PDS (Niedermeyer, Epileptic Seizure Disorders, 2005). GABA is a crucial neurotransmitter that is responsible for depressing the excitability of neurons. Without GABA acting to provide negative feedback it is reasonable to think that a PDS is possible. With the anatomical/physiological predisposition for

PDS in place, an increase in the influx of afferent signals could cause a massive depolarization and consequently rapid bursts of neuronal spiking. It is still unclear as to whether the intracortical propagation of the seizure activity is across dendrites or somas (Niedermeyer, Epileptic Seizure Disorders, 2005). It is however clear that this propagation can result in relatively localized seizure activity becoming much more widespread throughout the brain.

As previously discussed, it is not accurate to think of epilepsy as a disease but rather a term used to describe the outcome (epileptic seizures) of a variety of diseases/conditions. For this reason the treatment of epilepsy is often focused on treating the seizures rather than some deeper root cause (treatment of the root cause does occur but would not typically be considered a treatment of epilepsy). AEDs for example, are primarily anticonvulsants and are usually effective at reducing seizure frequency but do little to nothing to halt epileptogenesis or cure the underlying disorder. Despite the obvious shortcomings of these drugs they are typically quite effective in the treatment of epilepsy. Levetiracetam, Zonisamide and Topiramate are three of the most recent and effective drugs to date (Shorvon, 2007). The administration of any of these three drugs results in a greater than 50% reduction in seizure frequency in approximately 25% of cases and "seizure freedom" (i.e. no seizures for at least 12 months) in an additional 25% of cases.

Like most drugs, however, there are side effects associated with each of these medications. These include: psychiatric or behavioral side effects (16% of those taking Topiramate), acute myopia, and "changes in hormonal levels" (Shorvon, 2007).

Unfortunately, the use of AEDs is ineffective for approximately 30-40% of those with epilepsy. This percentage amounts to approximately 300,000 people in the United States alone (Amar, Levy, & Liu, 2008). Assuming that there is a focus that generates seizures, and the clinicians and/or researchers are able to locate it with some degree of accuracy, a patient who does not respond to AEDs may be eligible for resective surgery. The focus or foci may be located using MRI techniques or through the analysis of EEG (Westbrook, 2000). Resective surgery is an inherently risky procedure and may not even be possible in patients where the focus is in a vital area of the brain. If however, all of the above criteria are met, namely the patient has focal seizures (as opposed to generalized), the focus is accurately located, the surgeon believes he/she is able to remove that portion of the brain without unreasonable adverse effects and the patient does not present any contraindications that would preclude surgery, resective surgery is highly successful. If the surgeon is able to remove the entire focus the patient typically exhibits no further seizures.

As one can glean from the above description, there are many conditions that must be met, including severe refractory epilepsy, for respective surgery to be recommended. It is estimated that between 200,000 and 270,000 people in the

United States suffer from refractory epilepsy but are not candidates for resective surgery (Amar, Levy, & Liu, 2008). For this group there is the option of vagus nerve stimulation (VNS). VNS was approved for use in the United States in 1997 and the company (Cyberonics) claims that over 40,000 patients have had a stimulator implanted and over 100,000 patient-years have be accrued (Amar, Levy, & Liu, 2008). This type of stimulator is typically implanted in the chest on the left side of the body. The lead is then fed subdurally up to the vagus nerve located in the neck. There a helical cuff electrode is placed around the vagus nerve. The implanted stimulator is capable of being programmed to the doctor's specifications from outside the body with a programming wand that comes with the device. Like medication, vagus nerve stimulation does not work for everybody but about 23% of patients saw a reduction in seizure frequency by 50% (Amar, Levy, & Liu, 2008). While this number is quite close to the results seen for many AEDs it should be noted that medication is typically the first treatment attempted and that those who opt for VNS have seen little or no results from the drugs. It is also worth pointing out that the longer VNS is administered the more effective it becomes. After three years 43% of patients note a 50% reduction in seizure frequency (Amar, Levy, & Liu, 2008).

Traumatic Brain Injury

While it is a bit outdated, the term "head injury" is still sometimes used to describe TBI. This term, while accurate, is far too broad to effectively portray the intended information. The term could just as well be used to describe a broken nose or a laceration on the face and provides no information as to whether or not

the brain has sustained damage. For this reason it is important to clarify what is meant when the phrase "head injury" is used.

Epidemiology

TBI is a major health concern as 180-250 of every 100,000 people per year in the United States seek medical attention for TBI. This figure does not take into account those that do not seek medical attention. In those younger than 45 years of age, injury is the primary cause of death, and in 1/3 to 1/2 of all traumatic deaths, TBI is the primary cause (Bruns & Allen, 2003). Those that are most at risk for suffering from TBI are those in early childhood, late adolescents/early adulthood and the elderly. It has been shown that males are uniformly at higher risk than females. This difference in risk is highest during adolescence and is about 2:1 at this time. (Bruns & Allen, 2003) Across all age groups, however, the ratio is closer to 1.5:1. It has also been shown that the incidence of TBI is higher among blacks than non-blacks. It is likely, however, that this difference has little to do with any inherent physiological difference but rather is a function of socioeconomic status (Bruns & Allen, 2003). In those areas where the average socioeconomic status is low violence related TBI is greatest. **Table 1** demonstrates the causes of TBI and their associated rate of occurrence.

Table 1. Distribution of Causes of Traumatic Brain Injury

(Bruns & Allen, 2003)

Causes of TBI	Transportation Related	Falls	Sports and Recreation	Firearms	Other
% of all TBI cases	50	20	10	6	14

Traumatic Brain Injury can cause numerous difficulties for those affected and their families. One such difficulty that may be encountered is post-traumatic epilepsy. The understanding of the development of epilepsy following traumatic brain injury is not well understood and is discussed in more detail below.

Classifications/Extent of Traumatic Brain Injury

There are two main types of TBI. The first is "penetrating injuries"; these are a result of a foreign body entering the brain (often times this foreign body is a bullet). These types of injuries are often localized and typically only cause damage to the areas of the brain through which the object travelled. Clearly, the signs, symptoms and repercussions of such an injury are highly dependent on the areas of the brain affected (American Speech-Language-Hearing Association, 2012).

The second classification of TBI is "closed head injuries". These types of injuries often occur as a result of a motor vehicle accident (MVA) or a fall. Because closed head injuries are far more common than penetrating injuries, the remainder of this section will focus on various aspects of closed head injury.

Upon sustaining a closed head TBI there are two types of brain damage that may occur. The first type is known as primary damage. This type of damage typically occurs within the first hour after TBI. The various forms of primary damage are detailed below in a list adopted from the American Speech-Language-Hearing Association. (American Speech-Language-Hearing Association, 2012)

- Skull fracture: breaking of the bony skull
- Contusions/bruises: often right under the location of impact or at points where the force of the blow has driven the brain against the bony ridges inside the skull
- Hematomas/blood clots: occur between the skull and the brain or inside the brain itself.
- Lacerations: tearing of the frontal (front) and temporal (on the side) lobes or blood vessels of the brain (the force of the blow causes the brain to rotate across the hard ridges of the skull, causing the tears)
- Nerve damage (diffuse axonal injury): arises from a cutting, or shearing, force from the blow that damages nerve cells in the brain's connecting nerve fibers

While the damage presented above may certainly present life threatening complications, a victory over these does not ensure a full recovery for the patient. Brain damage does not solely take place immediately following the insult but rather evolves over time. The later stages of this evolution are classified as secondary damage. Again, the American Speech-Language-Hearing Association has developed a list of various types of secondary damage (American Speech-Language-Hearing Association, 2012).

- Brain swelling (edema)
- Increased pressure inside of the skull (intracranial pressure)
- Epilepsy
- Intracranial infection
- Fever
- Hematoma
- Low or high blood pressure
- Low sodium levels
- Too much or too little carbon dioxide
- Abnormal blood coagulation
- Cardiac abnormalities
- Lung abnormalities
- Nutritional changes

It is secondary damage to the brain that is the leading cause of hospital deaths post TBI. Although there are many problems that can result from the aforementioned "evolution" of brain damage, swelling of the brain (i.e. edema) can cause the most damage. When the brain swells intracranial pressure builds. This increased pressure can lead to an ischemic condition in the brain (Ghajar, 2000). Without sufficient blood flow to the brain it goes into a state of oxygen deprivation and the neurons begin to die (Lipton, 1999). It is only a matter of minutes before oxygen deprivation to the brain has permanent consequences; as such, it is crucial to alleviate the intracranial pressure as soon as possible. The alleviation of pressure is discussed in more detail in the subsequent section.

To evaluate the extent of a TBI a common tool among neurologists and hospital staff is the Glasgow coma scale. This tool is used to score the patients on a scale from 3-15 based on their ability to open their eyes, motor response and verbal

response. **Table 2** provides the criteria by which Glasgow coma scores are given (Ghajar, 2000).

Table 2. Glasgow Coma Scale

(Ghajar, 2000)

Eye Opening	**Score**	**Motor Response**	**Score**	**Verbal Response**	**Score**
Spontaneous	4	Obeys	6	Oriented	5
To Speech	3	Localizes	5	Confused	4
To pain	2	Withdraws	4	Inappropriate	3
None	1	Abnormal Flexion	3	Incomprehensible	2
		Extensor Response	2	None	1
		None	1		

Typically, if a patient receives a score of 14 or 15 he/she can receive adequate care in an emergency room. A score of 9-13 warrants a trip to a trauma center while a score of less than 9 requires care in a trauma center with TBI resources (Ghajar, 2000).

Treatment

Effective treatment for TBI must start prior to arrival at the hospital if for no other reason than to transport the patient to a facility equipped to deal with the situation at hand. This treatment begins at the scene of the accident with an assessment of the injury(ies) sustained. An accurate assessment at the site of the injury is crucial for prehospital care, transportation to the appropriate facility as well as initial hospital treatment. On-scene treatment may consist of the administration of a short acting neuromuscular blockade followed by endotracheal intubation (the

neuromuscular blockade is to aid in the intubation) (Ghajar, 2000). Once intubated, ventilatory assistance is often required. The optimal rate of respiration is 10 breaths per minute (bpm) for adults, 20 bpm for children and 25 bpm for infants. In extremely severe cases, these rates may be increased. It is also common place to treat for shock (i.e. systolic blood pressure of <90 mm Hg whereas normal systolic blood pressure is 120mm Hg) (Ghajar, 2000). It has been shown that administration of hypertonic fluids immediately following TBI not only treats the immediate threat of shock but also may provide a survival advantage for those with severe injuries. (Ghajar, 2000) **Figure 1** depicts a flow chart that may be used by paramedics to provide accurate assessment and effective treatment of TBI.

