Summer 8-2021

Epileptogenesis in rodents leads to neural system dysfunction and loss of associative memory measured by auditory event related potentials.

Sai Mohan Rudrashetty
Louisiana Tech University

EPILEPTOGENESIS IN RODENTS LEADS TO NEURAL SYSTEM DYSFUNCTION AND LOSS OF ASSOCIATIVE MEMORY MEASURED BY AUDITORY EVENT RELATED POTENTIALS

by

Sai Mohan Rudrashetty, B. Eng., M.S.

A Dissertation Presented in Partial Fulfillment
of the Requirements of the Degree
Doctor of Philosophy

COLLEGE OF ENGINEERING AND SCIENCE
LOUISIANA TECH UNIVERSITY

August 2021

LOUISIANA TECH UNIVERSITY

GRADUATE SCHOOL

May 14, 2021
Date of dissertation defense

We hereby recommend that the dissertation prepared by

Sai Mohan Rudrashetty, B. Eng., M.S.

entitled **Epileptogenesis in rodents leads to neural system dysfunction and loss of associative memory measured by auditory event related potentials.**

be accepted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Biomedical Engineering

Dr. Levi. B Good.
Supervisor of Dissertation Research

Dr. Steven A. Jones
Head of Biomedical Engineering

Doctoral Committee Members:
Dr. Levi B. Good.
Dr. Leon D. Iasemidis.
Dr. Steven A. Jones.
Dr. Jane Jacobs.
Dr. David K. Mills.

Approved:

Hisham Hegab
Dean of Engineering & Science

Approved:

Ramu Ramachandran
Dean of the Graduate School

ABSTRACT

Epilepsy is a common and disabling neurological condition affecting 1-2% of the world's population. Individuals suffering from epilepsy are prone to cognitive dysfunctions with detrimental effects in neural processing and memory resulting in decreases in quality of life. An evaluation of inherent neural processes is valuable information to diagnose and clinically assess cognitive function, which could significantly improve the treatment possibilities and thereby the quality of life for epilepsy patients.

An evaluation of cognitive functions during epileptogenesis was performed by experiments using auditory event related potentials (ERP) in rats before and after induction of status epilepticus (SE) using the Lithium-Pilocarpine model (LP) of epilepsy. The aim of this study was to assess changes in neural system function during epileptogenesis by evaluating inherent responses to auditory stimuli in three ERP tasks at different time periods: before SE (control state), one week-, one month- and two months- after SE (epileptic state). 1. Habituation- (a.) evaluate the ability to habituate to repeated auditory stimuli using the N70 peak response, (b.) examine the time-frequency response through inter-trial coherence (ITC) and event-related spectral perturbation (ERSP); 2. Chirp- evaluate the auditory steady state responses through ITC; and, 3. Mismatch-Negativity (MMN)- evaluate associative memory through ERP responses to regular or odd tones.

Habituation tasks showed increased N70 peak magnitude during epileptogenesis from 1-week, 1-month, and 2-months after SE using repeated measures analysis of variance (rANOVA) with significant differences before and after SE ($p<0.05$, 1-week, 2-months).

ITC showed significant differences between groups during habituation from 0.5-20 Hz and ERSP from 60-100 Hz and 0.5-15 Hz, with baseline corrected ERSP revealing differences from 1-30 Hz. The habituation results indicate a diminished ability to properly habituate to repeated stimuli with abnormal neuronal firing in the epileptic state compared to the non-epileptic control state linking a possible mechanism with imbalances in neuronal inhibition and excitation during epileptogenesis. Chirp response ITC showed increased synchronous activity in high gamma band (>40 Hz) during epileptogenesis indicating the neuronal response in epileptic groups are phase locked to the chirp stimuli at a higher incidence than controls. Epileptic MMN ERP responses for odd and regular tones exhibited a decrease in the response curves from 250-350ms post-stimulus indicating a loss of ability to distinguish tones and difficulties with their associative memory during epileptogenesis.

Our results indicate that a proper EEG-based analysis of auditory ERPs are useful in evaluating neural systems during epileptogenesis showing clear imbalances in excitatory: inhibitory function, as well as an indication that associative memory is detrimentally affected. The ERP methods employed may help in the diagnosis of the epileptic patients with cognitive disabilities as their epilepsy progresses, as it is simple, non-invasive and cost effective.

APPROVAL FOR SCHOLARLY DISSEMINATION

The author grants to the Prescott Memorial Library of Louisiana Tech University the right to reproduce, by appropriate methods, upon request, any or all portions of this Dissertation. It is understood that "proper request" consists of the agreement, on the part of the requesting party, that said reproduction is for his personal use and that subsequent reproduction will not occur without written approval of the author of this Dissertation. Further, any portions of the Dissertation used in books, papers, and other works must be appropriately referenced to this Dissertation.

Finally, the author of this Dissertation reserves the right to publish freely, in the literature, at any time, any or all portions of this Dissertation.

Author _____

Date _____

DEDICATION

This thesis is dedicated to all these people for their endless support, love and encouragement, without which I could not have accomplished it.

Maternal Grandparents: Srinivas Rao (late) and Shyamala Bhai Pyata (late),

Paternal Grandparents: Padma Rao (late) and Andalamma Rudrashetty (late),

Mom: Swetha Mohan Rudrashetty,

Dad: Chandra Mohan Rudrashetty,

Sister: Deepti Rudrashetty

Brother: Manikanta Rudrashetty.

All my other family members and friends.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

At the successful completion of my PhD dissertation, I take the opportunity to express my sincere gratitude to many who have made this possible. Firstly, I would like to thank my mentor Dr. Levi B. Good for trusting me with this project. His constant encouragement, guidance and valuable advice throughout the process of my research have immensely contributed to my professional advancement. I would also like to thank especially Dr. Leon D. Iasemidis, for supporting me financially in the fulfillment of this research, Dr. Steven A. Jones for his teaching and support, Dr. Jane Jacobs and Dr. David K. Mills, for willingness to serve on my committee.

I would like to thank the members of the Dr. Good's Neural System Lab and Brain Dynamics Lab, for their collaboration throughout this work. I would like to thank Dr. Kevin Holly for his constant assistance wherever needed. I would also like to thank Dr. Theresa Murray and the members of her lab (Kayla Ponder and Sadie Villarrubia) for performing the rat surgical procedures and status epilepticus inductions which were instrumental to my project. Last but not least, I would like to thank my parents, my sister and my twin for all their support and blessings, without which I would not have achieved anything in my life. I would also like to thank my other family members and friends for their constant encouragement.

CHAPTER 1

INTRODUCTION

1.1 Epilepsy and Cognition

A most common and disabling neurological condition is epilepsy, characterized by recurrent, unprovoked seizures, where abnormal electrical discharges interfere with the brain's normal function [1-3]. More than 65 million people (1-2% of the world's population) worldwide and over 2.2 million people in America have epilepsy. It is estimated that 150,000 new cases of epilepsy are diagnosed in the United States alone each year and 1 in 26 individuals develop epilepsy at some point in their lifetime [3].

Epileptic patients have unprovoked seizures, where a "seizure" is caused by the excessive, hypersynchronous firing of neurons in the brain and results in a rapid change of neurological function. Epilepsy is diagnosed if the patient has two or more unprovoked seizures [1]. Typically, seizures are classified into two categories, a) partial seizures, where seizure activity takes place in a constrained area/portion of the brain (the epileptogenic focus) and b) generalized seizures, where the seizure appears to start at widely spread brain locations. Depending on the location and extent of the epileptogenic focus/foci, seizures could cause loss of consciousness and irregular movement of portions of the body (convulsions) for a considerable period (typically up to 5 minutes) [4]. Seizures are self-terminating by the activation of internal inhibitory mechanisms. Failure of these mechanisms lead to prolonged seizures that may last for greater than 30 minutes. This

condition is called status epilepticus and may cause long term brain injury and even death [5].

Impairment of neurotransmitters could cause seizures as neuronal activity depends on the balance of excitatory and inhibitory neurotransmitters. The transmission of signals from one neuron to another through presynaptic neuron's membrane depolarization depends on the level of the presynaptic neurons' neurotransmitters. For example, seizures could be triggered by an imbalance between excitation and inhibition cased by loss/decrease of the GABAergic inhibition and/or increase of glutamatergic excitation [6].

The term "epileptic dementia" which is progressive cognitive decline caused by epilepsy, was coined in the 19th century by German psychiatrist Emil W. G. M. Kraepelin and neurologist Oswald Bumke. About 70-80% of patients suffering from chronic epilepsies have cognitive deficits and are more prone to cognitive and behavioral dysfunctions than the general population [7]. Cognitive impairment is considered to be caused by prolonged or recurrent seizures and the common comorbidities in patients with epilepsy include cognitive dysfunction, such as memory, processing or attention difficulties, and mental health problems including depression, anxiety etc. Among such co-morbidities, the most troublesome are cognitive abnormalities such as memory failure, which is a common clinical concern raised by patients with epilepsy, irrespective of the use of anti-epileptic medication or the presence of brain lesions [8]. Some of the cognitive difficulties associated with epilepsy decrease the patients' and their families' quality of life, decrement their educational progress and achievements throughout life, accelerate memory impairment (primarily in individuals with temporal lobe epilepsy) [6,8, 9]. Poorer cognitive status, with longer duration of epilepsy, are seen mostly in patients with temporal lobe

epilepsy [7].

While significant studies of pharmacologic therapies (such as acetylcholinesterase inhibitors donepezil and galantamine) have been made to improve cognitive function in disorders, such as attention deficit hyperactivity and neurodegenerative disorders, the efficacy of these agents in patients with epilepsy has received relatively less attention [10]. Anti-seizure drugs (ASDs) are still the mainstay therapeutic solution. However, some studies reported few cognitive adverse effects (CAEs) with the use of ASD-phenobarbital [11]. Another study showed that phenobarbital had worse cognitive effects than valproate or carbamazepine. One study reported that primidone causes more adverse effects on motor performance and attention/concentration tests [12,13]. Therefore, more effective ways (deep brain stimulation and seizure prediction) are needed to prevent seizures or cure epilepsy, thereby preventing cognitive impairment in these patients. These methods are costly, invasive and sometimes may worsen mental or emotional status of the patient.

In 1929, German psychiatrist Hans Berger used scalp electrodes and demonstrated the practical and diagnostic value of electroencephalography (EEG) in humans, and it has been the most widely used signal in the clinical assessment of brain activity [14]. Thus, a method capable of diagnosing and clinically assessing the cognitive function could significantly improve the treatment possibilities and thereby the quality of life for epilepsy patients. One approach is to record and analyze event related potential (ERP) responses from the EEG. A candidate ERP, is auditory evoked potentials (AEPs) on the brain, where different aspects of cognitive function, such as attention span, memory recall, and speed of mental processing, can be qualitatively and quantitatively processed and assessed. These

auditory ERP responses are characterized by long latency and low amplitude in acute and chronic diseases [15-18]. They are time-locked to the auditory stimulus and can be recorded through the electrical response of the brain (synchronized activity of a network of neurons in different regions of the brain including the auditory cortex that can be recorded by EEG). These auditory ERPs, elicited by tones or sound pulses, are divided into early (within the first 10 ms after stimulation), middle (between 12 – 50 ms after stimulation, which contains a series of positive and negative peaks in the EEG), and late latency (four peaks: P50, N100, P150, and N200, between 50 and 250 ms after stimulation) ERPs [19]. Evoked potentials can be further classified as exogenous (elicited by physical parameters of the external stimulus, such as frequency, intensity, and duration) and endogenous (elicited by brain and respond to the stimulus) [20]. Endogenous ERPs are used to study cognitive dysfunctions that could be evaluated once and also followed up over a period of time. This type of study is an unbiased and non-invasive electrophysiological method for measuring cognitive dysfunctions [21]. Targeted stimulation leads to a positive ERP peak at 300 ms after stimulation, known as P300, which is larger in central and posterior locations of the brain. This P300 is task-dependent and has longer latency. It is related to cognitive processes such as memory updating, attention, signal matching, recognition, and decision making [20]. The degree of cognitive dysfunction is typically assessed by the increase or decrease in P300 latency. Thus, we can study the brain mechanisms related to cognition and information processing in control versus cognitively disabled groups [22,23]. These ERP techniques are used in diagnostics of several diseases such as mild cognitive impairment, Alzheimer's disease, epilepsy, craniocerebral trauma, Parkinson's disease, autism, and other neurodegenerative diseases.

Few studies have indicated changes in P300 in patients with epilepsy [18]. The advantages of AEPs are the easiness of their implementation and their non-invasive nature. Thus, they can be performed over time and compared to other diagnostic methods to monitor the progress of a disease [21].

The observed event-related changes in the EEG are in the form of ERPs, event-related desynchronization (ERD) or event-related synchronization (ERS). The responses of cortical neurons caused by changes in afferent activity of neurons constitute the ERPs, while the changes in the activity of local interactions between neurons and interneurons constitute the ERD/ERS [24].

ERD/ERS are specific to frequency bands. Certain events can block or desynchronize specific frequencies. These effects can be detected by frequency analysis of the EEG, where we can see an increase or decrease of power in particular frequency bands, caused by increase or decrease in synchrony of the involved neuronal populations respectively [24].

1.2 Dissertation Contribution

1.2.1 Biomarkers for Habituation Tasks

In this study, we developed an algorithm that separates all EEG epochs across trials and averages them. Signal processing techniques were employed on ERP-EEG responses to extract from the quantitative features related to habituation tasks. These features could potentially serve as biomarkers, to study the differences between control and epileptic groups. Two biomarkers were estimated. Firstly, the ERP responses were quantified by the average N70 (negative peak at 70 ms from the onset of first stimulus in the ERP) over the trials and then normalizing the N70 onset from 1st for the 2nd, 3rd, and 4th N70 peaks.

Secondly, we applied advanced signal processing techniques i.e. time-frequency analysis like inter trial coherence (ITC) and event related spectral power (ERSP) measures from the EEGLAB's toolbox in MATLAB. The N70 biomarker distinguished between control and epileptic groups, revealing that the epileptic rats do not habituate to the auditory stimulus, while the controls did. The ITC gave a view of the frequency response, where statistical significant differences were observed between control and epileptic groups. Similarly, the ERSP results too showed that the spectral powers at certain frequencies were increased after the rat became epileptic.

1.2.2 Biomarkers for Chirp Modulation Tasks

We also developed potential biomarkers for chirp modulation signal by analyzing ITC from the ERP epochs of rats. The auditory steady-state responses to different frequencies of stimulation ('chirp' tone of increasing frequencies from 1-100Hz) is studied to see the responses in different frequency bands for inter-trial phase locking. ITC analysis on the ERP responses showed the synchrony of the neuronal populations at different frequencies that can be used as a biomarker to distinguish between control and epileptic groups. The epileptic rats were synchronized to frequency of 60-100 Hz when chirp signal was introduced and this could be seen from ITC, where the coherence values increased after the rat became epileptic, which tells us that the inhibitory function is not normal in the epileptic rats when compared with controls.

1.2.3 Biomarkers for mismatch negativity task

We developed an algorithm that separates all EEG ERP epochs across subjects (rats) that received an auditory discrimination task with standard/regular tones and deviant/odd tones and averaged them. In this task, repeated trains of standard or regular

tones/stimuli were interrupted by infrequent stimulus (deviant/odd) tones, that differed in frequency, with standard tones of 1000 Hz and deviants of 2000 Hz. The ERP responses of the deviants and standards were averaged separately and plotted on the same graph to see the mismatch negativity in control and epileptic rats. MMN ERP responses were evaluated by taking the area under the curve (AUC) from 250-350 ms beyond the initial tone for each of the regular and odd tones in eight rats. During epileptogenesis, the rats appear to lose their ability to distinguish the odd and regular tones by the declining AUC. When the analysis was done on all rats, we could see the impairment of cognition over time as epilepsy is progressed.

1.3 Dissertation Outline

In Chapter 2 we provide background material on the electroencephalogram (information on EEG signals and common artifacts), epilepsy and its effects on cognition, early approaches to the study of epilepsy, and evoked potentials. Information is also provided on currently available diagnostic tools for cognition in epilepsy, and other diseases like Alzheimer's are presented. The data acquisition and methods of data analysis employed in this project are described in Chapter 3. The methodology includes description of feature/biomarker estimation, and statistical analysis, e.g. repeated measure ANOVA (rANOVA).

Results for habituation, potential biomarkers of chirp modulation, and odd ball task are presented in Chapter 4 for both the control case (before the rat became status epilepticus state) and the epileptogenesis cases (one week, one month and two months after SE state).

We discuss the results of Chapter 4 in Chapter 5. In Chapter 6 we present our conclusion and suggestions for future work.

Additional plots and other resourceful information that are deemed helpful in further support of our hypotheses and results were added in the Appendix.

CHAPTER 2

BACKGROUND

2.1 Electroencephalography

The first recording of electrical brain activity in a human, was made in 1924 by the German psychiatrist Hans Berger [25]. The human brain can generate electric and magnetic fields as a result of summation of electrical activity, through flow of ions as neurons inside the cerebral cortex. In the brain, 10^9-10^{10} neurons are present in the cerebral cortex, and the summated electrical field is measured either from scalp (on surface of the head) or directly from brain using intracranial electrodes implanted in the brain. This measured electrical activity is the EEG, and it represents the summated electrical activity of brain. Clinicians and researchers use the EEG to see specific patterns or characteristics that are unique to specific disorders, and it has become one of the widely used neurophysiological tool in diagnosis of neuropsychiatric disorders, epileptic studies, and studies related to cognitive dysfunctions [26].

Scalp EEG is a noninvasive recording approach which uses electrodes placed along the surface of the head. In this type of recording, the pyramidal neurons within the cortex are orientated radially to the scalp surface as indicated in **Figure 2-1**. The postsynaptic potentials generated by excitatory and inhibitory pyramidal neurons (arranged in parallel) overlap in time, which are represented in the EEG as the signal that propagates towards the electrode present on the scalp [27&28]. Artifacts (e.g. movement artifacts by the subject)

and environmental noise (e.g. noise from AC power lines) are common during recordings but other artifacts may also arise as physiological artifacts, which are unwanted signals that can alter the EEG. Examples include interference of electrocardiogram (ECG) or eye movement. Technical artifacts can be caused by changes in instrumentation hardware or electrode quality such as low battery in amplifier, broken wire contacts in electrodes, and increases in electrode impedance.

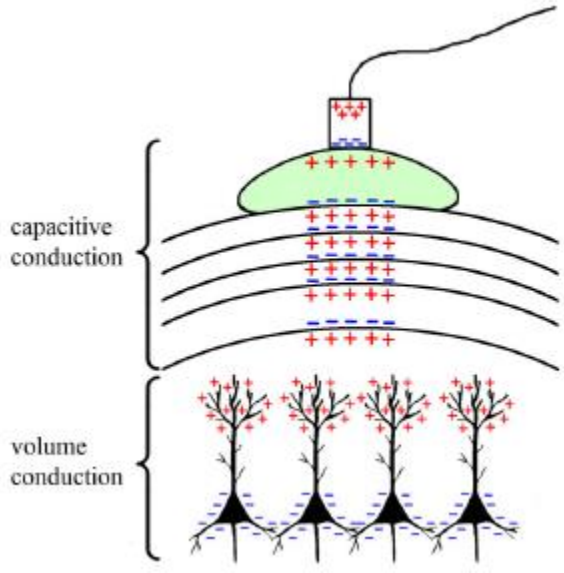


Figure 2- 1: Neural signal path from the pyramidal neurons through layers of brain, dura, skull and the electrode (Figure taken from Jackson and Bolger, 2014).

Intracranial EEG (iEEG), is an invasive method, where the electrodes (e.g. depth electrodes) are placed directly on the cerebral cortex, to probe neuronal electrical activity at deeper layers in the brain. We can place micro or depth electrodes at specific regions under study to record local field potentials (LFPs). iEEG is considered to be the most informative-neurophysiological recording technique [29]. This approach eliminates many of the artifacts mentioned earlier. We can target specific areas in the brain, thereby

acquiring local information that can be useful for clinical studies. The EEG from the LFP depends on the synchrony of the local oscillatory neurons surrounding the electrode [30].

LFP signals could give information about the resonance properties of neurons, which can be seen from frequency analysis such as power spectral density and ITC [31]. Reimann et al. used neocortical LFPs in biophysical computationally detailed model of rodents and showed that the active membrane currents (i.e., resonant currents) direct the generation of LFPs, and the LFPs are caused by the activity of local interactions between neurons and interneurons [32]. These neurons oscillate synchronously and contribute significantly to the recorded LFP signal, where the resonating property is both voltage and frequency dependent. This feature occurs mostly in inhibitory interneurons such as fast spiking interneurons (FSIs) [33]. The transmission of frequency dependent information via neural networks, can be modulated by controlling which subset of neurons are in a resonant state [34]. This neuronal property plays a critical role in shaping the dynamics of brain activity in various pathological and neurophysiological conditions [35].

2.2 Brain Wave Classification

A normal EEG signal has different frequency bands and is characterized by rhythmic activity. **Figure 2-2 shows** EEG waveforms of different frequency bands plotted over time for five seconds. The specific frequency bands in EEG signals are, in order of increasing frequency:

Delta Rhythm: EEG activity from 0.5- 4 Hz frequency is categorized as delta rhythm. This activity is prominent in frontal regions in adults and in the posterior in children. It is observed during sleep and attention tasks, and has high amplitude.

Theta Rhythm: EEG activity from 4-8 Hz frequency is categorized as theta rhythm. It occurs most often during sleep, but is also found during meditative state. This frequency range of EEG activity has been associated with learning, memory, and intuition.

Alpha Rhythm: EEG activity in a frequency range of 8-13 Hz consists of sharp peaks occurring periodically and is known as alpha rhythm. It is prominent in posterior regions of brain, and observed while the subject is in a resting state. It was designated alpha because it was the first activity recorded, by Hans Berger in 1924.

Beta Rhythm: EEG activity with a frequency range of 13-30 Hz is categorized as beta rhythm. These waves are faster and small and are associated with mental, intellectual activity, high alert and concentration. They are prominent on both hemispheres of brain, having asymmetrical distribution and mostly evident in frontal region.

Gamma Rhythm: EEG activity above 30 Hz frequency is categorized as gamma rhythm. Gamma waves are associated with perception, attention and cognition, as they promote coherence and 'temporal binding' of local and distant neuronal activity. A decrease in gamma activity is associated with cognitive decline. Apart from cognitive disorders, gamma oscillations are involved in other neurological disorders, and these oscillations are generated by fast spiking interneurons (FSIs) in brain, which resonate spontaneously between 30-100 Hz range [36–41]. The gamma frequencies (30-100 Hz) are generated naturally by GABAergic fast-spiking interneurons (FSIs) and can propagate to the hippocampal cornu ammonis 1 (CA1) subfield [42,43]. Because pyramidal cells are highly connected by interneurons, the FSIs can entrain the synchronous firing between them [44].

High Frequency Oscillations (HFOs): HFOs are short duration neural oscillations with a frequency greater than 80 Hz. These signals can be used to determine seizure onset [45,46].

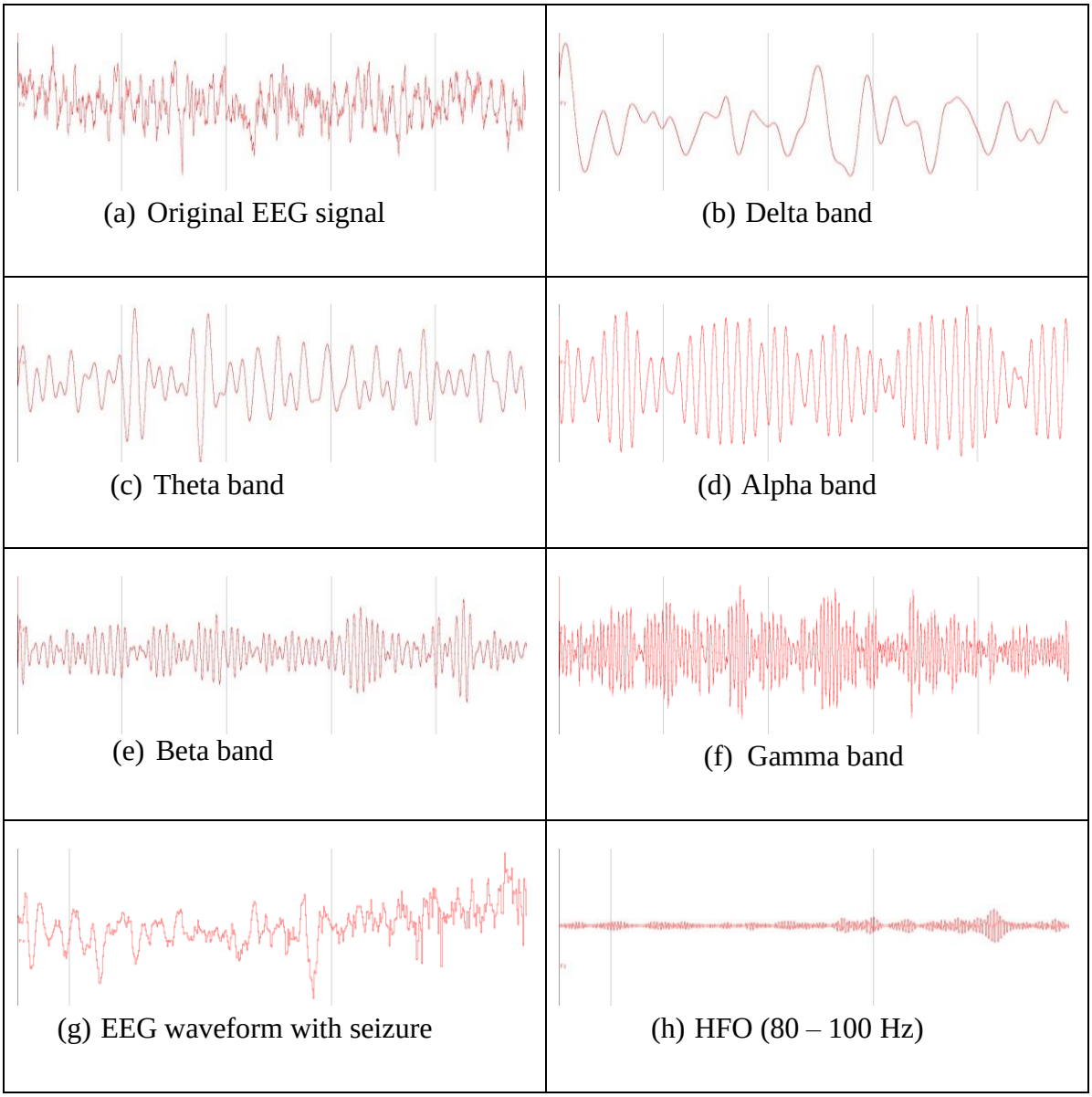


Figure 2- 2: EEG waveform at different frequency bands (Figure taken from Karamuri, 2017) [31].

2.3 Epilepsy

Epilepsy is common and disabling neurological condition, affecting more than 65 million people worldwide. It is a syndrome, characterized by recurrent, unprovoked seizures [1,2]. A common assumption is that seizures occur when neuronal excitability reaches a threshold combined with a high level of synchronization between neurons [47,48]. A patient is diagnosed with epilepsy, if "two or more unprovoked seizures" are observed. Seizures are self-terminating with the impairment of neurons, as neuronal activity depends on the imbalance of excitatory and inhibitory mechanisms caused by loss/decrease of the GABAergic inhibition and/or increase of glutamatergic excitation [6]. Figure 2-3 shows EEG waveforms from different electrodes of a rat during a seizure.

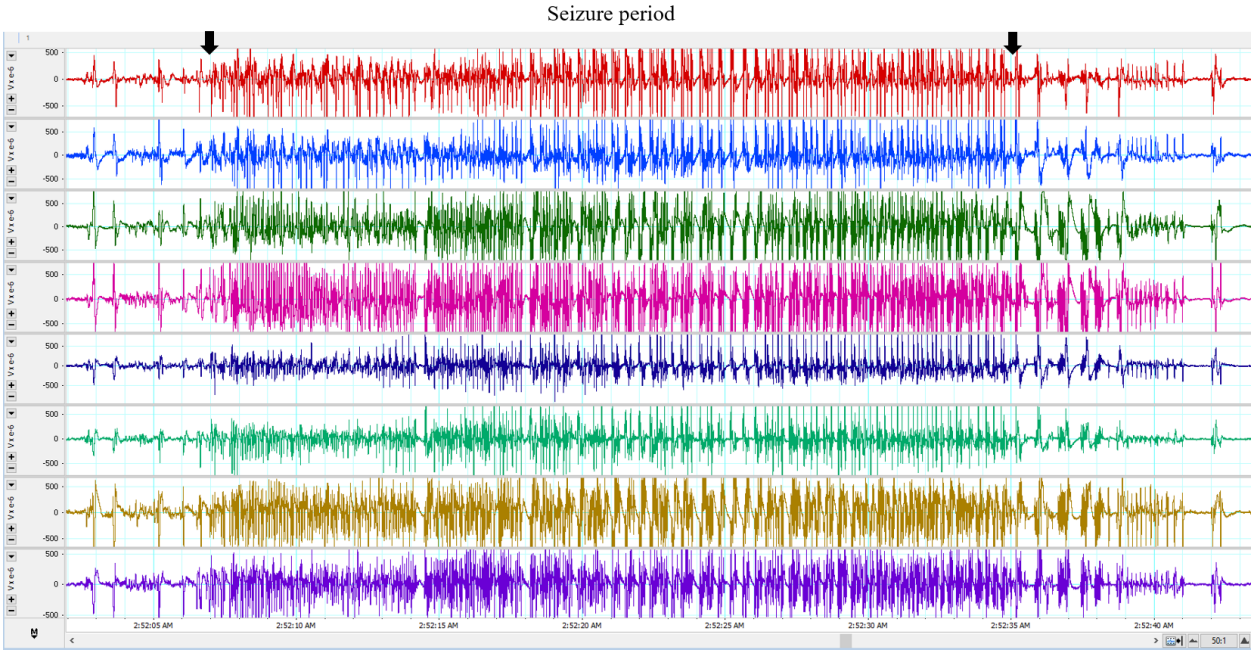


Figure 2- 3: Example of a rat iEEG recording during a seizure period.

About 70-80% of patients suffering from chronic epilepsies have cognitive deficits and are more prone to cognitive and behavioral dysfunctions than the general

population [7]. Cognitive impairment is considered to be caused by prolonged or recurrent seizures, and the common comorbidities in patients with epilepsy include cognitive dysfunction, such as memory, processing or attention difficulties, and mental health problems including depression and anxiety. Among such co-morbidities, the most troublesome are cognitive abnormalities such as memory failure, which is a common clinical concern raised by patients with epilepsy, irrespective of the use of anti-epileptic medication or the presence of brain lesions [8]. Poorer cognitive status, with longer duration of epilepsy, is seen mostly in patients with temporal lobe epilepsy [7].

2.4 Event Related Potential

2.4.1 Early Approach

Electroencephalogram (EEG) recordings were the first noninvasive brain activity measurements developed from human subjects [24,49-51]. The early studies examining the EEG helped to characterize the changes in electrical activity when stimuli and tasks were presented [52,53]. In cognitive neuroscience, ERPs or evoked potentials, became a primary tool, when scientists understood the importance of signal averaging of the EEG's [54-58]. The ERP method has several advantages, and is one of the most widely used methods in cognitive neuroscience, despite the rise of modern neuroimaging methods. It allows scientists to study specific cognitive processes, by observing human brain activity [58,59]. In recent years, the study of ERPs has increased dramatically, where the use of signal-averaged EEG recordings that are time-locked to a particular event, provides information regarding cognition [60]. Also, ERPs have been used extensively in neurophysiological research to study areas such as cognition, perception, and human

sensation, and its non-invasive nature and the ease of implementation has facilitated those studies [61].

ERPs have good temporal resolution, which makes them a primary tool in measuring the brain activity in milliseconds, where we can study the general and specific aspects of individual's response to an external stimulus, thereby revealing information regarding attention, cognition and perception (since these operate on a scale of tens of milliseconds). Also, given the tissue in which ERPs are generated and propagated, no measurable conduction delay occurs between the scalp potentials recorded from EEG and the brain activity (inside the head) [62].

In early days, the electrophysiologists proposed that the scalp EEG and intracranially-recorded field potentials (local-field potentials- LFPs), were generated by the postsynaptic activity of group of neurons [49]. The view is widely accepted today is that the EEG and averaged ERPs are measuring the electrical potential produced in the extracellular fluid, where ions stream across cell membranes and neurons convey the information with one other through neurotransmitters [62,63]. A large number of neurons should be active simultaneously, in order to generate electrical fields that are large enough to propagate through the brain, dura, skull and skin [64]. In addition, this large group of synchronously active neurons should have a geometry that is perpendicular to brain so that they are not cancelled by other group of neurons that are active during the same time [59,62]. These simultaneously active neurons should approximately have the same orientation for the electrical potentials to summate, and by this, we can say that ERPs are primarily generated by the postsynaptic potentials of cortical pyramidal cells. We can

predict the voltage pattern that will be created across the head, depending on the area and orientation of a specific neural generator in the brain [63].

Davis, in 1939 showed that the voltage fluctuations were elicited when the stimuli were presented [42]. ERP research gained popularity when Walter et al., demonstrated in 1964 that cognitive activity could be measured [58]. Kornhuber et al., in 1965 found out the first of the ERP components indexing cognitive processes [65]. Another group of studies took the average of evoked potentials, which are time-locked to an observable event, and thereby recorded multiple trials to see voltage fluctuations (common to each trial) [56, 66-68]. By the invention of 10/20 EEG system, the ERP tasks became easier to replicate across studies and were much easier to integrate [69].

AEPs are the potentials in response to auditory stimuli. These auditory ERPs, are divided into (1) early-latency/auditory brain stem responses, which occur within the first 10 ms after stimulation and represent the activity related to signal propagation through auditory nerve [70], (2) middle latency responses, which occur between 12 and 50 ms after stimulation and contain a series of positive and negative peak in the EEG and represents cortical and thalamus activity [71], and (3) late latency, which consists of four peaks: P50, N100, P150, and N200, between 50 and 250 ms after stimulation and are of cortical origin [72]. Transient AEP (tAEP)-evoked potentials are obtained from slow frequency stimulation, where the single responses do not overlap in time [73].

Furthermore, the evoked potentials are classified as exogenous and endogenous. Exogenous ERPs are elicited by physical properties/parameters of the external stimulus, such as frequency, intensity, and duration. P1, partially N2, and P2 are the exogenous components. Endogenous ERPs depend on stimulus categorization,

subject's attention [67, 72]. The studies related to cognitive dysfunctions use endogenous ERPs, which are evaluated once or can also be followed up over a period of time. These evoked potentials can be measured non-invasively and are an unbiased electrophysiological method for measuring cognitive dysfunctions [78].