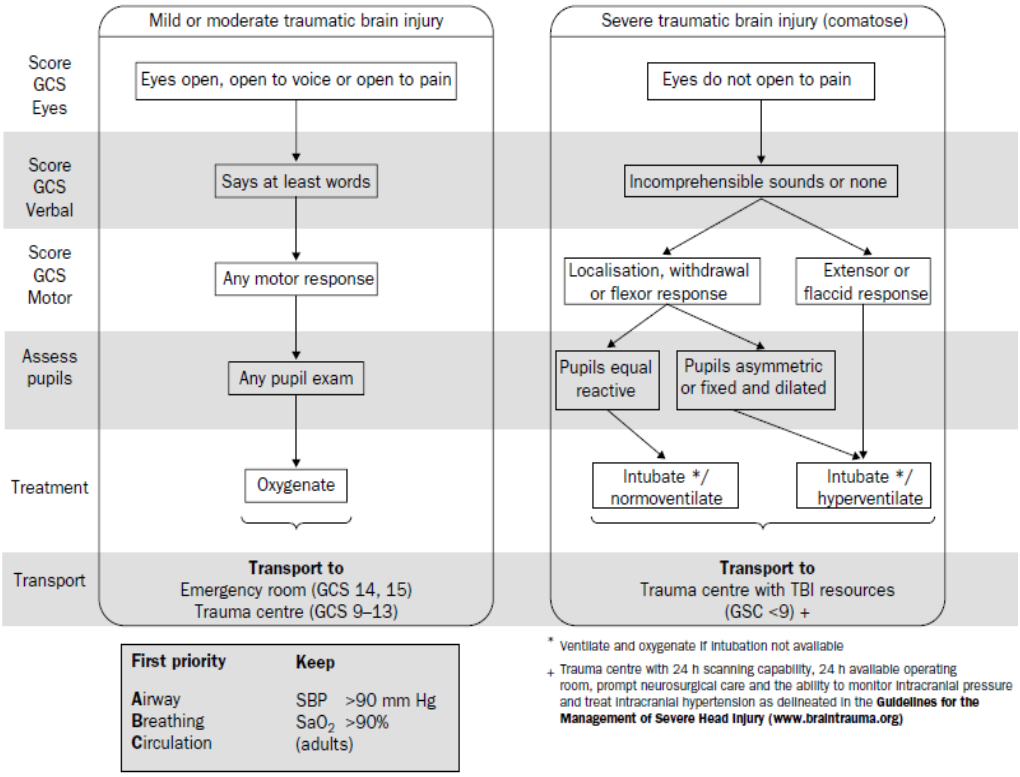

Figure 1. Triage of TBI patients (Ghajar, 2000)

It is often necessary to perform surgery immediately after a TBI patient has stabilized. Surgery may be performed if computed tomography (CT) scans provide evidence that there are mass lesions below the skull (Ghajar, 2000). If parenchymal hematomas are found it may be efficacious to resect them prophylactically. Resection of such abnormalities may aid in the reduction of intracranial hypertension and dramatically affect mortality rates. It has been shown, in fact, that resection of acute subdural hematomas greater than four hours after injury resulted in a 90% mortality rate. When the abnormality was resected earlier than four hours the mortality rate decreased to 70% (Ghajar, 2000).

Also critical to the treatment of TBI is the monitoring of intracranial pressure. As discussed above, edema and the intracranial pressure that comes with it, is the leading cause of secondary damage. Unfortunately, studies have found that only approximately 28% of U.S. hospitals do not practice regular intracranial pressure monitoring of TBI patients (Ghajar, 2000). This type of monitoring is done using a catheter fed into the ventricles and a pressure transducer connected outside the skull. This method of monitoring not only is cost-effective and sufficiently serves to monitor pressure, but also acts to relieve pressure itself. The normal range of intracranial pressure is 0-10 mm Hg (Ghajar, 2000). Treatment typically begins when the pressure reaches 20-25 mm Hg but may begin as soon as 15 mm Hg. While there are certainly complications that may arise from the aforementioned monitoring (approximately 6% of patients suffer from bacterial colonization and 1% from a significant hemorrhage (Ghajar, 2000)) the risks are minimal when compared with the benefit of a drastically reduced mortality rate. The treatment of increased intracranial pressure may involve draining of cerebrospinal fluid. If this alone is not effective, it may be necessary to use diuretics or hyperventilation (Ghajar, 2000). However, hyperventilation is not the optimal treatment as it has the risk of causing a hypocapnic state (low levels of carbon dioxide in the blood).

Post-Traumatic Epilepsy

Before delving into this topic it is important to be clear as to the definition used for PTE. Epilepsy in general can be defined as the occurrence of repeated unprovoked seizures. (Beghi, 2003) Epileptic seizures differ from acute seizures in that acute seizures occur soon after a “systemic, toxic, or metabolic insult”.

The occurrence of seizures immediately following TBI does not warrant the diagnosis of PTE. By definition, seizures that occur within 1 day of TBI are, "immediate seizures" and those that occur within 1 week are "early posttraumatic seizures". Each of these types of seizures is considered acute. Only when recurring seizures occur more than one week after TBI can the term PTE be applied.

Epidemiology

As discussed above, there are different types of TBI and estimation as to the level of severity is often relatively crude (Glasgow Coma Scale) when compared to various imaging techniques that are currently available. From these arise two issues that make broad-based epidemiological studies difficult. The first being that there are many types and severity of TBI and to effectively study each combination would take many subjects. This results in studies typically focusing on one type of TBI within one age group. The second issue is that despite a set of patients having been injured by the same mechanism, receiving the same Glasgow coma score and being the same age, sex, weight etc., the patients may still have very different injuries and certainly have very different anatomical/physiological organizations. This means that even if researchers attempt to study a very specific type/severity of injury the study population may not be homogenous.

Despite the aforementioned difficulties that may arise during epidemiological studies concerning PTE, there have been a large number of such endeavors. In large part due to the sheer number of potential subjects available during times of

war, many studies have included solely those injured during combat. **Table 3**, adopted from Frey, details results compiled from two such studies. It includes the nature of the injuries categorized as well as the percentage of individuals that developed PTE as a result (Frey, 2003).

Table 3. Incidence of PTE following various forms of TBI

(Caveness, Walker, & Ascroft, 1962) (Caverness, Meirowsky, & BL, 1979)

Series	Injury severity					
	Dura mater intact				Dura penetrated	
World War I	26.1%				47%	
World War II	—				43.4%	
Korea	23.8%				42%	
	Scalp or skull only		Single lobar injury		Multilobar injury or worse	
	Early	Late	Early	Late	Early	Late
Vietnam	3.3%	18.5%	3.5%	25.7%	4.6%	41%

As one may expect, the likelihood of developing PTE is related to the severity of the injury. It is also comforting to notice that (at least for these two studies) the reported chances of developing PTE given severe injury are similar across studies/conflicts. It is, however, necessary to note that TBIs obtained during military conflicts are typically more severe than those that civilians may obtain. Civilian rates of TBI range from approximately 1-25% while military rates may range from 25-50%. This idea is supported in **Tables 4 and 5** which show the age and population characteristics of the subjects as well as the length of study and the frequency of post- traumatic seizures (PTS) (Frey, 2003). Note that only those classified as having “late PTS” can be diagnosed as having PTE.

Table 4. Overview of studies concerning civilian post-traumatic epilepsy (Annegers, Grabow, & Groover, 1980) (Desai, Whitman, & Coonley-Hognson, 1983) (Jennet, 1975) (Haliner, Temkin, & Dikmen, 1997) (Hendrick & Harris, 1968) (Hahn, Fuchs, & Flannery, 1988)

Reference	Length of study	Overall frequency of PTSs	Frequency of early PTS	Frequency of late PTS	Population characteristics
Adults or mixed series					
(n = 4,541)	10+ yr	4.4%	2.6%	2.1%	Population based
(n = 702)	7+ days	–	4.1%	–	Hospital admissions
(n = 1,000)	1–4 yr	–	4.6%	5%	Hospital admissions
(n = 490)	5+ yr	–	16.9%	24.5%	Required intensive rehabilitation
Pediatric series					
(n = 4,465)	4–12 yr	7.03%	6.5%	1.3%	Pediatric hospital admissions
(n = 937)	7+ mo	9.8%	9.6%	–	Pediatric hospital admissions

Table 5. Overview of studies concerning military post-traumatic epilepsy (Caveness, Walker, & Ascroft, 1962) (Caveness W. , 1976) (Caverness, Meirowsky, & BL, 1979) (Weiss, Feeney, & Caveness, 1983) (Salazar, Jabbari, & Vance, 1985)

Reference	Length of study	Overall frequency of PTSs	Frequency of early PTSs	Frequency of late PTSs	Population characteristics
World War I (n = 317)	7–20 yr	34%	–	–	Gunshot wounds
World War II (n = 820)	5 yr	43%	–	–	Gunshot wounds
World War II (n = 739)	7–8 yr	28%	–	–	Unselected
Korean War (n = 356)	8–11 yr	30%	10% within first month	–	Unselected
Vietnam War (n = 1,030)	4+ yr	33.4%	4.4%	27.9%	Required neurosurgical attention
Vietnam War (n = 1,221)	4+ yr	35%	3.9%	31%	Required neurosurgical attention
Vietnam War (n = 421)	15+ yr	53%	–	–	Penetrating missile injuries

Beyond the severity of the injury, there are several other risk factors that have been identified as increasing ones likelihood of developing PTE. One such risk factor is the occurrence of early post traumatic seizures (Frey, 2003). These

seizures are classified as occurring within one week of the initial injury. It should be kept in mind, however, that this timeline was arbitrarily decided upon and it may be that the epileptogenic process occurs more rapidly (possibly within one week) in some individuals. If this is the case, then part of the reason that early post traumatic seizures seem to have some predictive value is that "full blown" epilepsy has already developed. Other risk factors include: acute intracerebral hematoma, brain contusion, posttraumatic amnesia lasting more than 24hrs. and being over the age of 65 at the time of the injury (Frey, 2003).

Treatment

There is no consensus among the epileptology community as to whether prophylactic anticonvulsant drug therapy ought to be offered to those who have suffered from TBI. Approximately 36% of clinicians do not provide prophylactic treatment to patients, 12% provide such treatment to all TBI patients and 52% decide on a case by case basis (Agrawal, Timothy, Pandi, & Manju, 2006). It has been shown that phenytoin is effective in reducing the number of post traumatic seizures. It is recommended, however, that phenytoin only be administered for the first week following TBI. This reduces the risk for any acute idiosyncratic reactions (Agrawal, Timothy, Pandi, & Manju, 2006). Only if the patient has early post traumatic seizures, "dural penetrating injuries, multiple contusions, and/or subdural hematoma requiring evacuation" should phenytoin be administered past the first week (Agrawal, Timothy, Pandi, & Manju, 2006). Although some anticonvulsants have proven effective in preventing early post traumatic seizures and there is thought that some may have neuroprotective

properties, there is no evidence that the prevention of early post traumatic seizures has any effect on a patient's prognosis (Agrawal, Timothy, Pandi, & Manju, 2006). Once it has been established that a person has PTE, their treatment is no different than any other epileptic patient.

Prediction

While there have been several attempts, there has been little success to accurately predict the patients that will develop PTE following TBI. This may be due, in part, to the fact that no study to date (excluding the study described in this thesis) has made use of quantitative analysis for prediction. The following sections discuss two of the more common methods of prediction and the results obtained from these methods.

EEG Based Prediction

The most recent study found that makes us of EEG in the prediction of PTE was by Jennet and Van de Sande in 1975. In this study, over 1,000 EEG recordings were analyzed from 722 subjects (Jennett & van de Sande, 1974). All subjects were followed for at least one year or until they had their first epileptic seizure. Each EEG recording was classified as either normal or abnormal. From this qualitative classification researchers attempted to test the predictability of PTE. **Table 6** shows the results of these methods (Jennett & van de Sande, 1974).

Table 6. EEG abnormalities in relation to development of PTE

(Jennett & van de Sande, 1974)

EEG	No late epilepsy (510) No.	%	With late epilepsy (391) No.	%	*p*
All abnormalities	349	68	282	72	NS
Local	266	52	193	49	NS
Diffuse	142	28	128	33	NS

The results displayed above provide evidence that prediction of PTE is not possible through a qualitative analysis of EEG. The results from this study were compiled in a number of different ways in an attempt to improve the accuracy of prediction. One such scheme looked at only those patients that had at least one normal recording within the first three months post injury. While the results do improve, this scheme focuses on only 184 of 722 subjects and still leaves much room for improvement. The results of this are shown in **Table 7** (Jennett & van de Sande, 1974). This table shows the fraction of patients in each category ("No late epilepsy" and "With late epilepsy") that were correctly classified based on EEG abnormalities.