2.4.2 ERP Components

ERP components are described as peaks and troughs, i.e. negative and positive peaks, which are time-locked to the auditory stimulus during a task, as shown in **Figure 2-4**. More information regarding the human sensation, cognitive processing, and speed of mental processing can be known from these ERP responses, during a trial, when different types of tasks are performed [74].

In **Figure 2-4**, negative voltage is plotted going up, as it has to do with the position of the electrode placement in the brain, while recording. When the traditional 1020 eeg system is used for the ERP recordings, the positive ERP components (P100, 200 P300), are deflected downwards because of the electrode placement. For example- if the reference electrode is on FZ and an active electrode is on the occipital region (OZ), an inversion in these will change the polarity of P1, so it will deflect downwards, as shown in Figure **2-4**. The plotting of negative voltage up and positive downwards, was followed by the majority of cognitive neuroscientists, as it became easier to interpret these ERP waveforms, to study various cognitive information processes [74]. In our research we followed the same approach.

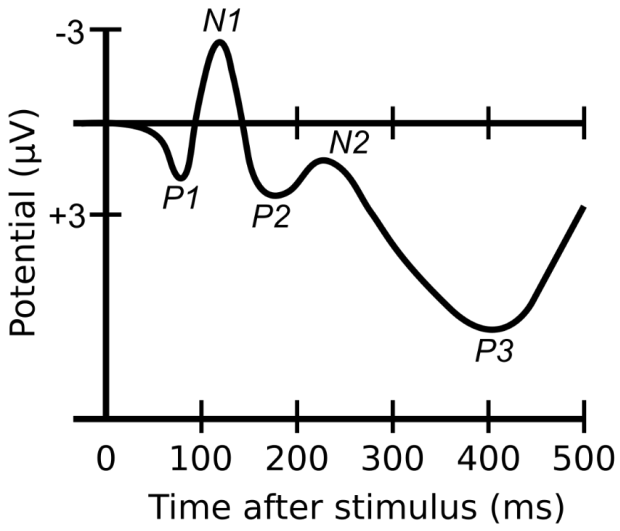


Figure 2- 4**:** ERP components during an auditory task.

The labelling of the ERP waveforms is a sequence of peaks, that occur at a different times indicating its polarity. For example, in Figure 2-4, P1, N1, P2, N2, P3 peaks, identify the negative and positive peaks by their latency, so P1, N1, corresponds to the first positive and negative peaks that occur 100 ms, and P2, N2 occur 200 ms after the stimulus onset. Similarly, P3 or P300 occurs at 300 ms, post stimulus [75]. We describe the most commonly identified auditory ERPs (shown in Figure 2-5), of an adult.

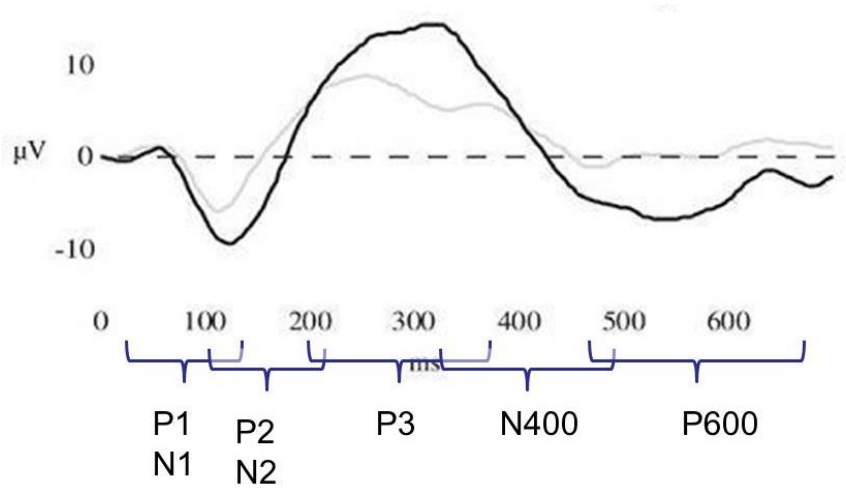


Figure 2- 5**:** Example of human auditory evoked potential.

P1: This feature is not easily identified in the ERP waveform, and appears earlier in time at 50 ms over posterior scalp electrodes. This component gives neurophysiological information regarding attention to sensory inputs. In 1992, Waldo, et al. found out that P1, is frequently associated with auditory inhibition and tested it in sensory gating tasks [76]. Multiple sclerosis and Parkinson's disease are often diagnosed, from the P1 latency [77].

N1: This feature is easily identified, regardless of specific tasks employed, and appears between 75130 ms over temporal areas [75]. In 1973, Hillyard et al. were the first to investigate this component in dichotic listening task [78]. With increase in inter-stimulus interval, and with increased attention to stimulus, the amplitude of N1 can be enhanced, where perceptual and early sensory information can be processed [78,79]. This N1 is most likely elicited in primary auditory cortex (temporal lobe of brain) [80]. It is related to sensory information processing, such as hearing [80].

P2: The P2 component is seen in cognitive tasks such as selective attention [81] and short-term memory [82], and it has low interindividual variability and high replicability [83]. In auditory modality, P2 is referred as the N1/P2 complex, as it occurs together with N1 and shares many characteristics with N1 [84]. It has maximum amplitude at a latency range of 150275 ms, and is typically noted from the central region of the brain, using earlobes as references [85].

N2: Polich and Bondurant, found that the N2 component has small amplitude and shorter latency when shorter inter-stimulus intervals are given [86]. This component is elicited over the central parietal region [24]. With respect to complexity of the task, the amplitude and polarity of N2 inhibition can change. It is noted over the fronto-central electrodes [87].

P3: This component is also called P300, since it has a latency of 300 ms after stimulus onset. The task for eliciting P300 component is oddball paradigm/mismatch negativity task, where occasional deviant tones are presented in-between frequent stimuli [88]. This P300 component seem to have larger amplitude in electrodes present in frontal and central regions [89]. In auditory modality, the MMN responses can be evoked by tones/sound pulses that differ in any characteristics, such as duration, intensity or frequency [90]. In 1988, Donchin and Coles found out that this P300 component acts as an indicator of memory updating [91]. P300 has gained immense attention in clinical studies of in subjects with attention deficits such as a-deficit/hyperactivity disorder (ADHD), where the amplitude varies with respect to stimuli. It reflects information related to various attentional functions. P300 with shorter latencies had better performance that was directly related to cognitive abilities [92&93]. It is not easy to identify the source of P300

component, but some researchers identified it as the medial temporal lobe [94], including the hippocampal region [95], thalamus, parahippocampal gyrus or amygdala [90], based on intracranial EEG recordings.

N400: The N400 ERP component occurs 400 ms after stimulus onset. This component is associated with visual and auditory sentence comprehension tasks and Kutas and Hillyard were the first to identify this phenomenon [96]. In order to generate N400 evoke responses, the words of a sentence were presented visually one after another at fixed intervals, and the N400 amplitude was larger over parietal and temporal regions in the right hemisphere [97]. The N400 correlates with familiarity of the stimulus presented [98].

P600: P600 has two functionality interpretations, one is related to language and other associated with memory processes. These peaks have same topographies but appear at different brain sites [75]. This ERP component ranges from 400 600 ms. When memory recall/recognition tasks are performed, the P600 waveform appears [99]. Intracranial recordings from anterior temporal lobe structures during continuous recognition tasks exhibited these responses [100].

Different procedures used to record ERPs, affect peak characteristics. Examples include differences in length of inter-trial intervals, number of trials, or different variations in intensity of stimulus. Therefore, to successful replicate the evoked potentials, researchers should follow a consistent approach while performing tasks [75]. Also, the ERP components are often smaller than normal EEG components, and one cannot recognize them from raw EEG traces. To improve the signal quality, one should rely on averaging the ERP components across the EEG epochs (for all trials), that are time-locked to the stimuli [101]. This procedure averages out the background EEG fluctuations, leaving only

the event-related brain dynamics. This evoked potential, represents only the activity which is consistently time-locked to that particular event, thereby giving a high temporal resolution, enabling the patterns of neuronal activity to be studied [102]. When looking at P1, N1 peaks (for studying about perception and attention), the researchers should conduct 3001000 trials per condition to get reliable data. For P300 component, which are usually large and slow waves, about 3560 trials per condition for each subject are necessary [74].

2.5 Auditory Steady-State Responses

The steady-state evoked response caused by repetitive auditory stimulation is known as auditory steady state response (ASSR). In ASSR, the frequency of auditory stimulation is higher than that of tAEP. This ASSR has infinitely stable amplitude, frequency and phase [103], and can be analyzed in shorter periods of time. The ASSR responses are driven by the traits of the external stimulation [104].

Adrian and Matthews reported in 1934 that alpha amplitude increased in response to a flashing light, and demonstrated the brain's ability to respond to external stimulation [105]. This response to flashing light is known as visual steady state response, which led to the assumption that similar responses could be traced/detected in the auditory system. By giving auditory tones, with a sound frequency of 40 Hz, Galambos et al. showed that auditory steady state response can be elicited [106]. Other researchers found that ASSR is strongest at 40 Hz frequency [104, 107, 108].

Two main theories have been proposed to explain the origin of ASSRs. Superposition theory proposes that the time period between auditory stimuli is shorter than the length of a single tAEP required to generate an ASSR. Therefore, the ASSR is summation of successive tAEPs, shifted in time. The alternate theory, entrainment, is based on ASSR studies, which involves the

effects of medication, ASSR propagation in time, and the behavioral significance to resonant frequency. In this theory, the response to external stimuli are generated by naturally existing brain oscillators that synchronize to particular frequencies of stimulation.

Ross et al. showed that in the first 100 ms of stimulation, transient gamma band response appears, and the stable amplitude and phase relationship with stimulus builds up in a time window of 80-250 ms [109]. He also showed that the phase shift or gap in a stimulus causes SSR to be rebuild around 200 ms, thus suggesting that ASSR is not an evoked potential, but is induced by the brain response [110]. During an internally or externally-paced events, the observed event-related changes in the EEG have a phase-locked response, known as ERP that is caused by changes in afferent activity of neurons, and non-phase-locked response, known as event-related desynchronization (ERD) or event-related synchronization (ERS). ERS/ERD is caused by changes in the activity of local interactions between neurons and interneurons [24, 111]. ERD/ERS are specific to frequency bands. Certain events can block or desynchronize specific frequencies depending on the subject's neurological condition. The ERP, ERD/ERS are responses of different neuronal structures in the brain. The ERD/ERS is highly specific to different frequency bands, and can occur either on the same or different locations on the scalp. So, this can be detected by frequency analysis of the EEG, where we can see an increase or decrease of power in particular frequency bands, that arises from an increase or decrease in synchrony of the involved neuronal populations respectively. ERD is a decrease in oscillatory activity during an internally or externally paced event, and it leads to decreased power in particular frequency bands, caused by desynchrony of the involved neuronal populations. ERS is the increase of rhythmic activity, and it leads to increased power in particular frequency bands caused by an increase in synchrony of the involved neuronal populations. Pfurtscheller, has shown that ERD in alpha and beta bands correlates to activated neural ensembles, and ERS in the alpha and lower beta band (<30 Hz) as correlate of deactivated/idling neural ensembles [111].

The various types of stimuli used to elicit ASSR are frequency modulation (FM), in which stimuli are given by modulating frequencies of a carrier tone at constant amplitude, amplitude modulation (AM) in which stimuli are given by modulating the amplitude of sound, and frequency-amplitude modulation or mixed modulation (MM) in which stimuli are given by modulating frequencies and amplitudes of a carrier tone. These types are classified as frequency specific, where the power is concentrated in a narrow band of frequencies as in tone bursts and band-limited chirps, and frequency unspecific, where power is concentrated in broad band of frequencies as in clicks, chirps, and noises [112,113]. Most of the ASSR studies use frequency or time-frequency analysis, but as ASSR is elicited during the stimulation time, time-frequency transformation is the most suitable approach for ASSR analysis. The most commonly used time-frequency (TF) analysis is the short time fast Fourier transform (stFFT) [110,114,115], and in this research we have used the ITC and ERSP analysis, which will be discussed later in Chapter 3.

Chirp-based stimuli: to test the brain's ability to elicit responses at different frequencies, one can use a chirp-based stimulus. In this type of stimulus, changing modulation frequency is used, where wide range of frequencies are covered [116]. AM-chirp with sine wave of changing frequencies is the most common type of chirps used, where one can assign the frequency modulation range, direction (up-chirp/down-chirp), duration [116,117]. Many investigators, have used, chirp-based stimulation in EEG and magnetoencephalography (MEG) research. Pérez-Alcázar et al. found that, when up-chirp was given in Parkinson's disease model rats, the frequency response in 40-60 Hz is reduced [118]. Poulsen et al. showed that the frequency of the peak response is increased in 25-55 Hz range for healthy adults [119]. In schizophrenia patients, the response amplitude was reduced in 30-50 Hz and 90-100 Hz range [120,121]. Ethridge et al. showed a decreased gamma phase-locking in the 30–58 Hz range in Fragile X syndrome mice [24].

CHAPTER 3

METHODS

3.1 Materials

In this research work, data from male Sprague Dawley rats were recorded over time and three types of acoustic stimulation tasks were employed on control (non-epileptic) rats and epileptic rats to study the EEG abnormalities related to their cognitive function over time. Details of the data acquisition, length of the data, and number of animals used are provided in the following subsections.

3.1.1 Data Acquisition

EEGs were recorded in control and epileptic rats at Louisiana Tech University, Ruston, LA in Dr. Levi B. Good's lab. The auditory event related tasks were performed on rats. Sampling rate for all modalities were all 2KHz. Adult male Sprague-Dawley rats aged 2-3 months (N=8) were anaesthetized and stereotaxically implanted with 8 intracranial tungsten electrodes (125-µm diameter) bilaterally in the frontal and parietal cortex, the hippocampus (CA1), and thalamus (CM), with one reference near bregma (Cz) and a ground electrode near lambda (Fig 3-1). Figure 3-1 shows the electrode placement in rats at particular positions, where, bilateral implantation coordinates in the hippocampus were anterior/posterior (**AP**), -5.6 mm, midline/lateral (**ML**) +/- 4.5 mm, Depth 5.9 mm. Bilateral implantation in the thalamus (central medial nucleus) was at AP -2.5 mm, ML +/- 1.5 mm, Depth 2.8 mm. Bilateral cortical electrodes were at AP 2.0 mm, ML +/- 3.0 mm,

Depth 1.0 mm and at AP -4.0 mm, ML +/- 3.0 mm, Depth 1.0 mm.

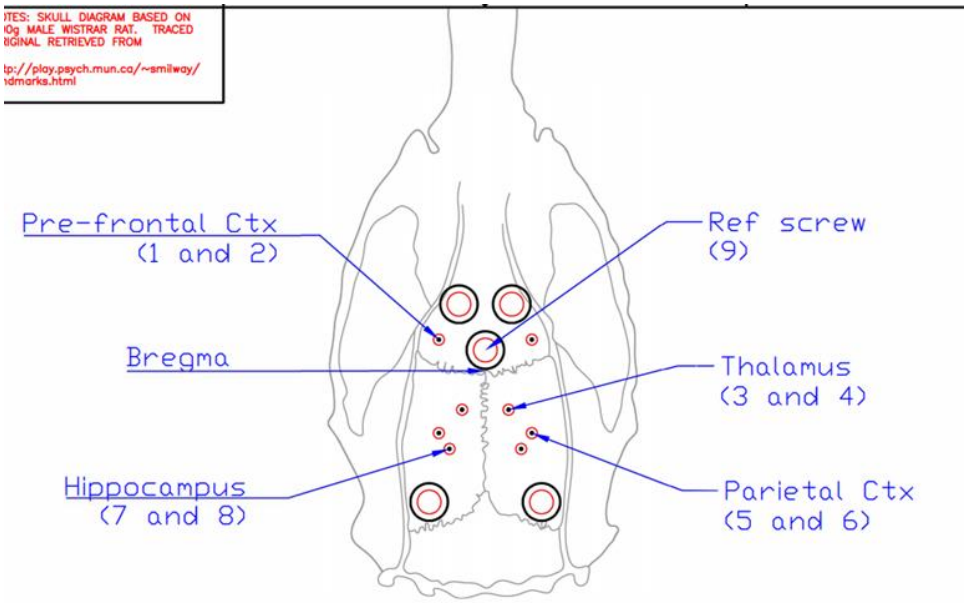


Figure 3- 1: Electrode placements on rats implanted with 8 intracranial tungsten electrodes bilaterally in the frontal and parietal cortex, the hippocampus (CA1), and thalamus (CM), one reference near bregma (Cz) and ground electrode near lambda.

Rats were allowed to recover for one day before recording 24 h of continuous EEG (sampled at 2 kHz). The lithium-pilocarpine animal model of epilepsy (LiCl-3mmol/kg 24 hrs. prior to 30 mg/kg pilocarpine) was used. One week after the electrodes were implanted, rats were pretreated with lithium chloride, and they were injected with pilocarpine one day after the lithium injection. These injections cause generalized convulsive status epilepticus (SE) for each rat. Rats were treated with diazepam 10 mg/kg and phenobarbital 25 mg/kg, approximately 3 hrs. into SE to aid in survival. SE causes an initial precipitating injury to the brain, and the rats exhibit spontaneously recurrent seizures (SRS) during the remainder of their life [122].

The experimental setup is shown in Figure 3-2. The rat was placed in sound isolation chamber and the ERP's were recorded during the auditory tasks. A Tucker-Davis

Technology's (TDT's) RZ6 high-frequency multi input/output auditory signal processor performed the auditory evoked response tasks. A subject interface (SI8) module was used to record the 32 channel EEG (from 2 rats at a time) at a sampling rate of 2 kHz. A MF1-Multifield magnetic speaker was placed inside the sound isolation chamber to deliver the sound tones and the setup consists of a monitor to record the ERP responses in rats.

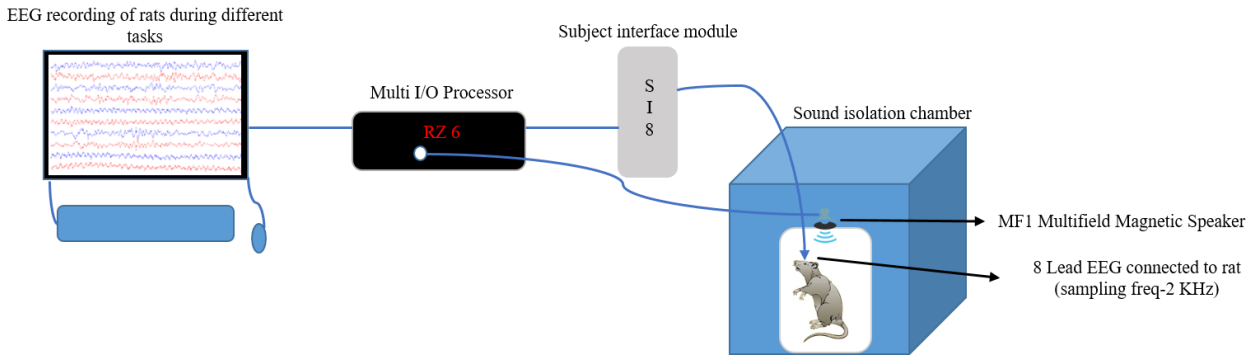


Figure 3- 2: Auditory ERP recording setup.

3.1.2 TDT System Overview

The recorded data were collected on the RZ6 multi-input/output processor from Tucker Davis Technologies (TDT). RZ6 is a core TDT system with a high-frequency auditory signal processor that can handle real-time signal generation, filtering, acquisition and analysis. It provides smooth signal generation and acquisition for frequencies up to 88 kHz and can sample the data up to 200 kHz. The analog inputs and outputs on the RZ6 are audio quality and have 24-bit resolution and over 115 dB of dynamic range. We used high quality signal amplifiers for data acquisition. The sound intensity for all the tasks were calibrated to 90 dB, inside the sound isolation chambers. All the recordings are made inter-ictally. The TDT system sampled the iEEG data at roughly 2034.5 Hz, and the sampled data were then converted into European Data Format (EDF) to be analyzed in MATLAB.

3.1.3 ERP Experiments on Rats

Habituation

The habituation test is a noninvasive spontaneous behavioral test used to study the ability to hear and discriminate between stimuli (such as sound) in humans and animals. The "response decrement as a result of repeated stimulation" is known as habituation, which was first reviewed by Harris in 1943. Thompson and Spencer in 1966 showed that, the behavioral response decreases (habituation), when any stimulus is repeatedly evoked, and without involvement of sensory or motor fatigue or sensory adaptation. This decrease in the response is usually a negative exponential function of the number of stimulus presentations [123]. When a stimulus is given repeatedly, the response decreases progressively through a decreases in its frequency content and/or its magnitude, towards an asymptotic level [124].

In this study, we used auditory stimulus and measured the ERP response by appropriate processing of the respective EEGs. The habituation task was delivered over 500 trials with a period of 4-sec between trials. Within each trial, four 4 Gaussian white noise sound pulses of 50 ms duration were administered. The bursts were separated by 500 ms (inter-stimulus) intervals. So each trial lasts for x50 ms + 3x500 ms = 1700 ms. Each stimulus trial/train is separated by a 4000 ms inter-trial interval. So, this test is completed in 500 x (4000+1700) ms, (47.5 minutes) per rat. Figure 3-3 shows an example of four trials for habituation is shown.

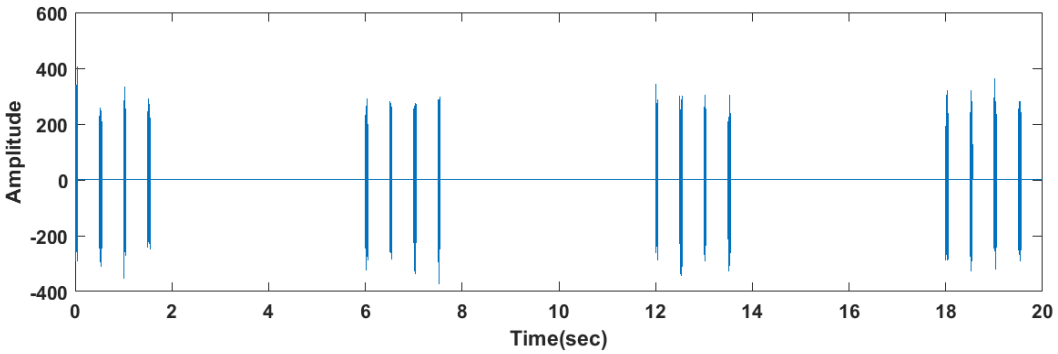


Figure 3- 3: Audio presentation of four trials for Habituation task, showing 4 Gaussian white noise sound pulses, with 50 ms duration, separated by 500 ms (inter-stimulus) intervals and each stimulus trial/train is separated by a 4000 ms inter-trial interval.

Chirp Modulation

A chirp signal is used as a stimulus to induce synchronized oscillations in animal EEG recordings across frequencies. The chirp was a sinusoid waveform, where its frequency increases or decreases linearly from 1 to 100 Hz [125]. A rapid transient change is observed in the EEG (from delta to high gamma frequency) as response to auditory stimulus of varying frequency, and it will be used to compare normal and abnormal groups of subjects in clinical and pre-clinical settings [126]. ITC analysis performed on the EEG responses measures the ability of the neurons to synchronize their oscillations to the frequencies present in the stimulus. This chirp stimulus has been used in studies with neurodevelopmental disorders like fragile X syndrome and Alzheimer's, as it is a fast and efficient way to measure multiple frequency modulations in a short period of time [125].

In this study, we used acoustic chirp stimulus, with a 2 sec Gaussian noise whose amplitude is modulated by a sinusoid waveform with an increasing frequency (Up-Chirp from 1 to 100 Hz). The goal is to study the auditory steady-state responses to different frequencies of stimulation and see whether the responses in gamma range (i.e. 30-50 Hz) are caused by an increase in the amplitude of the response at this particular frequency or

whether by an increase in the inter-trial phase locking [110]. Up chirp trains were presented 500 times (trains/trials of stimuli), separated by a 4000 ms inter-trial interval. This test takes about 50 minutes to complete per session. After the end of each session, inter trial coherence analysis was estimated on these ERP responses and the synchrony of the neuronal populations at different frequencies in response to the presented auditory stimulus was investigated. Figure 3-4 shows the Gaussian chirp modulated from 0-100 Hz over a 2 second interval. Figure 3-5 shows an example of four trials for chirp modulation task.

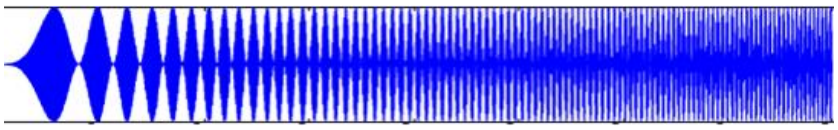


Figure 3- 4: Gaussian chirp modulated 0-100 Hz over 2 sec.

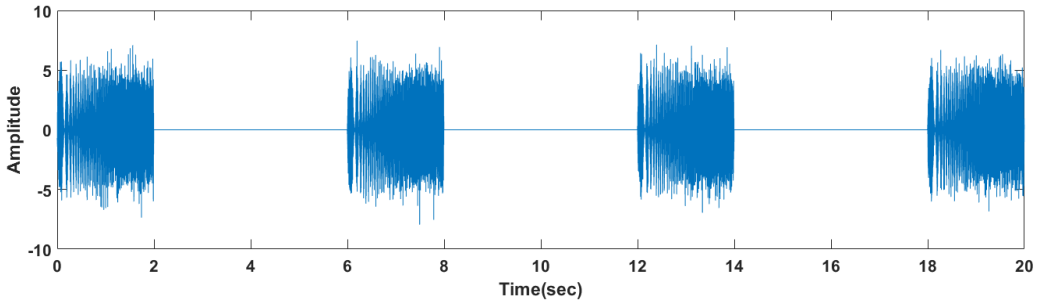


Figure 3- 5: Audio presentation of four trials for Chirp Modulation task, with a 2 sec Gaussian noise amplitude is modulated by a sinusoid waveform with a frequency of 1 to 100 Hz, separated by a 4000 ms inter-trial interval.

Odd Ball Paradigm for Associative MMN Task

In the event-related potentials, their P300 component (positive peak at 300 ms latency after onset of the stimuli) is related to decision making, attention, and memory updating and therefore is a valuable measure of the impairment of these processes in the human brain. P300 is the result of the summation of electrical activity from various widely distributed areas in the brain [127]. To measure pre-attentive information processing, the event related potential MMN will be used, which is a measured response to oddball sequences of auditory stimuli. In such an auditory ERP test, a repeated train of standard tones/stimuli is interrupted by infrequent stimuli (deviant/odd) tones. Deviant tones that differ in any characteristics, such as duration, intensity or frequency, are among the most frequently used stimuli in such investigations [127-129]. The MMN paradigm requires a certain number of trials for deviant tones, because the MMN amplitude can decrease with the number of deviant trials, as the subject may habituate to that particular stimulus [130].

In this study, subjects (rats) received an auditory discrimination task with standard tones of 1000 Hz and deviant tones of 2000 Hz with 45 ms duration. A total of 500 trials of auditory stimuli were conducted per session, out of which 400 were with standard stimuli and 100 with deviant stimuli. The presentation of standards and deviants was random with a 2000 ms inter-trial interval. Each session of this test takes about 16 minutes to complete. After each session, the ERP responses of the deviants (100 trials) and standards (400 trials) were averaged separately and plotted on the same graph to see the mismatch negativity in control and epileptic rats around 200-250 ms after the onset of the stimulus. All the three tasks take about 2.5 hrs. to complete per session. Each session is repeated biweekly over a

period of 3 months to monitor and quantify the impairment of cognitive networks over time as epilepsy is progressed. Figure 3-6 shows an example of ten trials for MN paradigm.

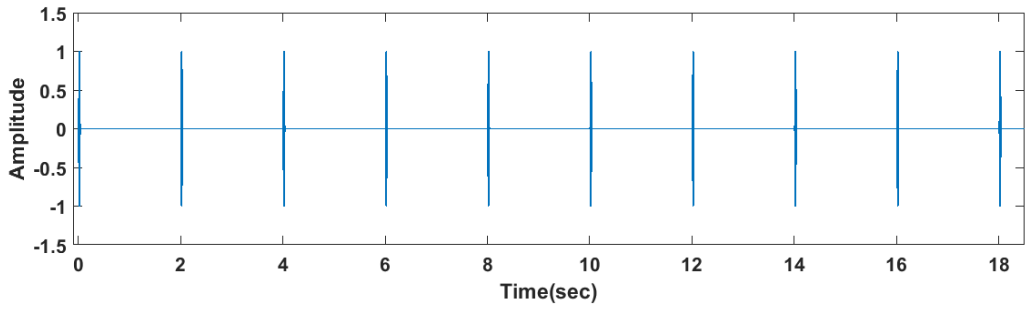


Figure 3- 6: Audio presentation of ten trials for MMN paradigm, with standard tones of 1000 Hz and deviant tones of 2000 Hz (seventh tone) with 45ms duration and the inter-trial interval is 2000 ms, between the trials.

Figure 3-7 shows an example of a series of standard tones (red-400 trials) and deviant tones (blue-100 trials) that were given randomly in the MMN task, and this sequence is constant across all the rats.

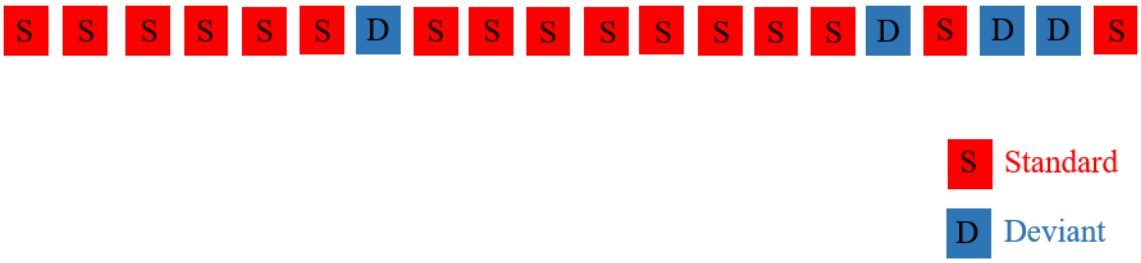


Figure 3- 7: Series of standard (16 trials) and deviant tones (4 trials) in an MMN task for the first 20 trials (showing a 4:1 ratio).

3.2 Data Preprocessing

The different artifacts in the auditory evoke response obtained while performing the ERP tasks necessitated filtering of the data and segmentation into epochs (with respect to audio signals played/trials) for computational analysis.

3.2.1 Preprocessing Rat Data

In order to distinguish between the control and epileptic groups, we have performed time-frequency analysis on auditory evoked responses from the EEG signal. We segmented the EEG-ERP data into segments (i.e. from 0-2.5 sec for each trial), that correspond with the auditory task for each trial. The ERP data for each of the eight rats were segmented according to auditory signals played in the different tasks (e.g. for habituation, chirp and odd ball tasks). While proper grounding and electrical shielding is done during analog recordings, the modern use of computers has suggested the investigation of digital approaches to solve power line interference problem. For the noise centered at 60 Hz, a notch 60-Hz filter was applied while recording the erp's from EEG to filter out any interference in the recordings from power sources (line noise). Post-segmentation, filtering techniques were employed; and a 2nd order digital Butterworth band pass filter (1 to 120 Hz) was applied. To correct the EEG baseline on the ERP segments, the average of the 0.5 sec EEG signal immediately before the auditory stimulus was determined and subtracted from the EEG data (to remove any normal neuronal activity during the stimulus times and only record the ERP responses). This process was applied to all 500 segments. Post filtering, we employed advanced and traditional signal analysis methods to extract measures (features) from ERP EEG segments, such as the time-frequency based features of inter trial coherence and event related spectral power (explained in next sections). These features were evaluated per frequency bin and averaged across all the 500 segments with respect to the bin size. We then used statistical methods to differentiate between controls and epileptic groups and see changes in cognitive processing (for example, attention span,

associative memory, and speed of mental processing) by experiments involving event related potentials (ERP's).

3.3 Methodology

3.3.1 Feature Estimation of the Generated ERPs:

The results from time-frequency analysis of single trial data were averaged across each frequency bin [131]. Time-frequency analysis provides a different perspective from time domain analysis alone [132]. Time-frequency decomposition of the EEG reveals individual brain oscillations. The ERD/ERS specific to different frequency bands is revealed and certain events that are amplified (synchronized) or blocked (desynchronized) in specific frequency bands are manifested by the increase or decrease of power of ERPs at particular frequency bands (spectral power), respectively. In addition to the spectral power, we will be looking into ITC. To implement these measures, we will use the EEGLAB toolbox in MATLAB programming software developed by Delorme and Makeig, 2004 [133]. Techniques for estimation of the different features from time-frequency domain are explained in the following subsections.

3.3.2 Inter-Trial Coherence (ITC)

Cortical oscillations play an important role in sensory and cognitive processes. Coherence of these oscillations tells us which groups of neurons communicate with each other [134, 135]. Through EEG analysis, these oscillations can be detected and patterns of brain activity can be examined from their identified relationships to specific stimuli (such as sound). Synchronous neural processes with rapid phase shifts (30-80 ms) and longer periods of phase locking (100-800 ms) of groups of neurons are contained within those ERPs. In addition, groups of neurons shift from one cluster of phase-locked neurons to

another during the test [136]. When a stimulus event is introduced to the brain, the event-locked distribution of EEG phase becomes one-sided, and the signal becomes "phase-locked" to that particular event [132]. This phase-locked EEG activity (characterized by the "phase locking factor") is measured by inter-trial coherence (ITC), and this temporal and spectral synchronization at particular times and frequencies provides valuable information about the underlying brain dynamics [137]. The formula to calculate ITC proposed by Delorme and Makeig [132], is

$$ITC(f,t) = \frac{1}{n}\sum_{k=1}^{n}\frac{F_k(f,t)}{|F_k(f,t)|}\ , \qquad \textbf{Eq. 3-1}$$

where n is the number of trials, $F_k(f,t)$ is the spectral estimate by short-time Fourier transform of trial k at frequency f and time t, and $||$ is the complex norm. The ITC measure takes values between 0 and 1, where 0 represents absence of synchronization between the EEG data across all channels and time locking events, and value of 1 indicates perfect synchronization.