Table 7. Patients with at least one "normal" recording in the first three months

(Jennett & van de Sande, 1974)

No late epilepsy	79/128	62%
With late epilepsy	11/56	20%
p	<0.001	

These results were disheartening to those in the field. This is evidenced by the following passages in the Jennet and Van de Sande paper: “Twenty years or so ago (this study was published in 1975) there were many claims for the value of EEG as a prognostic guide in traumatic epilepsy, but in the last 10 years there has been increasing doubt cast on its usefulness in practice.” and “The analysis of the present series lends weight to the view and indicates that the EEG does not contribute materially to the problem of predicting late epilepsy in individual patients.” (Jennett & van de Sande, 1974).

Mathematical Model-Based Prediction

A study by Feeney and Walker in 1979 presented a very simple mathematical model that could be used to aid in prediction of PTE (Feeney & Walker, 1979). The researchers analyzed the data from previous studies and fit a model to identify the risk factors and then use them to determine the likelihood that a patient develops PTE at a later point in their life. The formula to calculate this probability (P) is shown in Equation 1 below (Feeney & Walker, 1979).

Equation 1: Probability of developing PTE

$$P = 1.2 - (1 - \theta_1)(1 - \theta_2)(1 - \theta_3)(1 - \theta_4)(1.2 - .01)$$

where 1.2 a constant that was found to work well. The values of θ used are based on the four most significant risk factors that a patient may exhibit (see **Table 8)**. The most significant risk factors have the highest θ values (Feeney & Walker, 1979).

Table 8. Significant Risk Factors and corresponding value of θ (Feeney & Walker, 1979)

Risk Factors and Values of θ	
Factors	**θ***
Related to agent	
Missile wound and/or dura penetration[2, 5, 6]	.20
Related to severity of injury	
Loss of consciousness or post-traumatic amnesia of >1 hr[3-5]	.05
Linear skull fracture[4]†	.05
Depressed skull fracture[4]†	.10
Persisting general or focal EEG abnormality[3, 4]	.10
Relative to neurological status	
Hemiplegia, hemiparesis, or aphasia[2]	.20
Related to location of brain damage (contusion, puncture by missile, laceration, hemorrhage, or abscess, or evidence on neurological examination)‡ (A.E. Walker, unpublished data)‡	
Prefrontal area	.10
Centroparietal area	.25
Temporal area	.15
Occipital area	.10
Related to complications	
Hemorrhage (extradural, subdural, or intracerebral)[4]	.20
Infection (CNS)[3]	.10
Related to early fits	
Presence of seizures (general or focal) in first week after injury[3, 4]	.15

Based on the values of θ shown above the range of P is .01≤P≤.74. From this value of P the researchers added to the model so as to account for change in probability over time. The addition to the model is seen in Equation 2 (Feeney & Walker, 1979).

Equation 2: Probability that no seizures occur before the nth month

$$P_n = P(.925)^n$$

Where .925 is a constant that represents the likelihood that a person will go seizure free in any given month and n is the time in months since the injury (Feeney & Walker, 1979). This modification to the model allowed the researchers to make better comparisons between their model and previous studies in which patients were only followed for n months. The results of the mathematical model with this modification were fairly accurate as demonstrated in **Table 9**.

Table 9. Comparison of Mathematical Model with Previously Conducted Studies

(Feeney & Walker, 1979)

Walker and Jablon[3]			Russell and Davies-Jones[10]			Caveness et al[6]			Phillips[8]			Russell and Whitty[9]		
Observed	Calculated	AD	Observed	Calculated	AD	Observed	Calculated	AD	Observed	Calculated	AD	Observed	Calculated	AD
...	...	...	24	22	2	34	12	22	...	...	...	36	28	8
30	42	12							59	30	29			
33	33	0	54	51	3	21	29	8	27	24	3	84	65	19
30	26	4							16	19	3			
16	21	5	42	46	4	16	26	10	9	15	6	61	56	5
32	48	16	38	46	8	15	26	11	6	35	29	46	59	13
17	18	1				9	10	1	15	14	1			
10	7	3	15	28	13	14	7	7	8	5	3	21	36	15
5	3	2							5	3	2			
29	2	27	22	2	20	...	...	...				...	...	...
202		43	195		30	109		59	145		76	248		60

Despite their accuracy these results provide little/no new knowledge about the epileptogenic process (Feeney & Walker, 1979).

Brain Dynamics

The human brain has approximately 100 billion neurons with each of those neurons having thousands of synaptic junctions. The network the hundreds of trillions of connections make up is most certainly different for each of the nearly 7 billion people of the world. This does not take into account the physiologic conditions that play a role in brain function. To say that the problem of understanding the brain is complex is an understatement. Not only are the number of connections and physiologic possibilities astounding but the brain is also a dynamic and non-linear system. As it is impossible to collect enough data to determine the exact dynamics of the brain, any study with the aim to understand the dynamics ought to be through either mathematical modeling or a time series approach. Mathematical models have an advantage in that if a satisfactory model were produced it could be used to quickly develop many theories that could then be tested on real data. If, however, a time series approach is used then one must develop testable theories on their own. The problem, however, comes in developing a satisfactory model. In a dynamic and non-linear system as complex as the brain it is impossible to develop a set of equations that have an analytical answer in closed form. Without, such set of equations it is highly unlikely that a satisfactory mathematical model can be produced.

CHAPTER 3. MEASURES AND METHODS

Experimental

Ten male Sprague-Dawley rats, weighing approximately 375 grams each, were subjected to a controlled cortical impact (CCI). A 6mm piston was pneumatically driven 3mm into the right parietal cortex with velocity of 5.5m/s. The rats were subsequently implanted with 6 intracranial electroencephalographic (EEG) electrodes, in the left thalamus, hippocampus, parietal and frontal cortex, and right occipital and frontal cortex (no electrodes in the ipsilateral to the impact hippocampus and temporal cortex). Following a 2-week recovery period, long-term (14-week) continuous EEG recordings were conducted. The rats were provided with food and water daily and were kept in a climate controlled room in which a 12hr. light/dark cycle was in place. Recordings were interrupted once every four weeks so as to collect .5ml of blood per rat. Upon completion of the experiment the animals were euthanized and their brains harvested for further histological examination. The focus of this thesis is not the work done regarding the hematological or histological aspects of the study. We rather focused on the acquisition and analysis of EEG. **Figure 2** shows a rat head just after the EEG electrodes were placed. **Figure 3** shows a rat with the completed head cap through which the EEG signals travel. **Figure 4** shows a rat that has been connected to the EEG machine and is ready for recording.

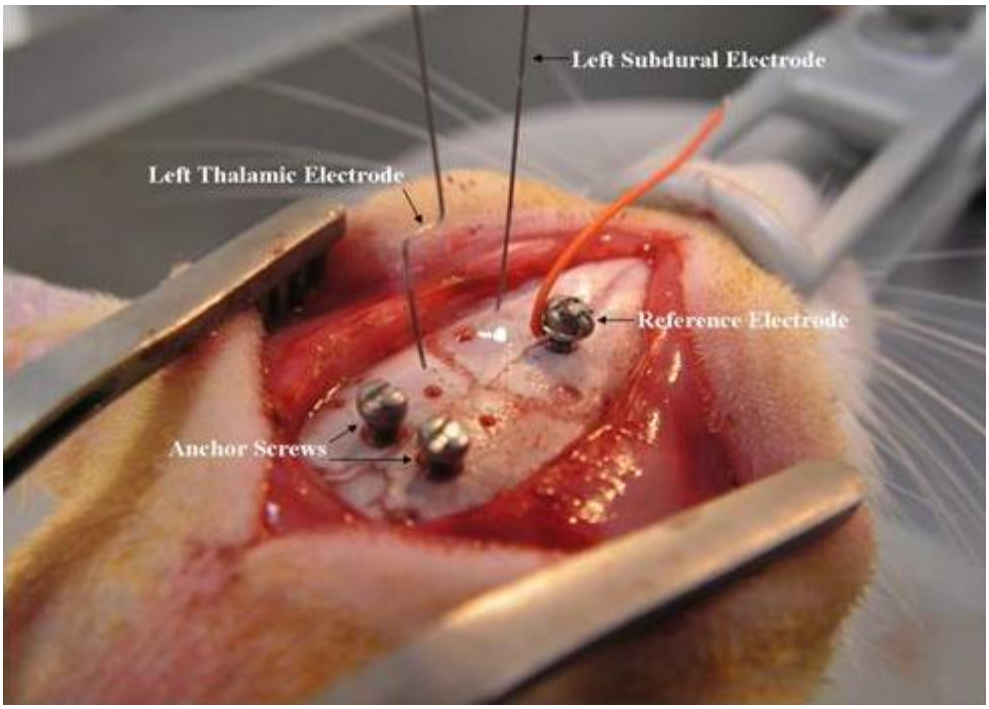

Figure 2 Rat mid-surgery showing placement of select electrodes

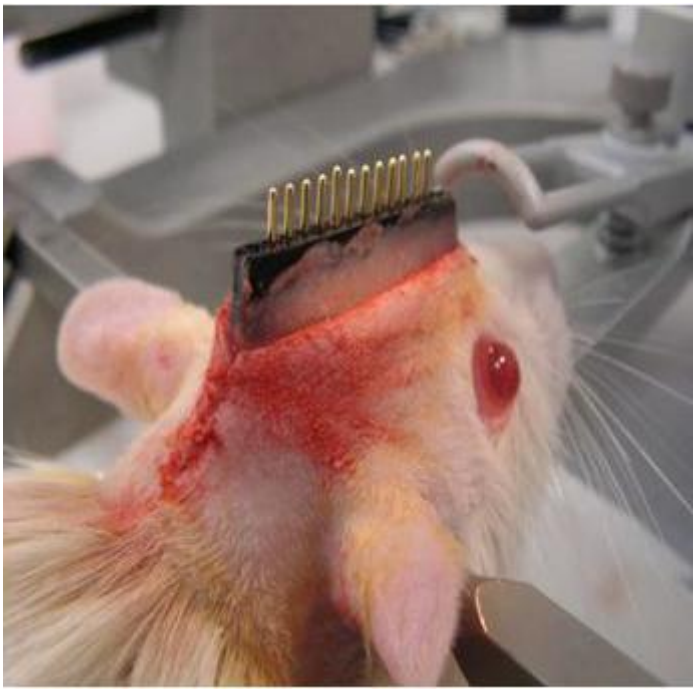

Figure 3 Rat mid-surgery showing completed headcap

Figure 4 Rat post-surgery ready for recording

The analog signals originating in the rat brain were low-pass filtered with a cut-off frequency of 70 Hz, pre-amplified and sampled at a sampling frequency of 256Hz. before reaching the PC of the XLtek EEG recording machine. There the signals were passed through a band-pass digital filter with cut-off frequencies of .3Hz and 70Hz as well as a notch filter (60Hz). The experimental protocol was approved by the local IACUC.

Prior to mathematical analysis, all data went through an additional zero-phase, 6^{th} order, digital Butterworth low-pass filter. The cut-off frequency for this filter was 40Hz.

Data Analysis

Dynamical Systems and Chaos

A dynamical system is one in which its states evolve over time. To better understand the temporal changes that take place in physical systems, mathematical models using differential equations are often used. Oftentimes

however, the dynamical systems that are studied are of a very high order and therefore extremely difficult or impossible to model with differential equations. This difficulty resulted in a lack of mathematical understanding of very complex dynamical systems such as the brain. For over 100 years mathematicians such as Poincare have studied and attempted to characterize the properties of nonlinear dynamic systems (Thietart & Forgues, 1995). Interest in such systems has grown in the last 30 years due to the ability to represent what once was thought as pure randomness or noise by non-probabilistic means (Thietart & Forgues, 1995).

This ability came about with the development and understanding of chaos theory. The definition of chaos, as given by Steven H. Strogatz, is (Strogatz, 1994):

"aperiodic long-term behavior in a deterministic system that exhibits sensitive dependence on initial conditions"

The first portion of this definition ("aperiodic long-term behavior") means that the trajectories of the system in state space do not approach fixed points or periodic orbits as time increases to infinity (Strogatz, 1994). The second portion ("deterministic") simply means that the system is non-random and that the irregular behavior is a result of the nonlinearity of the system rather than "noisy driving forces". The final part of the definition ("sensitive dependence on initial conditions") means that nearby trajectories diverge exponentially fast (Strogatz, 1994). Since the trajectories quickly diverge, starting from a minutely different point in state space (i.e. having slightly different initial conditions) will result in a vastly different trajectory. This idea was described well in a presentation given

by Edward Lorenz in 1972 titled, “Predictability: Does the Flap of a Butterfly’s Wings in Brazil Set Off a Tornado in Texas” (Lorenz, 1972).

Lyapunov Exponent

Since the EEGs have been shown to not merely reflect stochastic processes, and instead manifest deterministic chaos (Swiderski, Osowski, Cichocki, & Rysz, 2007). Lyapunov exponents can thus be used to measure this chaotic behavior. Furthermore, some studies suggest that Lyapunov values and their similarity across recording sites can be used to predict epileptic seizures (Iasemidis, 2003). It seems reasonable that a similar measure may be used to predict the onset of epilepsy. For this reason we calculated the Lyapunov exponent for every ten seconds of data in each channel recorded. To estimate how similar two Lyapunov time series are to one another we conducted a simple T-test using a ten minute moving window.

It is also hypothesized that prior to the first epileptic seizure following TBI cortical areas of the brain tend to be synchronized. The cross correlation between EEG channels provides information about this degree of synchrony in the underlying cortical areas and thus may provide predictive information.

Our goal was to use the aforementioned linear and non-linear measures of EEG dynamics and network connectivity to identify a distinct trend, difference or pattern that distinguishes the EEG signals of rats that eventually developed epilepsy from those that did not. We hoped to identify such distinguishing features a minimum of one week prior to the first epileptic seizure.

The first measure estimated was the maximum Lyapunov Exponent. The Lyapunov exponents give an indication as to how much chaotic the signal is (Pesin, 1977). A positive Lyapunov value indicates that the signal being analyzed is chaotic, while a Lyapunov value of zero indicates a periodic signal and a negative value corresponds to a signal ever-decreasing in amplitude (e.g. a decaying exponential).

Lyapunov values are calculated by first projecting the data into a higher dimension state space. For this study that dimension was 7. This was done by creating a 7-dimensional vector for each time point. The vector takes the form of that shown in **Figure 5,** where t is the current time point, x(t) is the signal analyzed and τ is the time delay chosen to be some positive number. For our analysis τ was chosen to be equal to 4 time points. For our analysis we chose non-overlapping EEG segments of 10 seconds in duration so that we have enough data points for accurate estimation of the Lyapunov exponent, and at the same time to allow for presence of non-stationarity in the data. From these vectors trajectories in the state space are reconstructed as time moves on. **Figure 5** displays a 7-dimensional epilepsy attractor (during a seizure) projected into 2-dimensions. (This projection was done only for the purpose of visualization here.) Nearest neighbors (in a Euclidean sense) can then be defined for each trajectory. The trajectory of x(t) as well as each of the nearest neighbors is tracked over a pre-determined short period of time. This is typically determined (and was for our analysis) by the following equation.

Equation 3:

$$\Delta t = (Dim-1) * \tau$$

Where Δt is the amount of time for which each trajectory is followed, τ is as previously discussed and Dim is the dimension of the reconstructed state space (Dim=7 here). With this equation we calculate that the number of points for which we ought to follow each trajectory (evolution time) was 24.

Upon the completion of this 24 point tracking, the distance between the original point and its nearest neighbor is calculated. This difference is compared with the distance between the two trajectories before they were allowed to evolve for 24 time points. As seen in **Figure 5**, if the distance between the two trajectories increases the argument inside the logarithm in the definition of the maximum Lyapunov exponent will be greater than one. Since Δt is always positive this results in a positive Lyapunov exponent.

Upon completing the estimation of all Lyapunov exponents over time (hundreds of thousands of 10 sec EEG segments in our case) per recording brain site, a simple T-test was conducted between the Lyapunov profiles of pairs of brain sites. (The results presented in the Results chapter do not include the Lyapunov exponents themselves but rather the T-index of said exponents.)

The average T-index values for every combination of pairs were also calculated. Because there were ten pairs of electrodes for which T-index was calculated and thousands of combinations of pairs whose T-index values were averaged together,

those pairs which include the RPV electrode (the electrode closest to the site of the impact) were the focus of the study. These pairs were chosen because it is thought that the focus of epileptic activity (in this case assumed to be the site of impact) may drive the activity of other brain sites resulting in high synchronization between the focus and all other brain sites (Sabesan, et al., 2009).

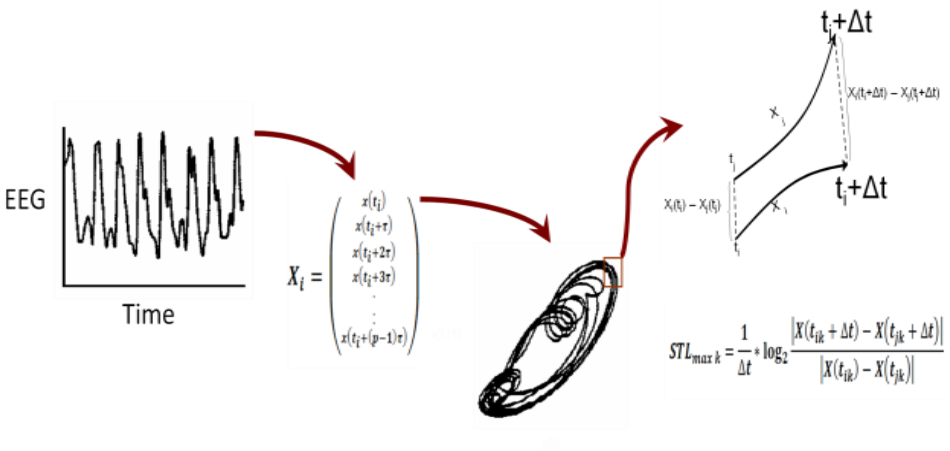

Figure 5. Depiction of the process by which the maximum Lyapunov exponents (STLmax) is estimated

Coherence

We also investigated the coherence between EEG from pairs of brain sites. Immediately before and during seizure the power spectra of EEG channels exhibiting the seizure discharge are similar. (Williamson, et al., 1998) It may be the case that prior to the onset of epilepsy coherence levels will rise. Estimating the Coherence between channels gives us this type of information as it determines the degree of correlation in the frequency domain. (Cabrerizo, Adjouadi, Ayala, Tito, & Lizarraga, 2009)

Coherence was calculated for each of six frequency bands. These bands are shown in **Table 10.**

Table 10. Frequency bands in the EEG for which coherence was calculated.

Band 1	.1-3 Hz
Band 2	3.5-7.5 Hz
Band 3	8-12 Hz
Band 4	13-20 Hz
Band 5	21- 30 Hz
Band 6	.1-30 Hz

The equation that can be used to calculate coherence is as follows.

Equation 4:

$$Coh_{xy}(j\omega) = \int R_{xy}(\tau) * e^{-j\omega t} d\tau$$

which is simply the Fourier transform of the cross correlation $R_{xy}(\tau)$. It can be easily shown that equation 4 reduces nicely to the following:

Equation 5:

$$R_{xy}(j\omega) = X(j\omega) * Y^{*}(j\omega)$$

where X(jω) is the Fourier transform of the signal x(t) and $Y^{*}(j\omega)$ is the complex conjugate of the Fourier transform of the signal y(t).

Similar to T-index the coherence was calculated for all pairs and the average of all combination of pairs was also calculated. Again the pairs with RPV were the

focus of the study. The focus was also narrowed in that the frequency band that has been shown to exhibit high levels of synchrony during seizures (8-12 Hz) was focused on (Ferri, et al., 2004).

K-Means Clustering

The K-means algorithm is one that works to cluster points in m-dimensional space. It is an unsupervised classification algorithm. The process begins with the user inputting a homogeneously described collection of data points and the number of sub-populations (k) in which the data are to be divided. Then k points (centroids) are randomly selected in m-dimensional space (m is the number of parameters used to describe each point to be classified). The algorithm consists of two primary steps (Davidson, 2002). During the first step each point is assigned to the closest centroid. The seconds step consists of a re-estimation of the centroid locations to the center of the newly developed clusters. These two steps are repeated until convergence occurs. This is when the centroid locations remain relatively unchanged. The algorithm works to minimize the point-to-centroid distances over all classes (Davidson, 2002). The sum of these distances is also known as distortion or vector quantization error. This error is mathematically expressed in Equation 6 below:

Equation 6:

$$E = \sum_{j=1}^{K} \sum_{i=1}^{N} \sum_{S_i \in C_j} \left| S_i - w_{Class(S_i)} \right|^2$$

Where k classes divide the instances into sub-populations denoted by C, w represents the centroid locations and S is the elements that are to be clustered.

While the algorithm itself is quite simple, there are a number of notes of caution that ought to be considered prior to implementation of this algorithm.

The first such note pertains to the "use of exclusive assignment". This exclusive assignment means that even if an instance could belong to two different clusters it must be assigned to only one (Davidson, 2002). Another ramification of this hard assignment is that overlapping classes cannot be accurately grouped and the centroids of these classes will be skewed from the true centers. Consider Figure 6 below as an illustration of this point (Davidson, 2002). Figure 6 shows two Gaussian distributions. Each distribution has a standard deviation of one. The mean of the first distribution is zero while the mean for the second distribution is two.

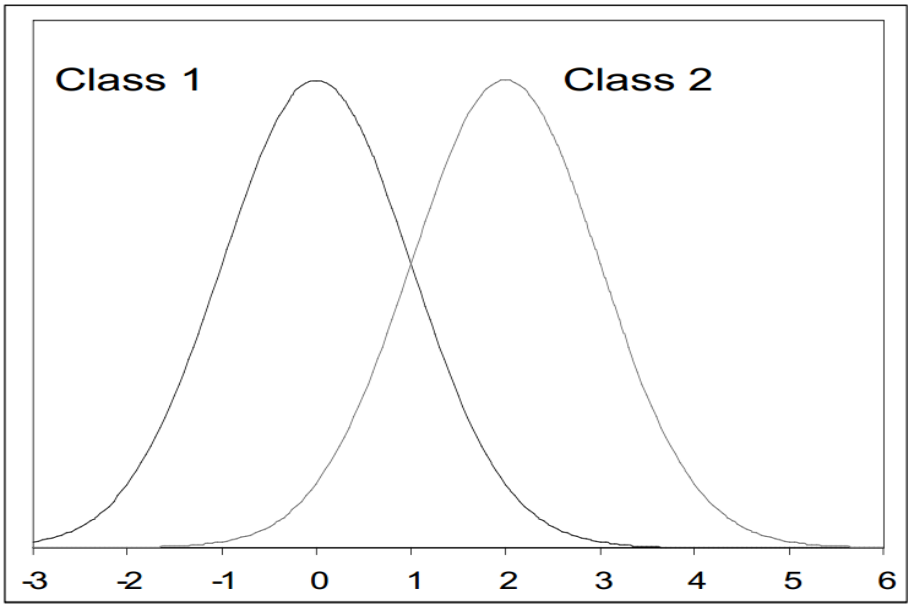

Figure 6. Two Overlapping Classes

(Davidson, 2002)

Those instances that fall into the right hand tail of the first class will be classified incorrectly into the second class (Davidson, 2002). Incorrect classification will also take place for those instances that fall in the left hand tail of class two. Not only will there be many misclassifications, but the centroid positions of the two classes will not reflect the means of the true distributions. The mean of the first class will be under-estimated while the mean of the second class will be overestimated (Davidson, 2002). These problems that arise from overlapping classes may be overcome by better choosing the attributes used to describe each instance. For example, adding an additional dimension to the situation described in Figure 6 may cause the distributions to no longer overlap.