3.3.3 Event-Related Spectral Power (ERSP)

Another method used to study the event-related brain dynamics of the EEG spectrum is the ERSP. The average dynamic changes in amplitude of the EEG frequency spectrum as a function of time relative to a set of external events/stimuli (such as sound) is measured by ERSP. These changes in frequency spectrum involve more than one frequency or frequency band. The ERSP was proposed by Delorme and Makeig [132] as

$$ERSP(f,t) = \frac{1}{n}\sum_{k=1}^{n}|F_k(f,t)|^2, \qquad \textbf{Eq. 3-2}$$

where n is number of trials, $F_k(f,t)$ is the short-time Fourier transform of trial k at frequency f over a sliding temporal time window t. The log-transformed measure is then derived by taking the log value of $ERSP$:

$$ERSP_{log}(f,t) = 10\ log_{10}(ERSP(f,t)), \qquad \textbf{Eq. 3-3}$$

The logarithmic scale offers two advantages, visualization of a wider range of power variations when compared to absolute scales (in absolute scale, the large changes in power at lower frequencies may mask changes in power at higher frequencies) and a more normal (Gaussian) distribution that is more conducive to statistical analysis (e.g. in assessing the statistical significance of the results). Parametric inference testing is often more valid when applied to log-transformed power values [138].

Figure 3-8, Panel (a) shows an example for estimating ITC and ERSP on three trials for a sine waves, with different amplitudes and phases. Figure 3-8 (b), shows an example of the values of ITC, and if all the trials i.e. ERP responses, are out of phase, then the ITC will have a value of 0.09. If all the ERP responses are in phase, then the ITC will have a value of 0.7 (concept adapted from Delorme and Makeig [132]).

Figure 3-8, Panel (a) shows an example for estimating ITC and ERSP on three trials for a sine waves, with different amplitudes and phases. Figure 3-8 (b), shows an example of the values of ITC, and if all the trials i.e. ERP responses, are out of phase, then the ITC will have a value of 0.09. If all the ERP responses are in phase, then the ITC will have a value of 0.7 (concept adapted from Delorme and Makeig [132]).

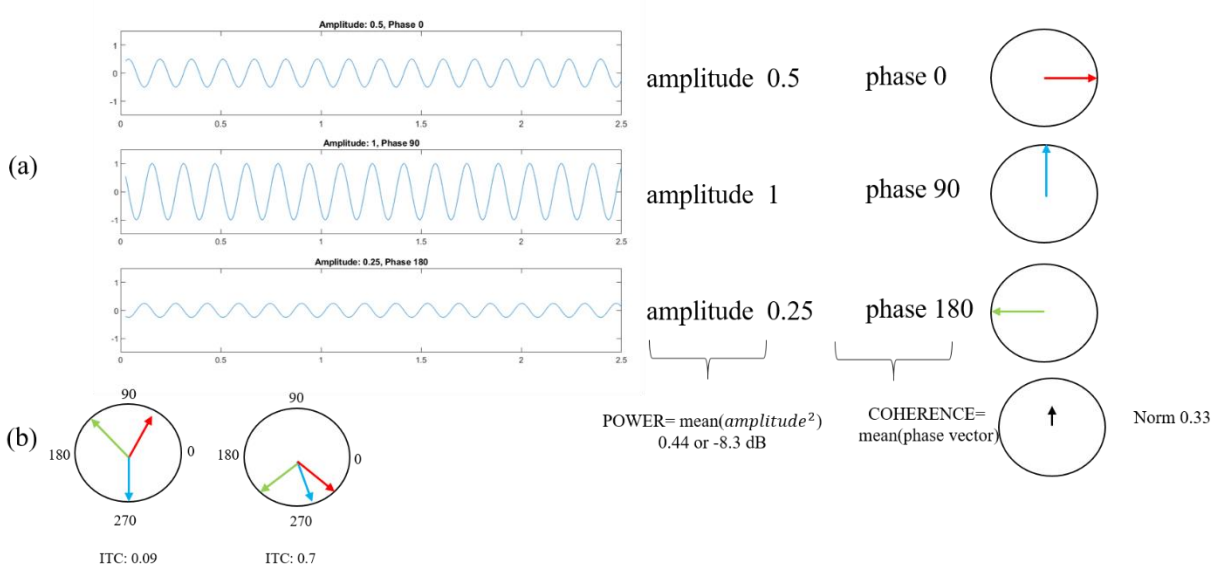


Figure 3- 8: (a) An example for estimating ITC and ERSP on sine wave for 3 trials with different amplitudes and phases (b) an example showing the value of ITC if all the trials are out of phase having a value of 0.09 and if all the trials are in phase having a value of 0.7 (concept adapted from (Delorme and Makeig [132])).

3.3.4 Baseline Correction:

In order to better visualize/capture the event-related changes in brain activity, the baseline is generally corrected to adjust the post-stimulus values by removing the values obtained for the normal EEG activity (pre-stimulus values i.e. baseline period), thereby leaving only the brain response activity caused by that particular stimulus. Some measures like ITC or phase coherence, may not have large significant values in the pre-stimulus (i.e. baseline) period, so a baseline correction may not substantially change the results. Also, when we have large values across trials, one can detect event-related changes easily when the baseline is corrected. So, in our study, the baseline was corrected only for ESRP values. This correction allows visualization of power changes across the frequencies (1-120 Hz for our research). Generally, in the time-frequency composition matrix (estimated with ERSP method described above), a baseline period is defined by the average of the values for each

frequency within a time window, prior to the time-locking event. Four methods of baseline correction are commonly used. The most common method is simply subtracting the baseline values from all the values in the TF matrix, so, for example, after estimating the ERSP, we subtracted the mean baseline log power spectrum values from each spectral estimate, thereby producing the baseline-normalized ERSP 2. In the second method, the baseline-subtracted values are divided by the baseline, thereby giving a percent change from baseline value. In the third method the value of each time point is divided by the baseline and this quotient is transformed to a logarithmic scale (base 10) and multiply it by 20, thus giving the output values in decibels (dB). In the fourth method the values are converted into z score (by subtracting the baseline from each value and then dividing the output by difference in the standard deviation of the values in the baseline period) [139].

One important consideration in defining baseline period is the duration of the baseline, as it will influence the EEG frequencies that are analyzed. Some studies showed that, use of longer baseline periods benefit the slower frequencies. Although no general rule of thumb governs the duration of baseline, if one wants to capture particular frequency of interest, one should use a baseline period that is long enough to capture that frequency. For example, to capture a 4 Hz frequency, one should use a baseline duration of at least 250 ms [139]. For our research, we have used the most common subtraction method, where the mean baseline activity is subtracted from each frequency within the epoch. We used a baseline duration of 500 ms.

3.3.5 N70 Peaks from Habituation

The ERPs elicited by auditory signals (i.e. evoked potentials) are described in a series of negative and positive peaks that occur at characteristic times [140]. The N70 is

the negative shift in the ERP waveform that occur at 70 ms after the onset of the stimulus and are related to inherent sensory information processing in all mammals that can hear [74,131].

For sensory information processing (i.e. hearing), habituation was the first task performed on rats/subjects, to see whether the rats can hear and habituate to the repeated tones. A decrease in the N70 peak amplitude following the first tone indicates proper habituation to the repeated stimuli. After the experiment is done, the ERP responses were quantified by the average N70 (70 ms peak in the ERP) over the 500 trials. An example of the average N70 peaks for four sound pulses for right hippocampus electrode (recorded before SE) is shown in Figure 3-9. Afterwards the 2nd, 3rd, and 4th N70 peaks are normalized to the 1st N70 peak, and this process is repeated across all rats (with good channels) and across all time points (before SE, one week after SE, one month after SE, two months after SE). Later, statistics are performed on the normalized data across all rats and across all time points.

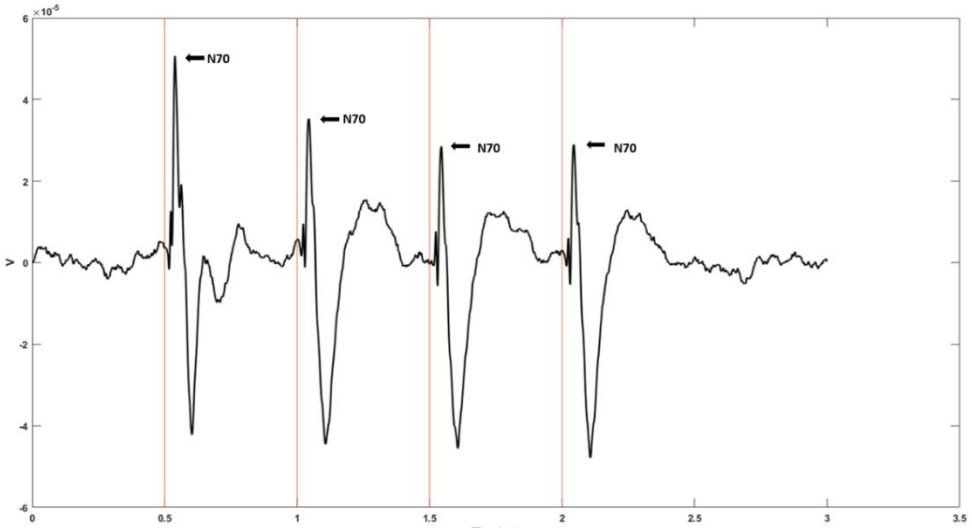


Figure 3- 9: An example of average of all 500 trials for one rat for right hippocampus electrode (recorded before SE). The arrows show the N70 peak for each sound pulse.

3.3.6 Area Under the Curve Around P300 Peaks from Oddball Task

P300 latency is the most common parameter used in research settings to infer potential auditory processing changes [141]. The P300, is a positive potential elicited by the recognition of a rare stimulus (oddball paradigm as described in the above sections) within a series of frequent stimuli. Kraus et al. showed P300 latency values between 250 and 350 ms in adults after the onset of the stimulus [142,143]. However, wider latency ranges of 220 to 380 ms were also reported by McPherson [144]. Researchers have shown that the P300 occurs at around 300 ms, and the peak latency will vary from 250 to 500 ms or more, depending on the individual subject's response. It depends on some abilities, such as memory, discrimination, and attention, and it reflects cortical activity [143].

The auditory mismatch negativity task was performed to access the associative memory, between control and epileptic groups. After the experiment was done, the average ERP response for each of the regular (400 trials) and odd (100 trials) tones is plotted on

the same graph. An example showing the average ERP response from MMN task for right hippocampus electrode (recorded before SE) is shown in Figure 3-10, where the red color is the regular tone and blue color corresponds to odd tone. Afterwards, the MMN ERP responses were quantified by the area under the curve (AUC) from 250-350 ms beyond the initial tone at 500 ms (Figure 3-10 around 750-850 ms) and the blue region in Figure 3-10 gives the area under the curve for one rat on left frontal electrode (from before SE). This process was repeated across all rats (with good channels) and across all time points (before SE, one week after SE, one month after SE, two months after SE) and statistics were performed on these areas across all rats and across all time points.

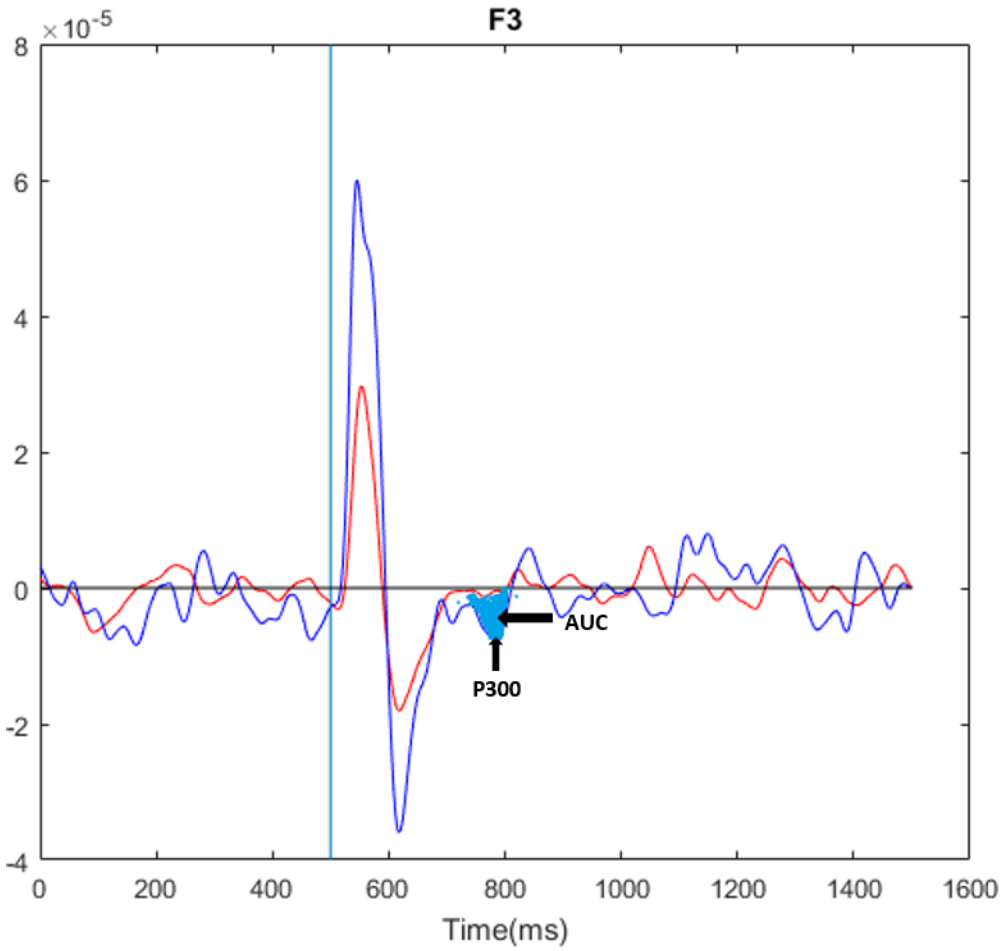


Figure 3- 10: An example of average of all 400 trials for standard tone (red line) and 100 trials for deviant tone (blue line), showing the area under the curve (AUC) (blue region), estimated at P300 (i.e. around 250-350 ms) for one rat on left frontal electrode (from before SE).

3.3.7 Repeated Measure Design

Repeated measure is a research design that involves measures of same variable taken on the matched or same subjects over two or more time periods or under different conditions [145]. Repeated measurements data are often called longitudinal data since they are collected in a way that allows change over time to be assessed. In such designs, the repeated-measure factor (i.e. biomarkers/measurements/responses) is the within-subjects factor, while the dependent quantitative variable on which each participant is measured is

the dependent variable [146,147]. In psychological and biomedical studies, this design is extremely common [146]. Behavioral science researchers often use repeated measure designs to assess treatment effects [148].

The advantages of this repeated measure design is that the subject can serve as its own control and it will decrease the number of subjects. For example, when studying the effects of treatment over time, it is desirable to observe the same subjects repeatedly rather than to observe different subjects at each time point [149]. This design, can be used efficiently, both in statistical sense, where we have less error variance with more statistical power, and in practical sense, where fewer subjects are needed [146]. The disadvantage of this model is that we cannot control the circumstances of obtaining the measurements, so the data may be unbalanced or partially incomplete [145].

In our study, the ERP experiments were performed before SE, one week after SE, one month after SE and two months after SE, for each of the 10 rats/subjects, and the biomarkers described above are estimated for all the rats and for all the channels. Figure 3-11 shows the general layout for the repeated measurements that are estimated, where n denotes the number of independent subjects from which repeated measurements/biomarkers are obtained, t_i denotes the number of time points for subject i, and y_{ij}be the response from subject i at time point j, where $j = 1, \dots, t_i$ and $i = 1, \dots, n$.

	Time Point				
Subject	1	...	j	...	t
1	y_{11}	...	y_{1j}	...	y_{1t}
⋮	⋮	⋱	⋮	⋱	⋮
i	y_{i1}	...	y_{ij}	...	y_{it}
⋮	⋮	⋱	⋮	⋱	⋮
n	y_{n1}	...	y_{nj}	...	y_{nt}

Figure 3- 11: An example of the general layout of repeated measure design experiment.

In our study we performed ERP experiments on 18 rats from 4 different cohorts, over 2 years, with each cohort being recorded for ~four months. From the set of 18 rats, 10 rats went through full three months of recording. Of the other 8 rats, 1 rat did not progress following for SE recordings, as it had low signal quality caused by a decrease in single to noise (SNR), 2 rats were removed from the study one month after the onset of SE recordings, as 1 lost its electrode implant and the other died in the middle of the recordings, 5 rats were removed from the study two months after the onset of SE recordings because 2 rats were losing their electrode implant, 2 rats died and 1 rat became disconnected. Table 3-1 summarizes the information regarding the ERP recordings on rat data over three months. While 10 rats went through full 3 months of recording, not all the channels had sufficient quality of EEG recordings, as some channels had high signal to noise ratio, and thus were omitted from our analyses. From Figure 3-11, our n size was 10, in which the ERP recordings were repeated at 4 time points, before SE, one week after SE, one month after SE, two months after SE, so $t = 4$.

Table 3- 1: Number of rats that were subjected to ERP experiments.

	Total	Before SE	One week after SE	One month after SE	Two months after SE
Total rats for ERP	18	17	15	10	10
Total rats that did not complete the study		1	2	5	0

3.3.8 Performance Metrics

Repeated Measure Analysis of Variance (rANOVA)

The equality of means between different groups is tested by performing ANOVA statistics, but for the repeated measure designs the commonly used statistical approach is repeated measures analysis of variance (rANOVA) [145]. As our experimental studies are done over a period of time, which involves study designs with repeated measurements (i.e. where for each subject, the response variable is measured at multiple points in time as described above), the repeated measure analysis of variance (rANOVA) statistical test is done, to test the hypothesis between different time periods [145]. The null hypothesis for an rANOVA assumes no significant difference among different time periods for within subjects, while the alternate hypothesis is that there is at least one significant difference among different time periods [146].