Another note of caution relates to algorithm being "inconsistent". This "inconsistency" can be thought of in the following way: In what situations would the point to centroid distances be smallest (or zero)? This would occur when the number of classes is equal to the number of instances to be classified. Each instance would also be a cluster centroid (Davidson, 2002). Looking at this extreme case helps to illustrate that the more classes to be created the lower the point to centroid distance can be and the more accurate the clustering may seem. This problem makes comparison of clustering results across number of clusters created impossible. For this reason it is extremely difficult to determine the true number of clusters in a data set using K-means.

As discussed above, the K-means algorithm continues to update the centroids of the clusters until convergence occurs. This means that the function described in Equation 6 has reached a local minimum (Davidson, 2002). It is quite possible

and in fact, probable that the algorithm has determined the clustering based on the global minimum for any give iteration. This issue may be mitigated by running the algorithm multiple times and saving only the clustering with the lowest point to centroid distance (Davidson, 2002).

The K-means algorithm was used so as to investigate the activity of the thousands of combinations of pairs, frequency bands, and measures that could not be examined individually. For each combination of pair, frequency band and measure two parameters were calculated and given as input to the algorithm:

1) A line was fit to the smoothed data and the slope of that line was calculated.
2) The values of the data were averaged over the first week and over the last week of recording. The percentage change from beginning to end was then calculated.

With these inputs the K-means would be tasked with clustering the rats into two groups. The clusters that correctly classified the most rats as either epileptic or non-epileptic are displayed in the results section below. It should be noted that despite correct classification of all rats for both T-index and coherence, there is no obvious physiological basis for the combination of pairs and frequency band selected by the algorithm.

CHAPTER 4. RESULTS

Rat 1

Rat 1 developed PTE and, as such, we expected to see increasing levels of functional connectivity before the development of epileptic seizures.

Lyapunov Exponent results

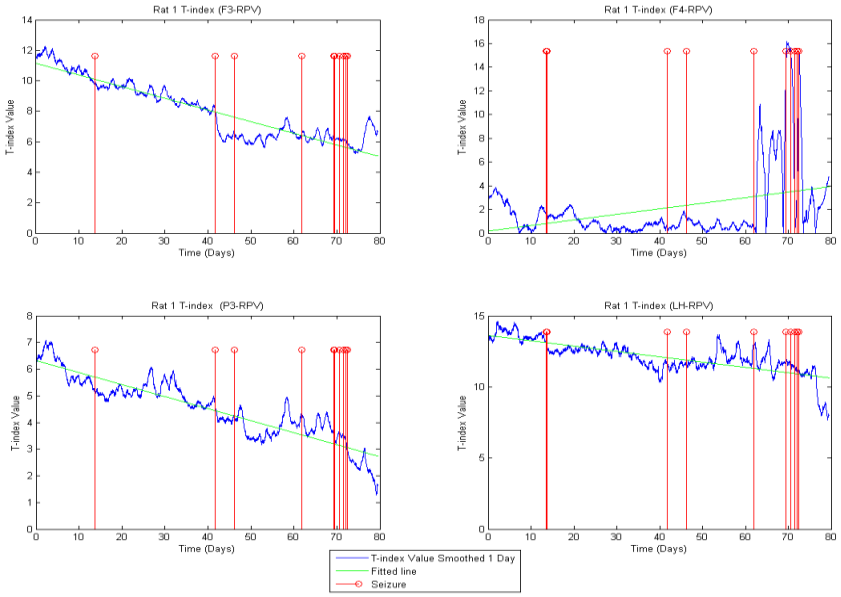

Figure 7. T-index of Lyapunov Exponents plotted over time

Table 11. Quantification of slope and statistical significance of change at the end vs the beginning of the EEG recording based on T-index of Lyapunov exponents as a measure of functional connectivity

Rat 1	Slope	Slope<0	Coefficient of Determination	T-test p-value	Rank sum p-value
F3-RPV	-.0765	Y	0.86	<.001	<.001
F4-RPV	.047	N	0.13	0.245	0.499
P3-RPV	-.0451	Y	0.83	<.001	<.001
LH-RPV	-.0374	Y	0.58	<.001	<.001

*The p-values were obtained by sampling one point every 4 hours from both the first and last week of recording and performing a Student's T-test as well as a Mann Whitney U-test (Rank sum) on the distributions.

Coherence results

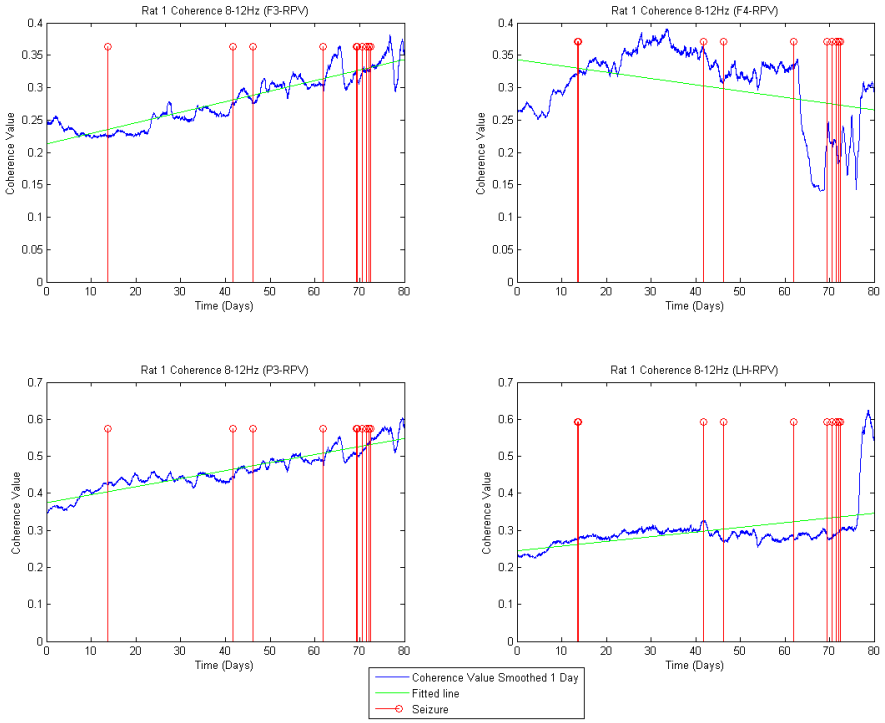

Figure 8. Coherence plotted over time

Table 12. Quantification of slope and statistical significance of change at the end vs the beginning of the EEG recording based on Coherence as a measure of functional connectivity

Rat 1	Slope	Slope>0	Coefficient of Determination	T-test p-value	Rank sum p-value
F3-RPV	.0016	Y	.86	<.001	<.001
F4-RPV	-.0010	N	.11	.037	.418
P3-RPV	.0021	Y	.88	<.001	<.001
LH-RPV	.0013	Y	.23	<.001	<.001

Rat 3

Rat 3 developed PTE and, as such, we expect to find increasing levels of functional connectivity.

Lyapunov Exponent results

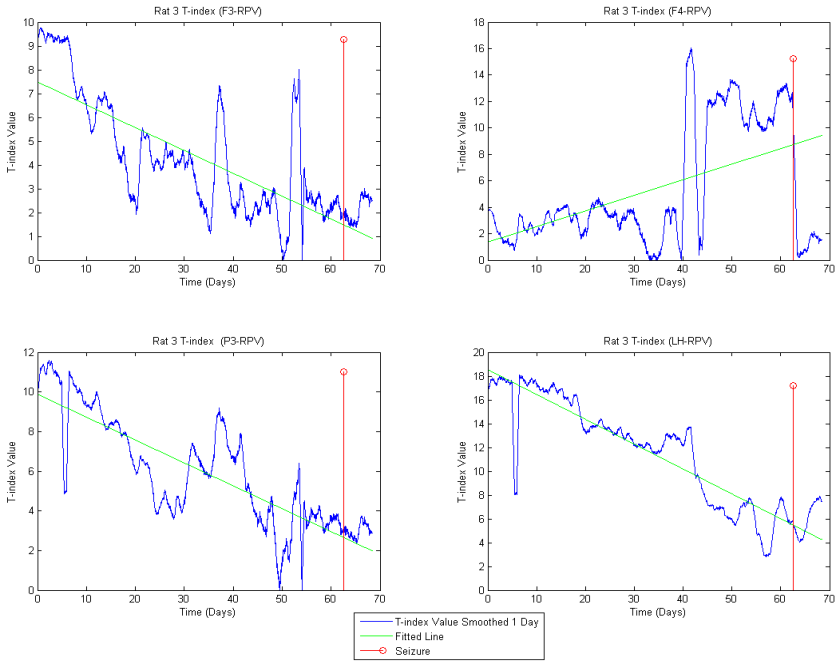

Figure 9. T-index of Lyapunov Exponents plotted over time

Table 13. Quantification of slope and statistical significance of change at the end vs the beginning of the EEG recording based on T-index of Lyapunov exponents as a measure of functional connectivity

Rat 3	Slope	Slope< 0	Coefficient of Determination	T-test p-value	Rank sum p-value
F3-RPV	-0.096	Y	0.59	<.001	<.001
F4-RPV	0.118	N	0.27	0.065	0.277
P3-RPV	-0.1154	Y	0.69	<.001	<.001
LH-RPV	-0.2089	Y	0.84	<.001	<.001

Coherence results

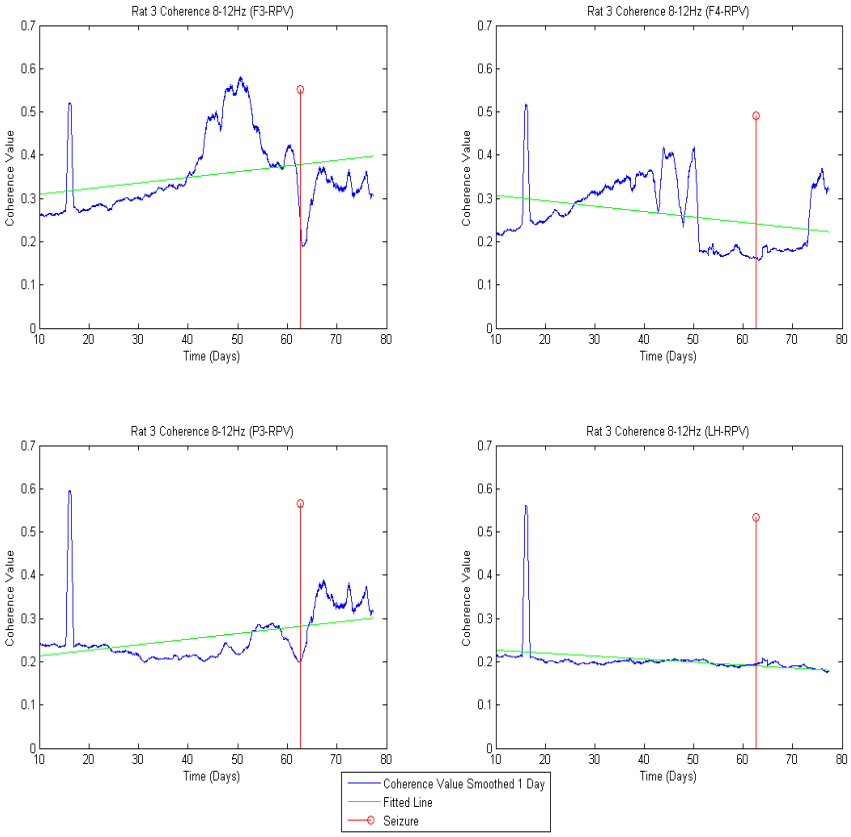

Figure 10. Coherence plotted over time

Table 14. Quantification of slope and statistical significance of change at the end vs the beginning of the EEG recording based on Coherence as a measure of functional connectivity

Rat 3	Slope	Slope> 0	Coefficient of Determination	T-test p-value	Rank sum p-value
F3-RPV	.0013	Y	.08	.049	<.001
F4-RPV	-.0012	N	.09	.76	.89
P3-RPV	.0013	Y	.17	.006	<.001
LH-RPV	-.0007	N	.11	<.001	<.001

Rat 6

Rat 6 developed PTE and, as such, we expect to see increasing levels of connectivity.

Lyapunov Exponent results

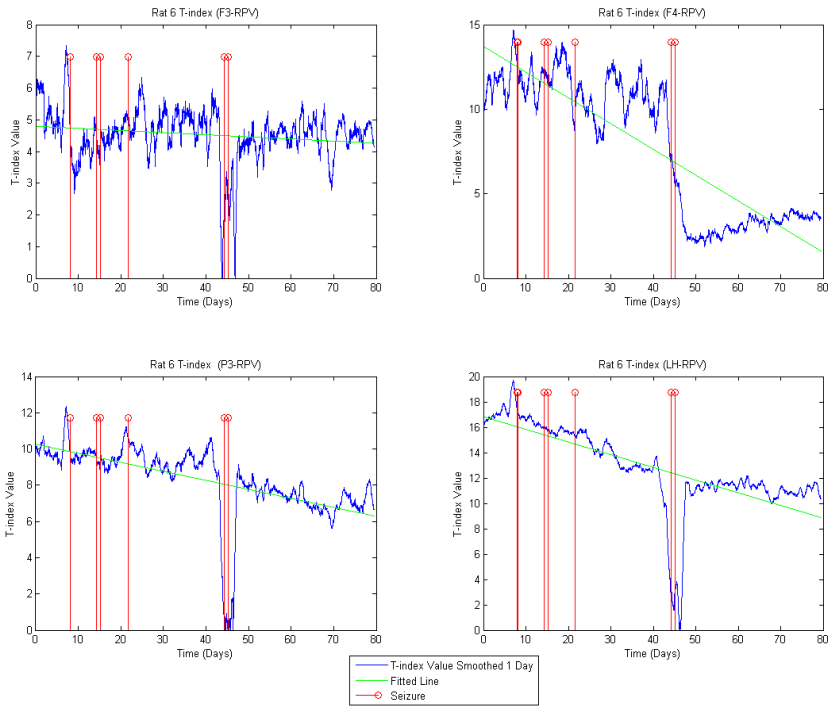

Figure 11. T-index of Lyapunov Exponents plotted over time

Table 15. Quantification of slope and statistical significance of change at the end vs the beginning of the EEG recording based on T-index of Lyapunov exponents as a measure of functional connectivity

Rat 6	Slope	Slope< 0	Coefficient of Determination	T-test p-value	Rank sum p-value
F3-RPV	-0.0067	Y	0.03	<.001	<.001
F4-RPV	-0.1523	Y	0.74	<.001	<.001
P3-RPV	-0.0497	Y	0.34	<.001	<.001
LH-RPV	-0.0999	Y	0.5	<.001	<.001

Coherence Results

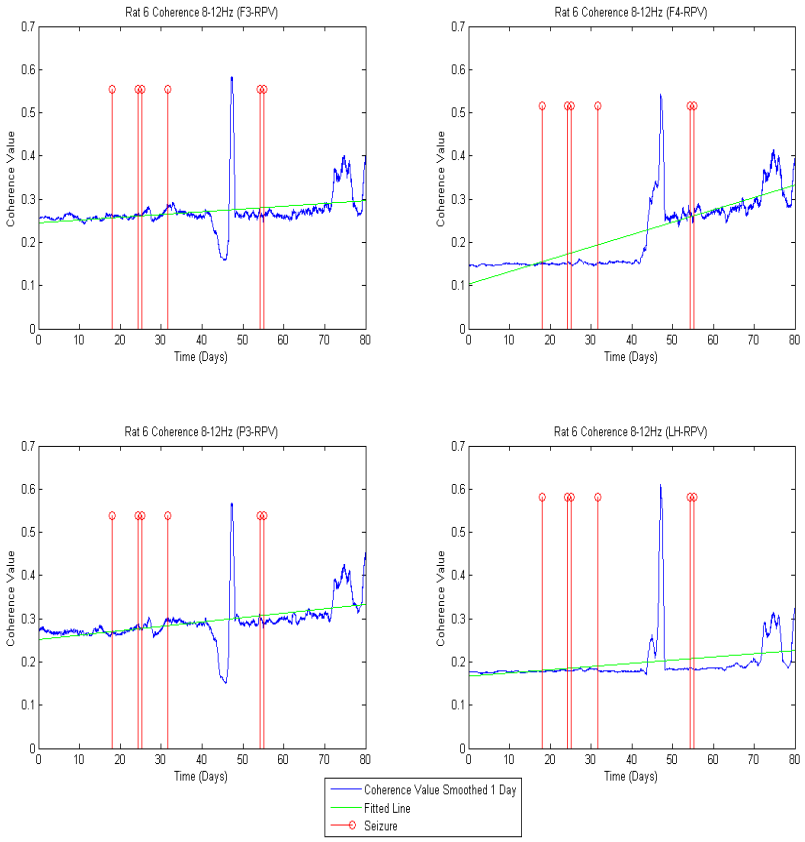

Figure 12. Coherence plotted over time

Table 16. Quantification of slope and statistical significance of change at the end vs the beginning of the EEG recording based on Coherence as a measure of functional connectivity

Rat 6	Slope	Slope> 0	Coefficient of Determination	T-test p-value	Rank sum p-value
F3-RPV	.0006	Y	.11	<.001	<.001
F4-RPV	.0029	Y	.67	<.001	<.001
P3-RPV	.0010	Y	.25	<.001	<.001
LH-RPV	.0007	Y	.12	<.001	<.001

Rat 7

Rat 7 did not become epileptic, and as such, we do not expect to see increasing levels of functional connectivity.

Lyapunov Exponent results

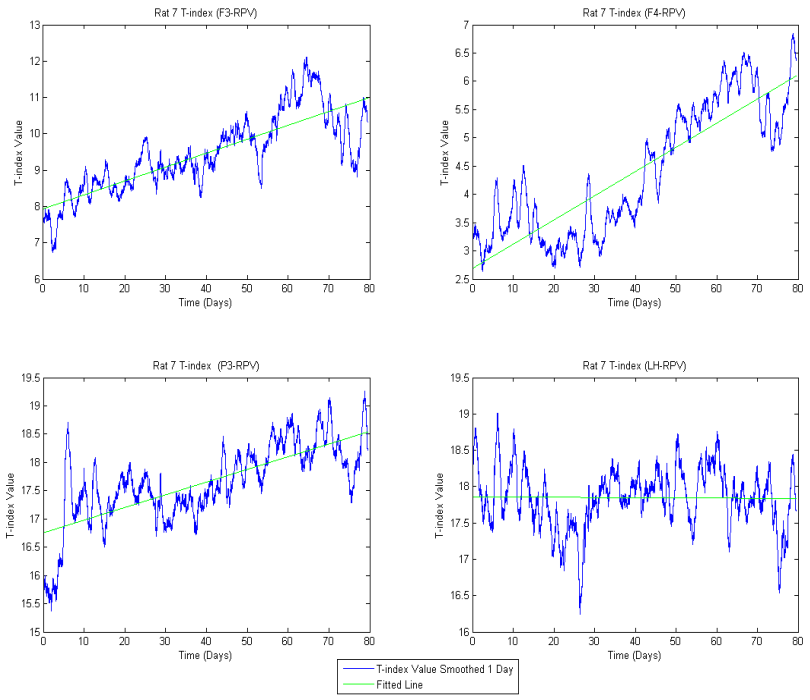

Figure 13. T-index of Lyapunov Exponents plotted over time

Table 17. Quantification of slope and statistical significance of change at the end vs the beginning of the EEG recording based on T-index of Lyapunov exponents as a measure of functional connectivity

Rat 7	Slope	Slope< 0	Coefficient of Determination	T-test p-value	Rank sum p-value
F3-RPV	0.0383	N	0.67	<.001	<.001
F4-RPV	0.0428	N	0.75	<.001	<.001
P3-RPV	0.0224	N	0.54	<.001	<.001
LH-RPV	-0.2923	Y	0.26	0.008	0.043

Coherence results

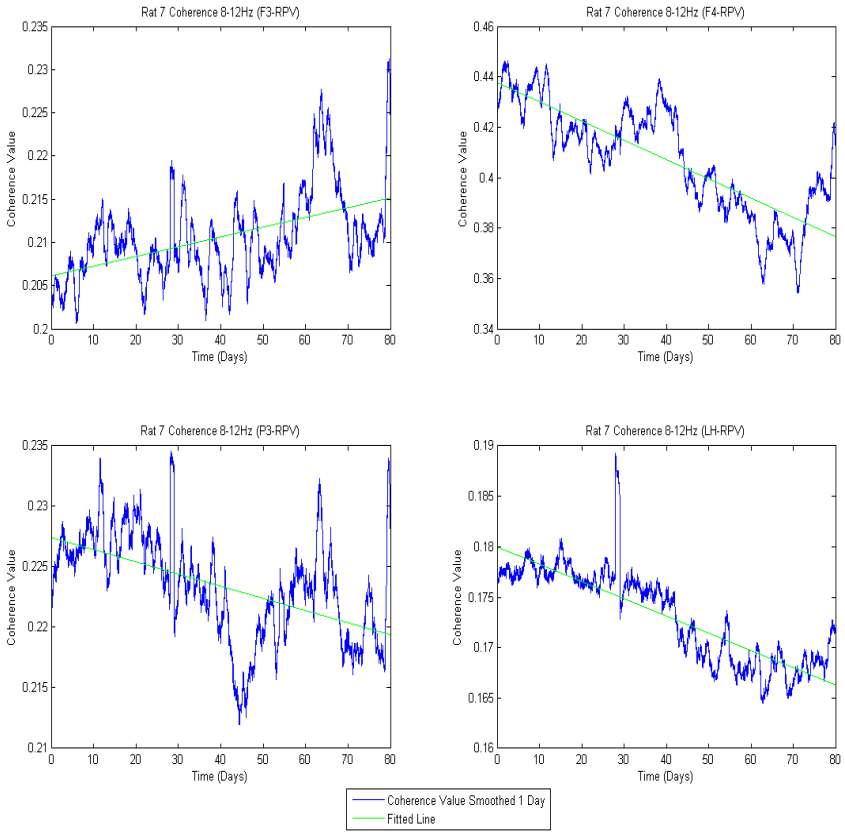

Figure 14. Coherence plotted over time

Table 18. Quantification of slope and statistical significance of change at the end vs the beginning of the EEG recording based on Coherence as a measure of functional connectivity

Rat 7	Slope	Slope> 0	Coefficient of Determination	T-test p-value	Rank sum p-value
F3-RPV	.0001	Y	.26	<.001	<.001
F4-RPV	-.0008	N	.67	<.001	<.001
P3-RPV	-.0001	N	.28	<.001	<.001
LH-RPV	-.0002	N	.73	<.001	<.001

Rat 8

Rat 8 did not develop PTE and, as such, we do not expect to see increasing levels of functional connectivity.