In this research for differentiating different time periods (i.e. before SE, one week after SE, one month after SE, and two months after SE, of rats, rANOVA was performed. The goal of this analysis was to determine whether the response/biomarkers were changing over time (i.e. controls vs epileptic rats), and rANOVA was used to assess statistically significant differences between the time periods for all rats were at the $\alpha = 0.05$ significance level. This rANOVA was performed across all subjects (n=8) and on all channels, for the

biomarkers, with the normalized N70 peaks/amplitudes (for second, third and fourth pulse in habituation task), area under the curve around 250-350 ms (i.e. P300) for odd ball mismatch negativity task, and point by point analysis for ITC and ERSP. *P* values < 0.05 were considered to denote statistical significance among different time periods.

3.4 Flowchart

The flowchart that outlines the various steps involved in our analysis, from data acquisition to feature extraction and statistical analysis for differentiating between control and epileptic groups using ERP responses from EEG of rat data is outlined in Figure 3-12.

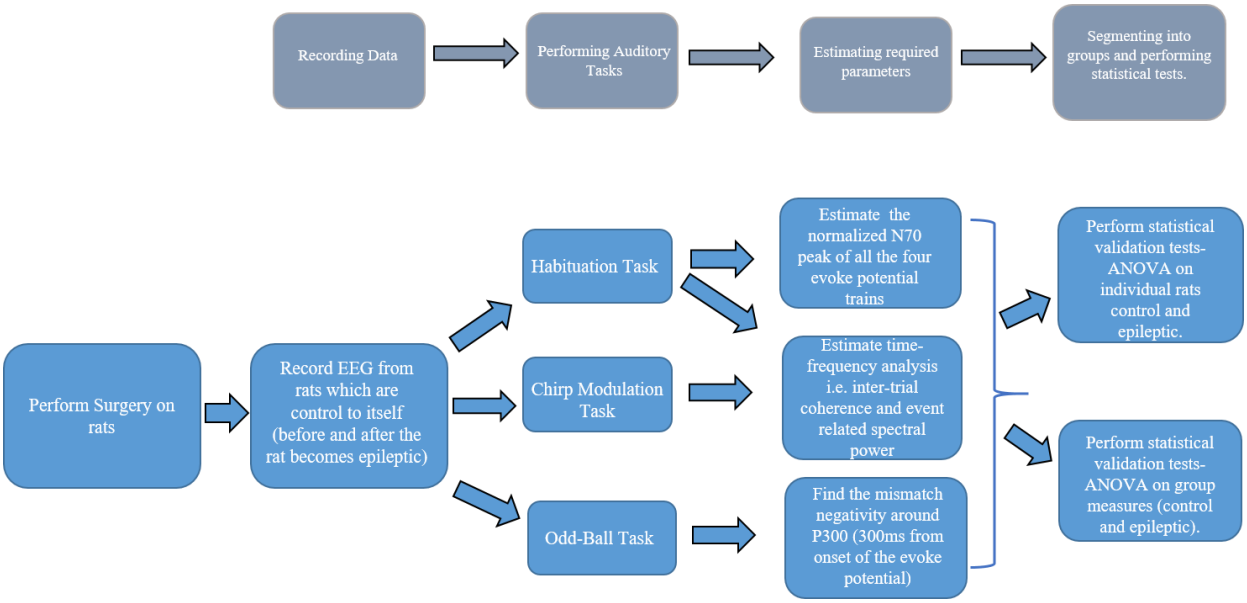


Figure 3- 12: Flow chart depicting the steps in our analysis.

CHAPTER 4

RESULTS

4.1 Habituation Results

The results from the habituation task are presented in three sub-sections. (1) Habituation ERP responses in the time domain are averaged across trials and subjects, and N70 peaks were quantified. (2) Time-frequency (TF) composition is used, where the inter-trial coherence is averaged across all trials and subjects. (3) The event-related spectral power, averaged across all the trials and across subjects, and baseline corrected event-related spectral perturbation are presented. We selected the electrode location that had the best signal quality, with a large N size, averaged across all subjects for the results. The results presented here are from right-hippocampal electrode with N = 8 subjects. The statistics were performed on the estimated biomarkers and a p-value less than 0.05 was deemed to show a significant difference across control and epileptic groups.

4.1.1 Normalized N70 Peaks from Habituation

Figure 4-1 shows the habituation ERP responses averaged across all 500 trials for before, one week after, one month after, and two months after SE for right-hippocampal electrode. These data are from one rat, and this process is followed across all subjects (N=8). The red vertical lines in the figure represent the sound pulses (at 0.5, 1.0, 1.5, and 2.0 secs). The N70 peaks are designated by the arrows following each pulse. These ERP responses were quantified by the average N70 (70 ms peak in the ERP) over the 500 trials,

for each subject and the 2nd, 3rd, and 4th N70 peaks were normalized by the 1st N70 peak. The mean and standard error of means (SEM) of four sound pulses recorded from right hippocampus from N=8 rats were evaluated and are presented in Figure 4-2.

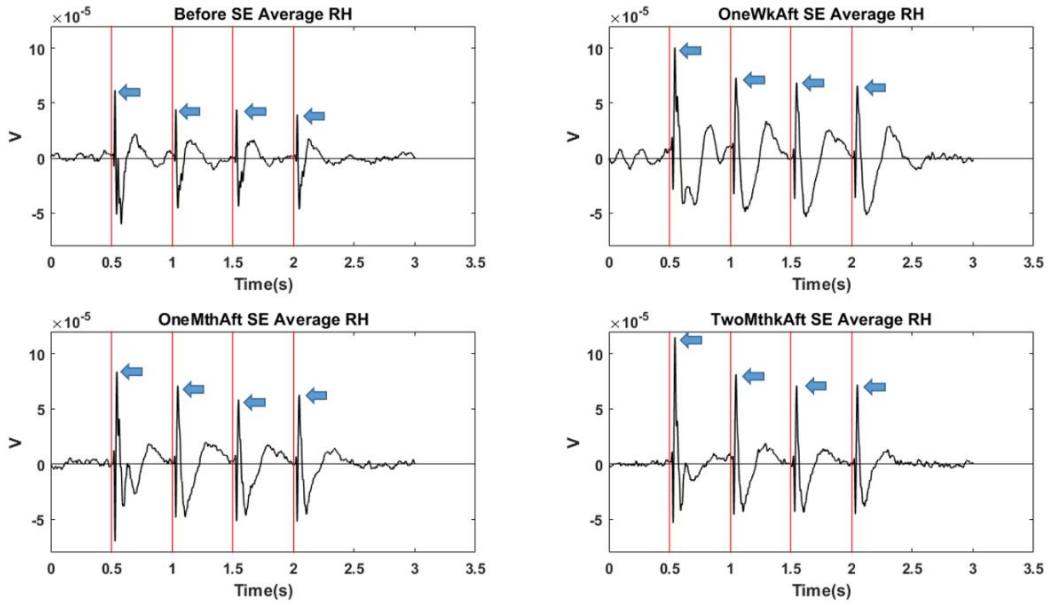


Figure 4- 1: Averaged ERP response (from 500 trials) for Habituation task performed (top left) before SE, (top right) one week after SE, (bottom left) one month after SE, and (bottom right) two months after SE, from right-hippocampal electrode (for 1 rat). Vertical red line represents the sound pulses at 0.5, 1.0, 1.5, 2.0 secs, with N70 peaks designated by the arrows following each pulse.

From Figure 4-2, the normalized N70 peak increases during epileptogenesis from 1 week, 1 month, and 2 months after SE, in the subsequent peaks for the second, third and fourth sound pulses. This indicates an imbalance between excitatory and inhibitory function, since the dampening in repeated sound pulses is diminished, thereby telling us that the epileptic rats cannot habituate to the repeated sound pulses. The normalized 2nd N70 peaks from 8 subjects at different times points- before, 1 week, 1 month and 2 months after SE is taken and the values are subjected to repeated measure ANOVA

statistical test, which revealed significant differences (p-value is less than 0.05) between the time periods-controls and epileptic groups. The same procedure was performed on the normalized 3rd and 4th N70 peaks from 8 subjects for before, 1 week, 1 month and 2 months after SE, and it revealed significant differences between time periods-controls and epileptic groups using the fourth pulse and it didn't show any significant difference using the 3rd pulse. The p-values are shown in Table 4-1. The normalized standard errors of mean for F3, LH, RT, LT electrodes is shown in the Figures A-1 to A-4 and its statistical significant differences and the p-values shown in Table A-1, in Appendix A.

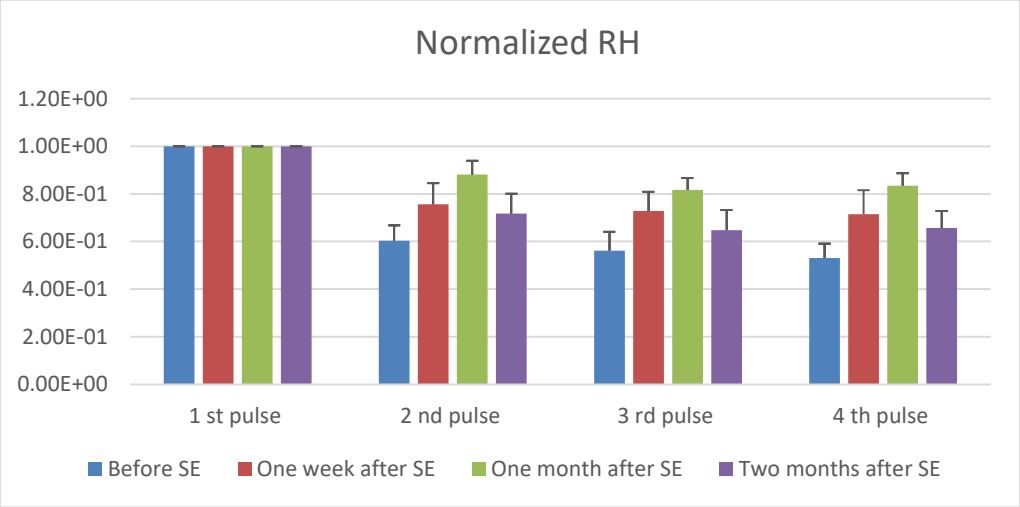


Figure 4- 2: Normalized N70 ERP response of four repeated pulses for before SE and during epileptogenesis (i.e. one week, one month and two months after SE) for N=8, mean±SEM.

Table 4- 1: Repeated measure ANOVA test with repeated measures of 2nd, 3rd, 4th pulses between the different groups (before SE, and one week after, one month after, two months after SE), for the habituation task.

Normalized N70 ERP response peaks	P-value
2nd pulse	0.0327
3rd pulse	0.091
4th pulse	0.0246

4.1.2 Inter-Trial Coherence (ITC) for Habituation

Time-frequency analysis was done on the ERP responses from habituation task and stFFT was used to estimate inter-trial coherence for every 100 data points (which is 50 ms) non-overlapping time window, at a particular frequency (from 1-120 Hz in a step of 1 Hz), which are averaged across all the 500 trials. This analysis was done to reveal the cortical oscillations of the brain and its ERP responses in time-frequency domain. This procedure was applied across all the rats (N=8) and the ITC values were averaged across subjects and plotted in the time-frequency domain for right hippocampal electrode, as shown in Figure 4-3. Figure 4-3a shows that the ITC values decrease in the frequency range of 20-60 Hz after the first pulse for the 2nd, 3rd and 4th pulses (during before SE state, i.e. control group, N=8), suggesting that the control group of subjects are habituating to the repeated stimuli, and the excitation is less for the 2nd, 3rd and 4th stimuli. Similarly, as the same group of rats progress through epileptogenesis, the ITC value increases after the 1st sound pulse for one month and two months after SE which can be seen in Figure 4-3 (c and d), for one month and two months after SE (when compared with before SE group), indicating more

excitation in the epileptic group of rats. This result indicates a lack of synchrony, and hence communication, in the neuronal populations as the rat's progress through epilepsy.

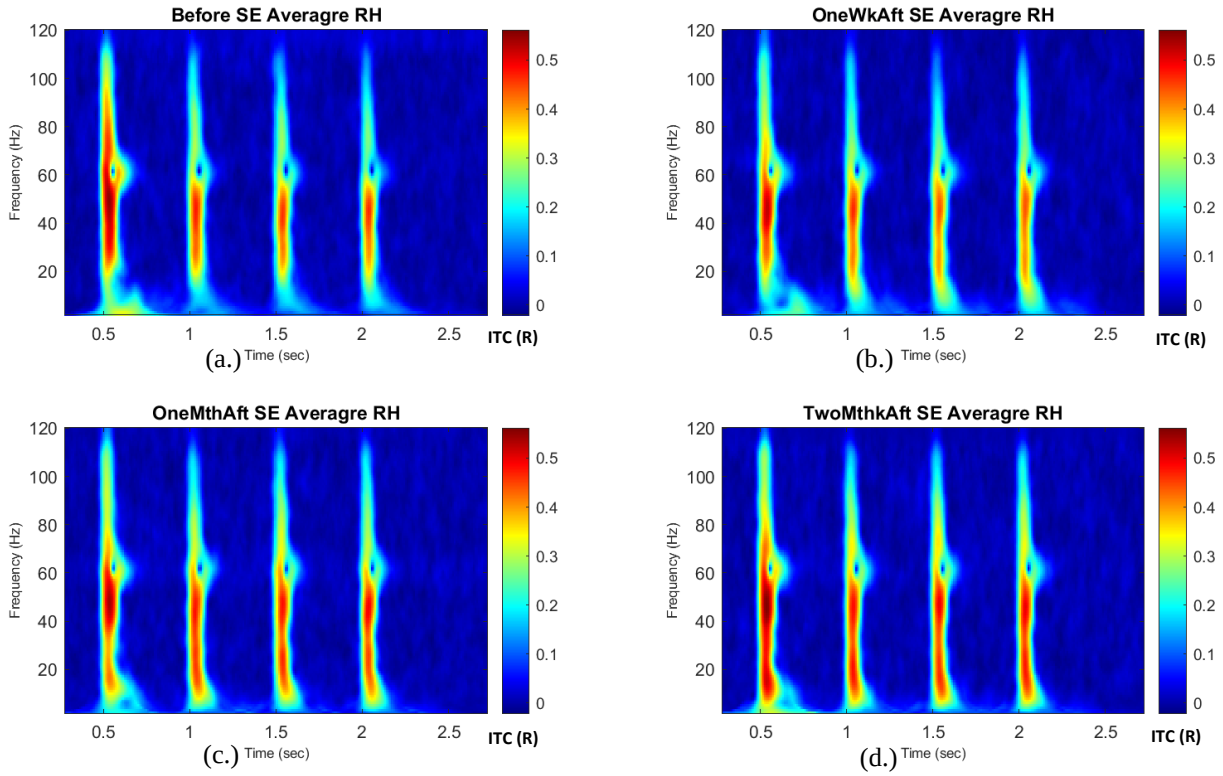
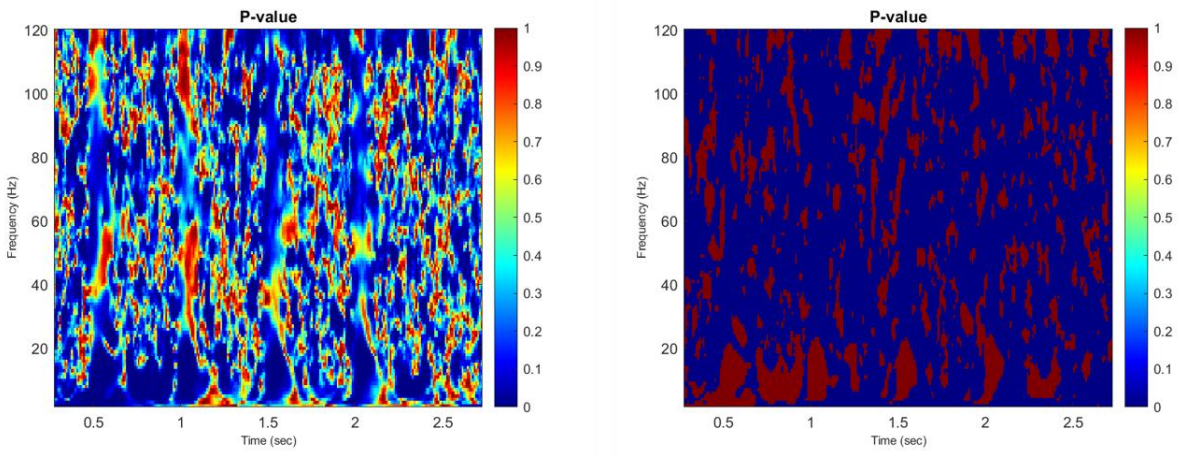


Figure 4- 3: Averaged ITC values (from 500 trials, across all subjects N=8) of four repeated sound pulses played at 0.5, 1.0, 1.5 and 2.0 secs for (a.) before SE and (b., c., d.) during epileptogenesis, for RH electrode.

To show statistical significant difference among different time periods- before, 1 week, 1 month and 2 months after SE, the ITC values (from all subjects N=8), at each time point (which is 50 ms of data) and for each frequency (from 1-120 Hz in steps of 1 Hz) were subjected to repeated measure ANOVA test. This is done by point-to-point group comparison (which consists of 3-d matrix of 119 by 250 by 8 dimensions, where 119 is frequency (y-axis), 250 is time points (x-axis) and 8 is number of subjects (z-axis)) across control and epileptic time periods and the same procedure is followed for the ERSP, base

line correction for habituation task and ITC for chirp modulation task. For each time point and each frequency, we get a p-value and the resulted p-values are plotted in time and at particular frequency in Figure 4-4 (a). When the sound pulses are played at 0.5, 1.0, 1.5, 2.0 sec, results show significant differences in 0.5-20 Hz frequency bands (delta, theta, alpha and low beta (<22 Hz)). It also showed slight significant differences in other frequency bands, when the sound pulses were played as seen in Figure 4-4 (b.). The red color indicates a significant difference at particular frequency bands. This plot indicates that the epileptic rats did not habituate to repeated stimuli as a result of either imbalance in inhibitory and excitatory neurotransmitters or increase in excitatory neurotransmitters in epileptic groups. ITC and p-values for other electrodes are shown in Appendix A (Figures A-5 to A-8).