Lyapunov Exponent results

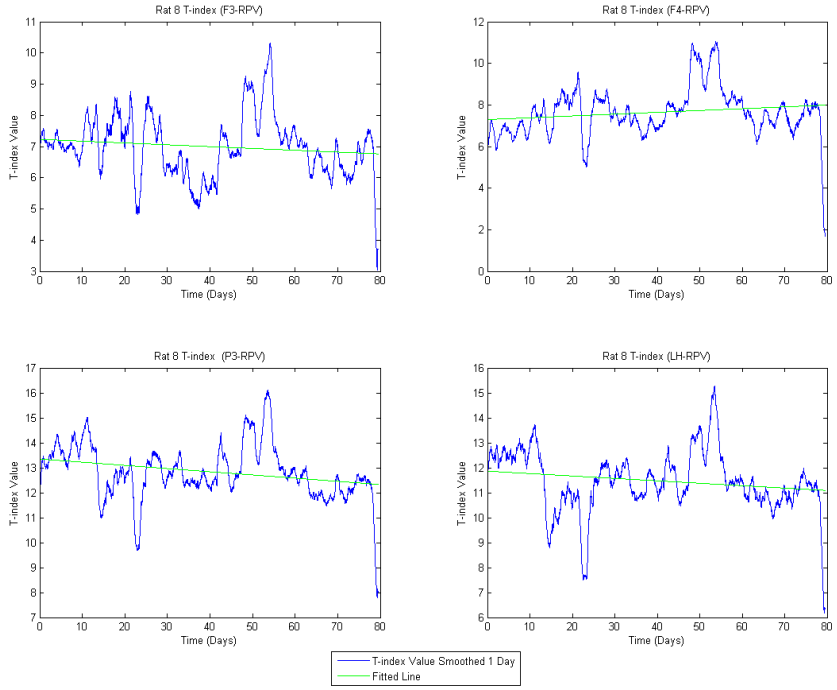

Figure 15. T-index of Lyapunov Exponents plotted over time

Table 19. Quantification of slope and statistical significance of change at the end vs the beginning of the EEG recording based on T-index of Lyapunov exponents as a measure of functional connectivity

Rat 8	Slope	Slope< 0	Coefficient of Determination	T-test p-value	Rank sum p-value
F3-RPV	-0.0058	Y	0.02	<.001	<.001
F4-RPV	0.0087	N	0.03	.070	<.001
P3-RPV	.0087	N	.03	.070	<.001
LH-RPV	-.0097	Y	.03	<.001	<.001

Coherence results

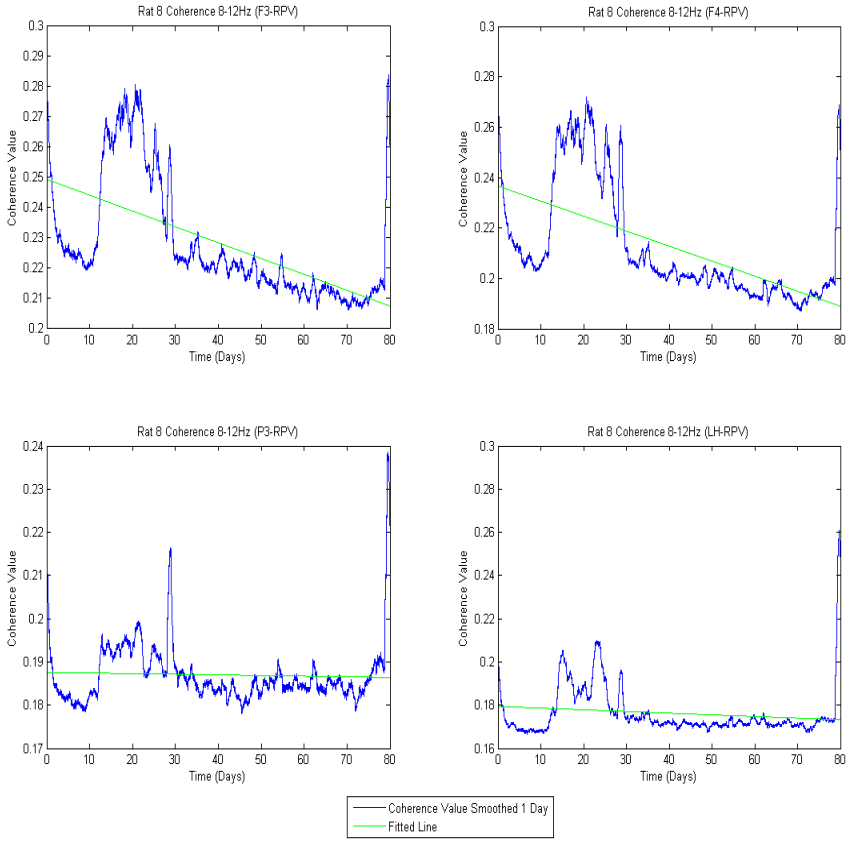

Figure 16. Coherence Values plotted over time

Table 20. Quantification of slope and statistical significance of change at the end vs the beginning of the EEG recording based on Coherence as a measure of functional connectivity

Rat 8	Slope	Slope> 0	Coefficient of Determination	T-test p-value	Rank sum p-value
F3-RPV	-.0005	N	.36	<.001	<.001
F4-RPV	-.0006	N	.36	<.001	<.001
P3-RPV	-.0000	N	.00	.003	<.001
LH-RPV	-.0001	N	.02	.023	<.001

Rat 12

Rat 12 developed PTE and, as such, we expect to see increasing levels of functional connectivity.

Lyapunov Exponent results

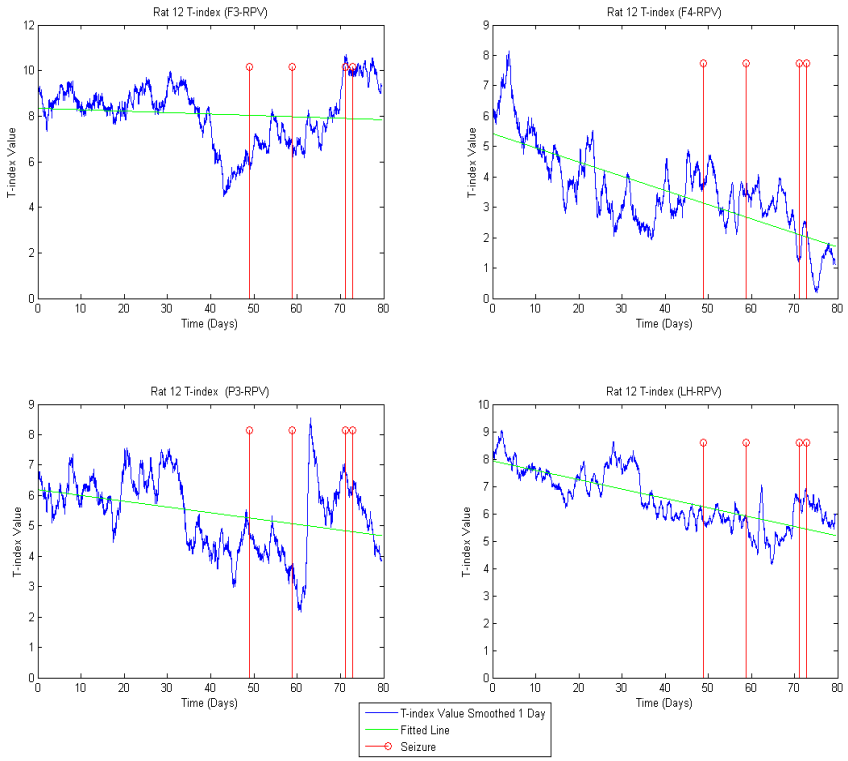

Figure 17. T-index of Lyapunov Exponents plotted over time

Table 21. Quantification of slope and statistical significance of change at the end vs the beginning of the EEG recording based on T-index of Lyapunov exponents as a measure of functional connectivity

Rat 12	Slope	Slope< 0	Coefficient of Determination	T-test p-value	Rank sum p-value
F3-RPV	-.0062	N	.01	<.001	<.001
F4-RPV	-.0467	N	.57	<.001	<.001
P3-RPV	-.0190	N	.13	<.001	<.001
LH-RPV	-.0342	N	.60	<.001	<.001

Coherence results

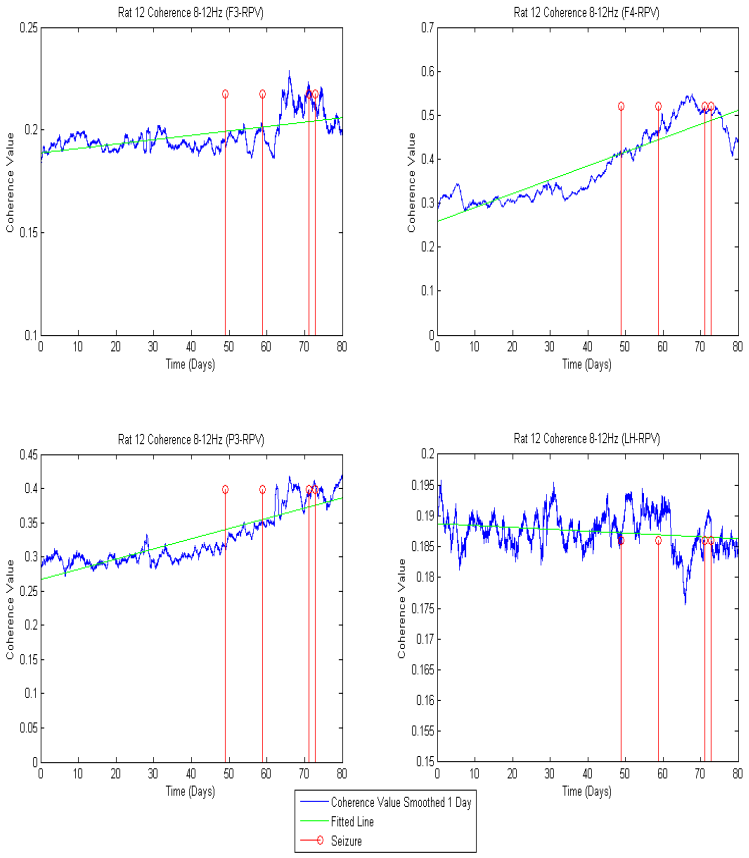

Figure 18. Coherence Values plotted over time

Table 22. Quantification of slope and statistical significance of change at the end vs the beginning of the EEG recording based on Coherence as a measure of functional connectivity

Rat 12	Slope	Slope >0	Coefficient of Determination	T-test p-value	Rank sum p-value
F3-RPV	.0002	Y	.37	<.001	<.001
F4-RPV	.0032	Y	.82	<.001	<.001
P3-RPV	.0015	Y	.78	<.001	<.001
LH-RPV	-.0000	N	.05	<.001	<.001

K-Means Classification

Based on Lyapunov exponent values

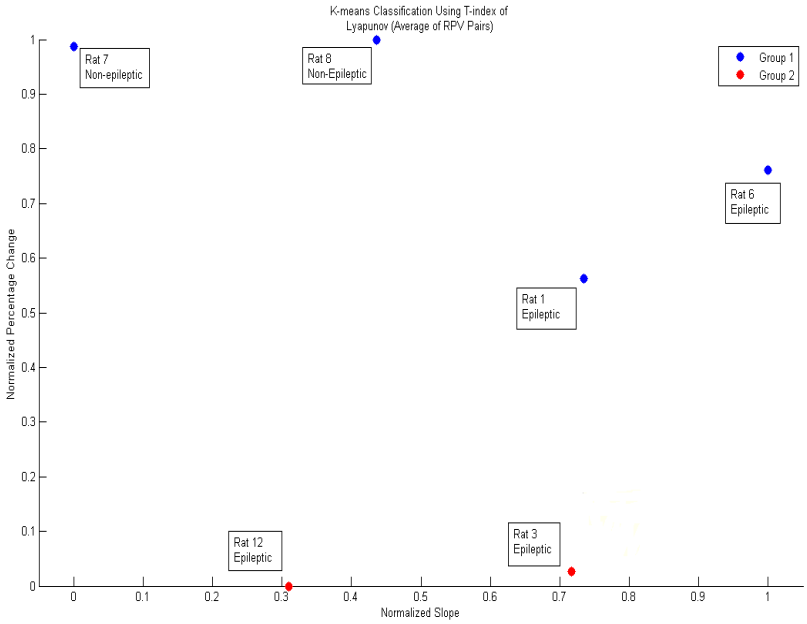

Figure 19. K-Means Classification of Rats using T-index values and only pairs involving the impact site (that is, all possible 4 pairs with RPV)

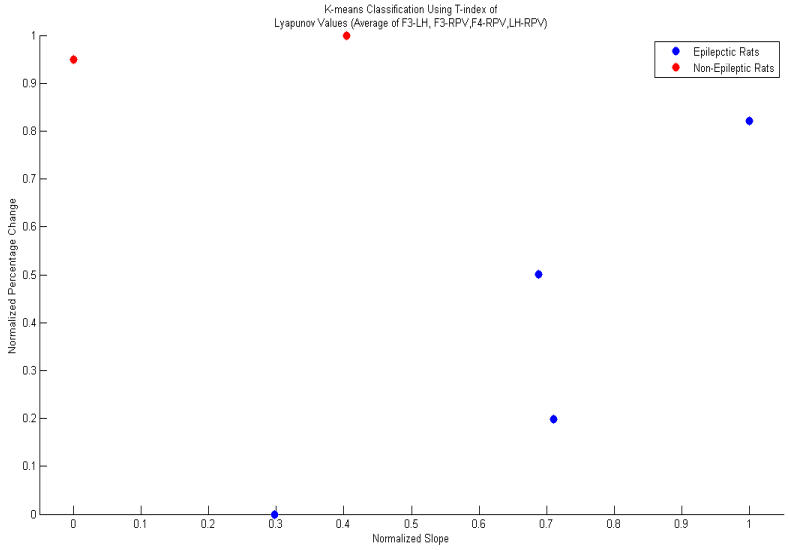

Figure 20. K-Means Classification of Rats using T-index values and all pairs selected by the algorithm for optimal classification (F3-LH, F3-RPV, F4-RPV, LH-RPV)

Based on Coherence values

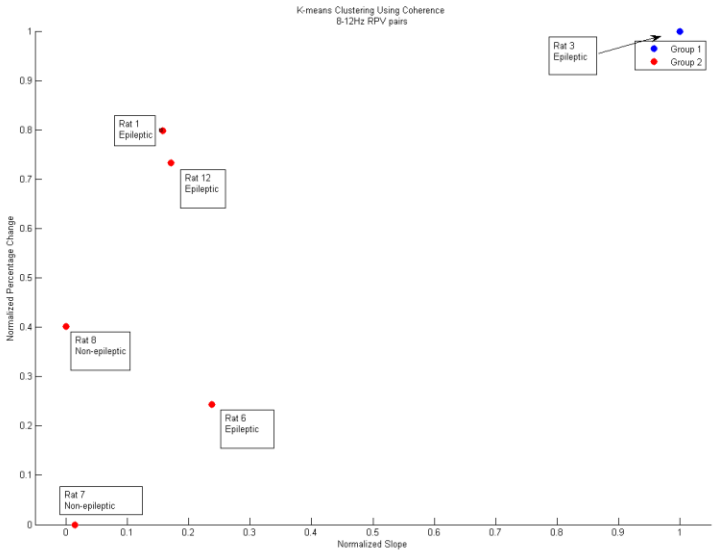

Figure 21. K-Means Classification of Rats using Coherence values and only pairs involving the impact site (that is, all possible 4 pairs with RPV)

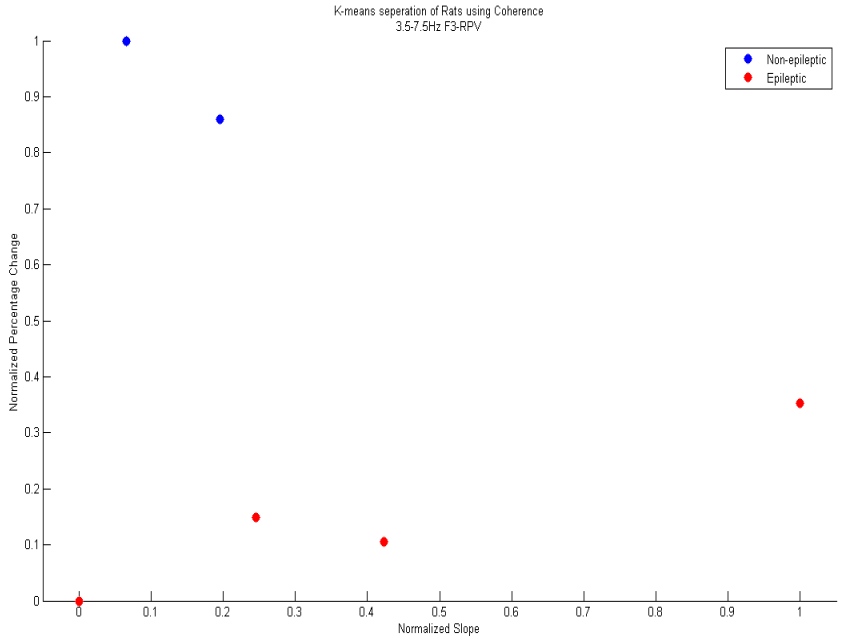

Figure 22. K-Means Classification of Rats using T-index values and all pairs selected by the algorithm for optimal classification (F3-LH, F3-RPV, F4-RPV, LH-RPV)

Discussion of Results

Rat 1 (see **Figures 7-8** and **Tables 11-12**)

Rat 1 did develop PTE. The plotted Coherence values for Rat 1 were very much in line with what we expected to find. Three of the total of four electrode pairs that involved the impact site demonstrated positive slopes. This indicates increasing functional connectivity between the impact site and the rest of the recording sites in the brain over time. The one pair that did not show this trend had relatively high p-values (.037 and .418) as compared with the other pairs and also showed an extremely poor fitting with a coefficient of determination of .11 and, as such, the trend of this pair was considered inconclusive or non-existent. The results from the T-index were very similar in that 3 of the four pairs demonstrated increasing functional connectivity while the fourth showed high p-values (.245 and .499) and a low coefficient of determination of .13. For both Coherence, as well as T-index, pair F4-RPV did not show favorable results.

Rat 3 (see **Figures 9-10** and **Tables 13-14**)

Rat 3 developed PTE. The coherence for Rat 3 showed a positive slope for two of the four considered electrode pairs. Unlike Rat 1, however, the pairs that did not show the expected results had similar p-values and coefficient of determinations with ones that did. While the results for coherence did not point to increase of functional connectivity over time for the majority of the considered pairs in our analysis, the T-index results were similar to the ones from Rat 1. Three of the four pairs showed the expected trend and the one pair that did not had much

higher p-values and a lower coefficient of determination that pointed to inconclusive results for this pair.

Rat 6 (see **Figures 11-12** and **Tables 15-16**)
Rat 6 developed PTE. All four pairs demonstrated increasing coherence over time. It should be noted that for two pairs (F3-RPV and LH-RPV) the slopes are small as are the coefficients of determination. The p-values, however, clearly indicate an increase in the distribution of coherence values from the beginning to the end of the study. The T-index showed similar results. All four pairs demonstrate a negative slope but one of these pairs had a very small slope and the fitted line did not fit the data well (R^2 =.03).

Rat 7 (see **Figures 13-14** and **Tables 17-18**)
Rat 7 did not develop PTE and therefore we expected to see either unchanging or decreasing levels of functional connectivity. Three of the four pairs demonstrated decreasing coherence levels. All p-values indicated significant changes in coherence from beginning to end of study. The T-index yielded positive slopes for three of the four pairs. Again, the pair that did not show the expected results had high p-values (.008 and .043) and a low R^2 value (.26) that pointed to inconclusive trend for this pair.

Rat 8 (see **Figures 15-16** and **Tables 19-20**)
Rat 8 did not develop PTE. Three of the four considered pairs showed decreasing coherence levels while the fourth pair exhibited unchanged level throughout the duration of the study. Two of four pairs showed T-index traces with positive slope, while the other two pairs demonstrated very small negative slopes (-0.0058

and -0.0097). It should be noted that the largest coefficient of determination for T-index for Rat 8 was .03.

Rat 12 (see **Figures 17-18** and **Tables 21-22**)
Rat 12 developed PTE. Three pairs demonstrated increasing coherence with very low p-values (<.001) and relatively well fit lines from which slope was calculated (lowest R^2=.37). The fourth demonstrated a miniscule negative slope calculated from a poorly fit line (R^2=.05). The T-index indicated increasing levels of connectivity in each of the four sites and all p-values indicate statistical significance.

Overall, using the T-index measure, we found consistency of our hypothesis across rats, sensitivity and specificity-wise, when connections of the site of impact with the rest of brain sites we recorded from were considered. In particular, rats that did not develop epilepsy post TBI (2 out of 6 rats) did not show any statistically significant increasing trends of synchronization over time after TBI (100% specificity) and the rats that did develop epilepsy (4 out of 6 rats) showed statistically significant trends of synchronization over time in the majority of the four pairs of brain sites considered in this analysis (100% sensitivity). The pair in the ipsilateral to the injury hemisphere and the one of the impact with the contralateral hippocampus were the most consistent ones in showing the above trends. In addition, results from the Coherence measure of synchronization were pretty much in line with the results from the T-index measure (specificity 100% and sensitivity 75%).

K-Means Classification Results (Figures 19-22)

We employed the K-Means methodology as an additional measure for validation of our hypothesis and of the measures we used to quantify brain's synchronization. After values of the measures and the parameters used in their estimation were given as input to the K-Means algorithm, the rats were classified correctly as having developed PTE or not with 100% accuracy for T-index as well as Coherence. Importantly, the accuracy achieved came from pairs of sites that were automatically selected by the K-Means algorithm under the classification criterion of maximizing the distance between two classes of rats. When given the pairs of sites that contained RPV the K-Means clustering was much less successful, correctly classifying only 2 of six rats.

CHAPTER 5. CONCLUSIONS AND FUTURE RESEARCH

Analysis based on Lyapunov exponents, estimated from continuous intracranial EEG recordings over 80 days in rats suffered from initial traumatic brain injury (TBI), consistently detected a progressive generalized synchronization of brain dynamics long (weeks) prior to development of post-traumatic epilepsy (PTE). This synchronization was more consistent and detectable between brain sites in the same hemisphere as the location of the injury (ipsilaterally), as well as between the site of injury and the contralateral hippocampus. Lyapunov exponents have been used by researchers in the field for prediction of seizures for over ten years (Iasemidis, 2003) and the findings herein further support their use in analysis of EEG dynamics. Analysis based on coherence (Cabrerizo, Adjouadi, Ayala, Tito, & Lizarraga, 2009) (Williamson, et al., 1998) produced similar results but with a bit less sensitivity. This implies the existence of nonlinear transitions of the injured brain over time that the linear measure of coherence may not be able to capture.

The generated results provide a strong indication that, in the brain of the subjects that develop epilepsy after TBI, a) a network pathology (hypersynchronization) is initiated and b) it worsens over time if untreated. Quantification of these observations could be investigated further through the development of new measures of functional connectivity, for example, detection of epileptic spikes in the EEG and analysis of their spatio-temporal synchronization. Also, having established the existence of precursors to PTE, future research in this area could

utilize additional, less-invasive, technologies to intracranial EEG, such as scalp EEG, MRI, fMRI and CT scans to further develop prediction strategies for PTE.

This animal model for epilepsy we used provided us with the unique opportunity to investigate aspects of epileptogenesis. Our results contemplate work in larger number of animals to further enhance their statistical significance. It is now conceivable that the progressive trend towards spatio-temporal synchronization of TBI brain we observed could constitute the basis for the development of predictors of PTE following TBI. The results from our correct classification of rats into epileptic and non-epileptic definitely point to this possibility. Such an early diagnosis of PTE could lead to better and early treatment of the impending epilepsy, and even to a reversal of the route of the traumatized brain towards development of epilepsy.

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