(a.) **(b.)**

Figure 4- 4: (a) P-values from repeated measure ANOVA, and (b) P-values < 0.05, where red shows significant differences at delta, theta, alpha and low beta frequencies and blue color shows no significant differences at other frequencies (among control and epileptic groups), when the four repeated sound pulses are played at 0.5, 1.0, 1.5 and 2.0 secs, for RH electrode.

4.1.3 Event Related Spectral Perturbation for Habituation

Apart from ITC, we estimated the ERSP, from stFFT for every 100 data points (50 ms of data) non-overlapping time window, from 1-120 Hz frequencies in a step of 1 Hz, which are averaged across all the 500 trials. The ERSP measures the mean event-related changes in the EEG power spectrum as a function of time relative to a set of four sound pulses/stimulus. This procedure is done across all the rats (N=8) and the grand average (across subjects) ERSP values are plotted in the time-frequency domain for right hippocampal electrode, shown in Figure 4-5. The power increases in 0.5-10 Hz (delta and theta) frequency bands and the 60-100 Hz (high gamma) as the rat's progress through epilepsy, when compared with the control group (see Figure 4-5 (a)). The increase in these frequency bands were further verified with rANOVA statistical test, which is discussed below.

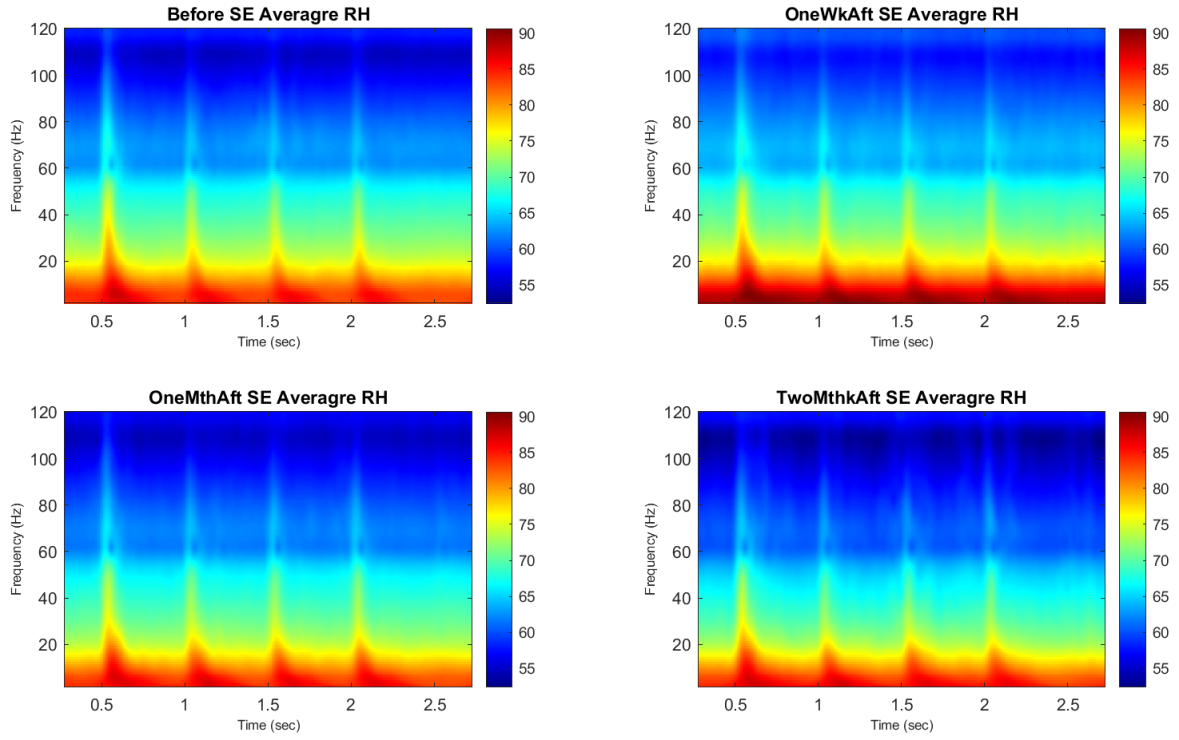
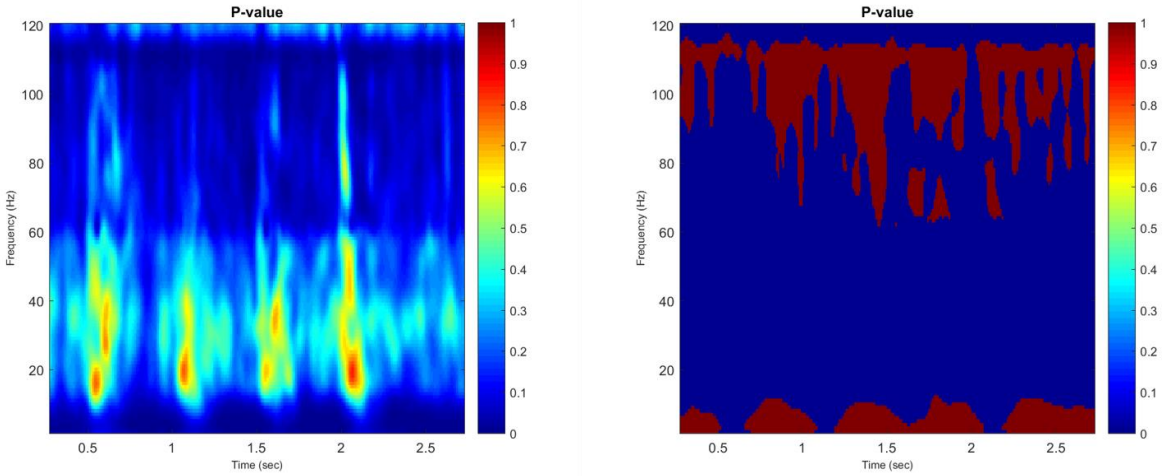


Figure 4- 5: Averaged ERSP values (from 500 trials, across all subjects N=8) of four repeated sound pulses played at 0.5, 1.0, 1.5 and 2.0 secs for (a) before SE and (b, c, and d) during epileptogenesis, for RH electrode.

The ERSP responses were compared between different time periods- before, 1 week, 1 month and 2 months after SE, that is control and epileptic rats in order to evaluate the spectral power. The ERSP values (from all subjects N=8), at each time point (which is 50 ms of data) and for each frequency (from 1-120 Hz in steps of 1 Hz) were subjected to rANOVA test, which is done by point-to-point group comparison (as described earlier) across control and epileptic time periods. For each time point and each frequency, we get a p-value and the resulted p-values are plotted in time and at particular frequency in Figure 4-6a. Figure 4-6b shows red as significant differences, where p-value is less than 0.05 and blue showing no difference. The figure shows phase-locking in the higher gamma range, associated with decreased spectral power, suggesting an overall increase in neural

background activity (in epileptic rats), which contributes to both hyperexcitability and disorganization (decreased ability to 'lock in' or synchronize gamma oscillations to the stimulus). This phase locking tells us that the neuronal activity in epileptic rats is high at higher gamma band (60-100 Hz) during the pre-stimulus (i.e. when no external auditory stimulus given in the baseline) and post stimulus activity (i.e. when an external auditory stimulus is given), indicating an increase in excitation of neuronal firing, irrespective of the pre and post stimulus. When baseline was corrected no statistically significant differences were seen in the higher gamma frequency (60-100 Hz) range, as the pre-stimulus EEG activity and post stimulus EEG activity in higher gamma frequencies cancel each other out (baseline correction), i.e. higher gamma frequency range power cancels out, leaving out only the lower frequencies below 30 Hz responding to the stimulus.

The plots for F3 and the left hippocampus electrode shown in Appendix A, from Figures A-9 to A-12, where F3 did not show significant differences among the groups, but the left hippocampus showed significant differences in the 55-75 Hz frequency range.



(a) **(b)**

Figure 4- 6: P-values when the four repeated sound pulses are played at 0.5, 1.0, 1.5 and 2.0 secs, for RH electrode, for repeated measure ANOVA. (a) P-values. (b) P-values < 0.05, where red color showing significant differences at (0.5-15 Hz and 65-100 Hz) and blue color showing no significant differences (among control and epileptic groups).

4.1.4 Baseline Corrected ERSP for Habituation

The ERSP values are baseline-corrected to see the power changes across the frequencies from 0.5-120 Hz. The correction was made across all subjects, where the average of the baseline corrected values were plotted in the time-frequency domain as shown in Figure 4-7 for right hippocampal electrode. The brain's response for repeated stimuli is visible only after the baseline EEG activity during the stimulus is removed. The baseline corrected values were increased in 5-20 Hz frequency range as the rats progress through epileptogenesis (seen in the first sound pulse at 0.5 sec). Similarly, the baseline-corrected values increased during epileptogenesis in the alpha frequency range for 2nd, 3rd and 4th sound pulses.

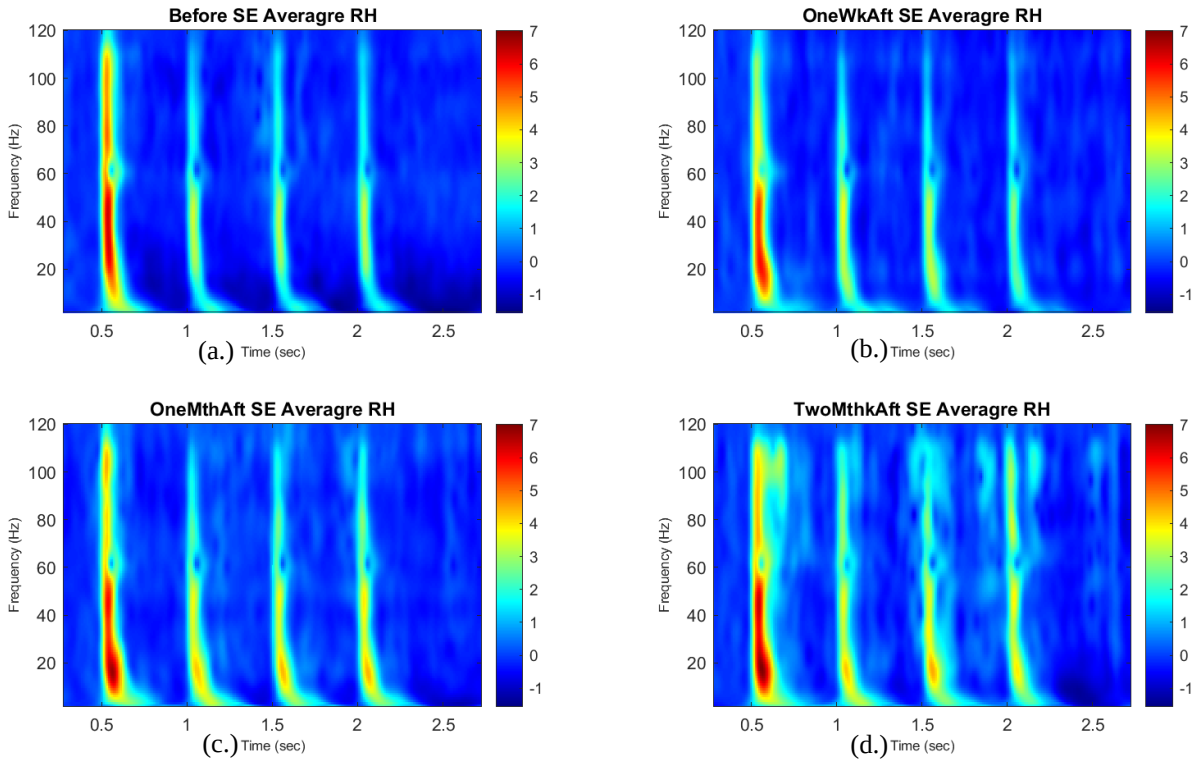
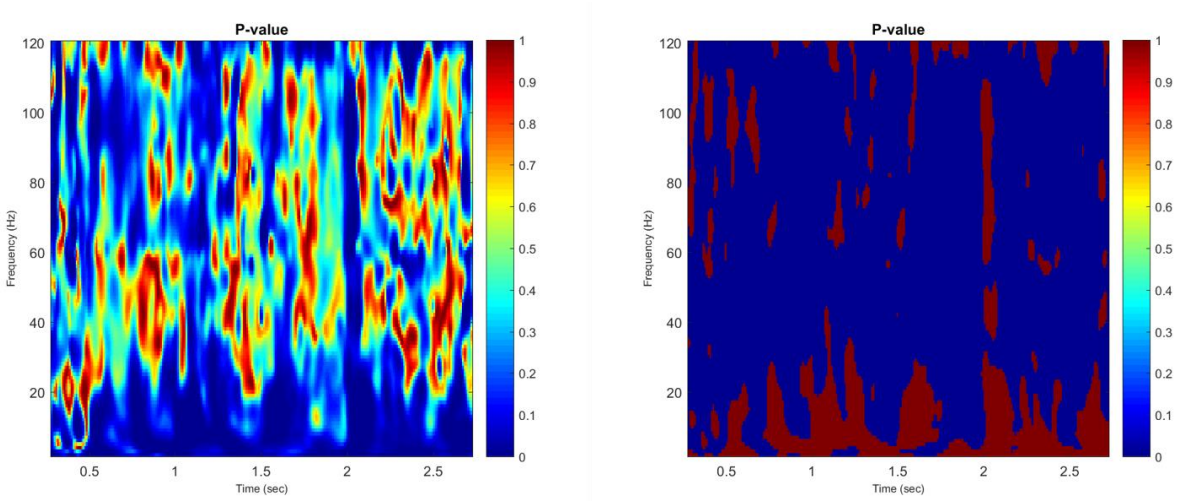


Figure 4- 7: Baseline corrected averaged ERSP values (from 500 trials, across all subjects N=8) of four repeated sound pulses played at 0.5, 1.0, 1.5 and 2.0 secs for (a) before SE and (b, c, d) during epileptogenesis, for RH electrode.

Statistically significant difference between different time periods- before, 1 week, 1 month and 2 months after SE (that is control and epileptic group of rats) is evaluated using the baseline-corrected values (from all subjects N=8). These values at each time point (which is 50 ms of data) and for each frequency (from 1-120 Hz in steps of 1 Hz) were subjected to rANOVA test, which is done by point-to-point group comparison (as described earlier) across control and epileptic time periods, and we get a p-value for each time point and each frequency, and the resulted p-values are plotted in time and at particular frequency, as shown in Figure 4-8a. The p-values < 0.05 shown red indicate significant differences between the controls and epileptic groups around 1-30 Hz (delta-beta), when

the sound pulses are being played at 0.5, 1.0, 1.5, 2.0 sec. This tells us that, the epileptic groups have higher power changes than the controls (seen from Figure 4-7 in the time-frequency domain), revealing that the rat's ability to discriminate between the repeated stimuli is diminished, thereby indicating that the epileptic group of rats are not habituating to repeated stimuli (due in increase in excitation).



(a.) **(b.)**

Figure 4- 8: P-values from repeated measure ANOVA (left fig (a.)), and P-values < 0.05, where red color showing significant differences at (0.5-35 Hz) and blue color showing no significant differences (among control and epileptic groups) (right fig (b.)), when the four repeated sound pulses are played at 0.5, 1.0, 1.5 and 2.0 secs, for RH electrode.

A similar pattern was observed in the left hippocampal electrode. Plots are shown in appendix A, from Figures A-13 to A-16.

4.2 Chirp Results

In this section, the results from the chirp task are presented in two different sub-sections, (1) the average Chirp ERP responses in time domain, and (2) time-frequency (TF) composition, by estimating the inter-trial coherence averaged across all the trials and across subjects. We selected the electrode location with the best signal quality, with a large N size, averaged across all subjects for the results. The results presented here are from left-hippocampal electrode with 7 subjects. The statistics were performed in a point by point analyses, on the estimated ITC values (i.e. biomarkers), and the p-value <0.05 was deemed to show significant difference at each frequency among control and epileptic groups.

4.2.1 Averaged EEG for Chirp Stimulus

Figure 4-9 shows the chirp ERP responses averaged across all 500 trials, for before, one week after, one month after, and two months after SE for left-hippocampal electrode. These responses shown in figure 4-9 are from one rat, and this process is followed across all subjects (n=7). The red vertical line in the figure represents the up-chirp sound pulse (from 0.5-2.5 secs). As, we cannot get much information regarding the EEG frequencies from the average chirp EEG in the time domain, we transformed to the time-frequency domain and investigated the auditory steady-state responses to different frequencies of stimulation and examined the responses in different frequency bands for inter-trial phase locking.

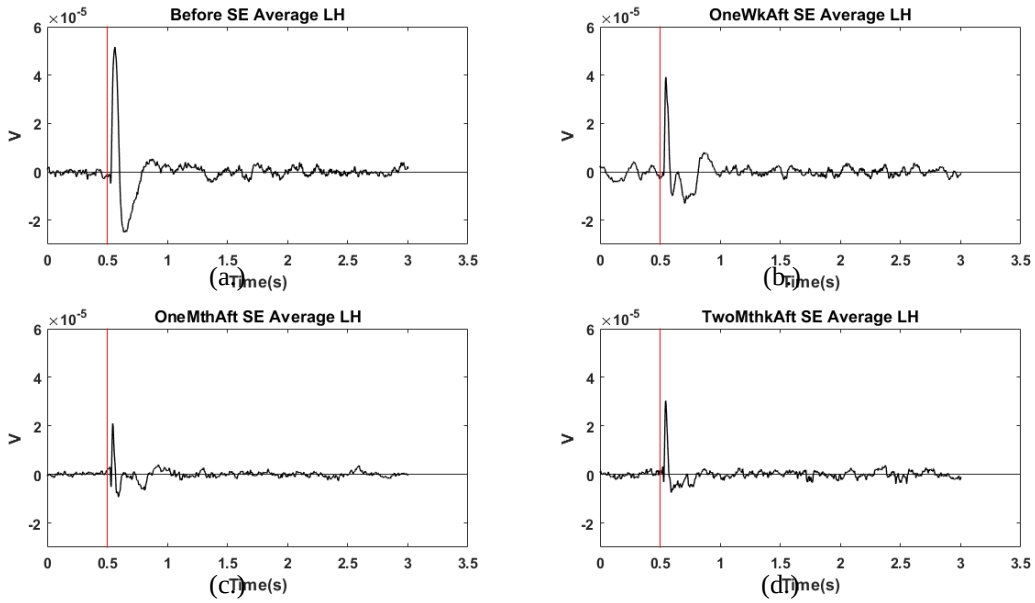


Figure 4- 9: Averaged ERP response (from 500 trials) for chirp task, performed (a) before SE, (b) one week after SE, (c) one month after SE, and (d) two months after SE from left hippocampal (LH) electrode (for one rat), where the vertical red line represents the start of the chirp stimulus at 0.5 secs trough 2.5 secs.

To perform time-frequency analysis on the ERP responses from the chirp task, the stFFT was used to estimate inter-trial coherence for every 100 data point non-overlapping time window at a particular frequency (from 1-120 Hz in a step of 1 Hz). Coherences were averaged across all the 500 trials to see the cortical oscillations of the brain. This procedure was done across all the rats (N=7) and the ITC values were averaged across subjects and plotted in the time-frequency domain for the left hippocampal electrode, as shown in Figure 4-10. The ITC values increases from 80-100 Hz during one week, 20-55 Hz and 60-100 Hz during one month and 40-55 Hz and 60-100 Hz during two months after SE, when compared with before SE group of rats, indicating synchronous activity in the neuronal populations within the high gamma band, 60-100 Hz, as the rats progress through epilepsy.

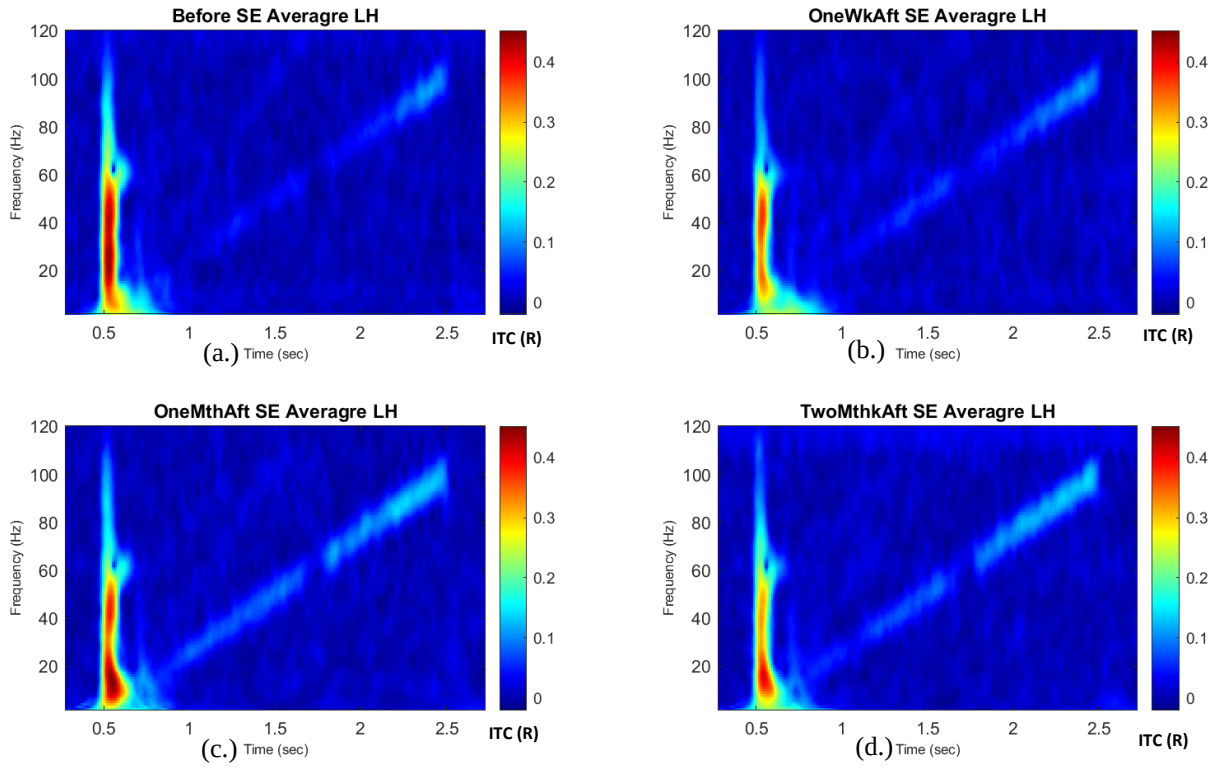
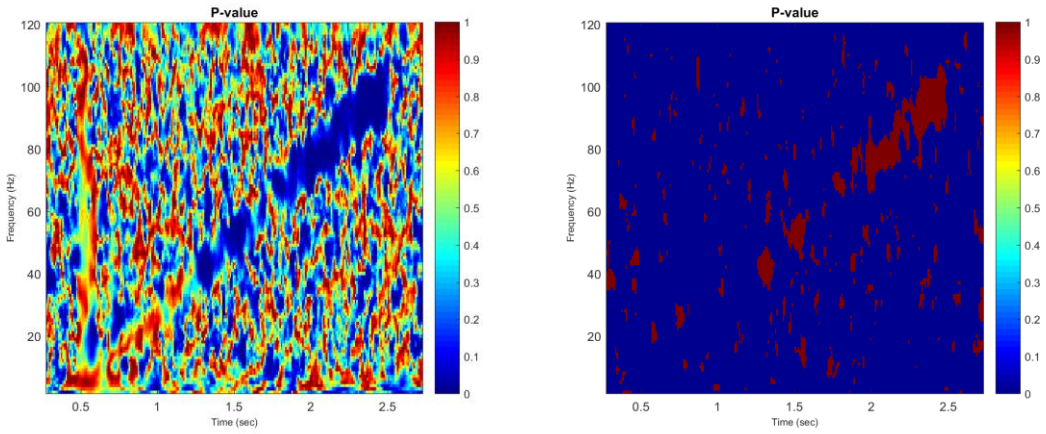


Figure 4- 10: Averaged ITC values (from 500 trials, across all subjects N=7) of chirp stimulus starting at 0.5 secs to 2.5 secs for (a) before SE and (b, c, d) during epileptogenesis, for LH electrode.

The ITC chirp-evoked responses were compared between different time periods-control and epileptic group of rats, in order to evaluate a potential of patterns from brief chirp stimulation to highlight the brain's impaired ability to synchronize to chirp stimuli. The ITC values (from all subjects N=7), at each time point (which is 50 ms of data) and for each frequency (from 1-120 Hz in steps of 1 Hz) were subjected to rANOVA test, which is done by point-to-point group comparison (as described earlier) across control and epileptic time periods. For each time point and each frequency, we get a p-value and the resulted p-values are plotted in time and at particular frequency, as shown in Figure 4-11a.

From Figure 4-11b shows that the controls and epileptics showed significant differences in high gamma band (35-60 Hz and 70-100 Hz), as p-values were below 0.05. This result indicates that the chirp modulated stimuli revealed impaired brain ability to synchronize at high gamma ranges in epileptic subjects as a result of lost inhibitory or increased excitatory neurotransmitters in epileptic groups. This pattern was also found in the right hippocampal F3 (left frontal) electrodes, as shown in Figures B-1 to B-4 of Appendix B.



(a) **(b)**

Figure 4- 11: (a) P-values using repeated measure ANOVA and (b) P-values < 0.05, where red color showing significant differences at (25-55 Hz and 60-100 Hz) and blue color showing no significant differences (among control and epileptic groups). The chirp stimulus is played at 0.5 through 2.5 secs, for LH electrode.

4.3 Odd-Ball Results

In this section, the results from the MMN/odd-ball task are presented, including (1) average odd-ball ERP responses in the time domain, and (2) the area under the curve at around the P300 peak (250-350 ms), after the onset of the stimulus. The results presented here are from left-hippocampal electrode and the statistics were performed on the standard

error bars of the means (N=5 subjects). A p-value <0.05 was deemed to show significant difference among control and epileptic groups.

4.3.1 Averaged EEG for MMN

Figure 4-12 shows the odd-ball ERP responses averaged across all 400 trials of standard/regular tones (red) and 100 trials of deviant/odd tones (blue), for before, one week after, one month after, and two months after SE for left-hippocampal electrode from one rat. The red vertical line in the figure represents the time at which the sound pulse is played (at 500 ms).

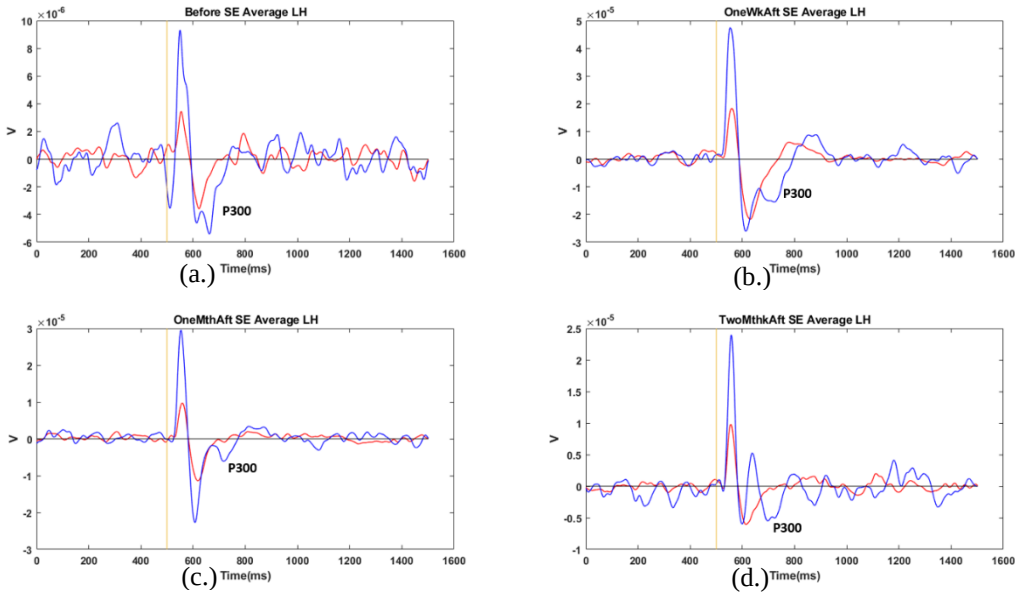


Figure 4- 12: Averaged ERP response ((Red: regular tone (400 trials), Blue: odd tone (100 trials)) for MMN/Odd-ball task performed (a) before SE, (b) one week after SE, (c) one month after SE, and (d) two months after SE from left-hippocampal (LH) electrode (for 1 rat), from left-hippocampal electrode (LH) (for 1 rat), where the vertical line represents tones played at 500 ms.

MMN ERP responses were quantified by the area under the curve (AUC) from 250-350 ms beyond the initial tone for each of the regular (400 trials) and odd (100 trials) tones in N=5 rats (Figure 4-13). As P300 is associated with memory, we looked at the area under the curve (AUC) around P300. A large AUC means the odd tone is distinguished from the regular tone and represents associative memory. During epileptogenesis, the rats appear to lose their ability to distinguish the odd and regular tones by the declining AUC (Fig 4-13). The rANOVA test, between the different groups (before SE, and one week after, one month after, two months after SE), for MMN task on left hippocampal electrode shows statistically significant difference (p-value is 0.0002, which is less than 0.05), between control and epileptic groups, which show that associative memory in rats is affected during epileptogenesis.

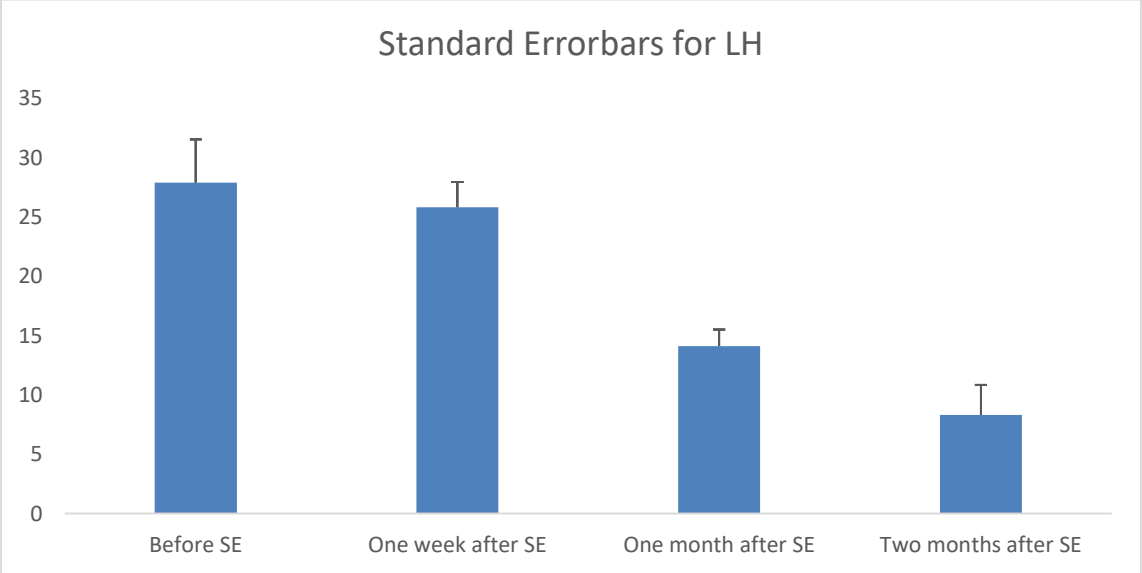


Figure 4- 13: MMN AUC around P300 peak (250-350 ms) for before SE and during epileptogenesis (N=5, mean±SEM).

The same procedure was repeated for all other electrodes. The area under the curve and the p-values for all of the electrodes are shown in Appendix C.

CHAPTER 5

CONCLUSIONS

Patients with epilepsy suffer from more cognitive and behavioral deficits than the general population. To study the long term effects of epilepsy, we developed a novel method where the subject is exposed to different auditory stimuli. The auditory ERP's are simple unbiased non-invasive tasks that are useful in evaluating neural systems for those suffering from epilepsy. Features (Normalized N70, ITC, ERSP and P300) were estimated from the EEG signals of each rat. The estimated values were averaged across rats and the repeated measure ANOVA statistics were evaluated. The statistics showed significant difference among the controls and epileptic rats (P-value < 0.05).

Habituation task: The normalized N70 peaks from the four pulses across all the rats showed significant differences among the controls (before SE) and epileptics (one week, one month and two months after SE), in the right hippocampus, thereby, showing imbalances in excitatory/inhibitory functions in epileptic groups, as these group of rats did not habituate to the repeated stimuli.

The ITC value increased, after the 1st sound pulse, for one week, one month and two months after SE in right hippocampal electrode, indicating an increase in excitation in epileptic groups. As the rat's progress through epilepsy, synchrony and communication diminished in the neuronal populations during the auditory tasks. Repeated measure

ANOVA revealed significant differences in 0.5-20 Hz frequency bands (delta, theta, alpha and low beta (<22 Hz)), when the sound pulses were played at 0.5, 1.0, 1.5, 2.0 sec.

With ERSP responses, we saw increases in power in the frequency ranges of 0.5-10 Hz and 60-100 Hz in the right hippocampus and in the frequency range of 55-75 Hz in the left hippocampus, as the rat's progress through epilepsy. Also, phase locking decreased in the higher gamma range, associated with decreased spectral powers. The decrease suggests an overall increase in neural background activity in epileptic subjects, which contributes to both hyperexcitability and disorganization (decreased ability to 'lock in' or synchronize gamma response to the stimulus). The repeated measure ANOVA revealed significant differences in 0.5-10 and 60-100 Hz frequency ranges.

When ERSP responses were baseline-corrected, the power difference values increased in in the 5-20 Hz range during epileptogenesis during the first sound pulse played at 0.5 sec. Similarly, the baseline corrected values increased during epileptogenesis in the alpha frequency range for 2nd, 3rd and 4th sound pulses in right hippocampal electrode. These increased power changes in the epileptic groups indicate that the rat's ability to discriminate between the repeated stimuli is diminished, thereby indicating that the epileptic group of rats are not habituating to repeated stimuli (due in increase in excitation). Repeated measure ANOVA shows significant differences between the controls and epileptic groups around the 1-30 Hz (delta-beta) frequency range. In summary, when the habituation tasks were performed, the rats did not habituate to repeated stimuli during epileptogenesis, due to imbalances in excitatory/inhibitory functions.

The normalized N70 peaks from the four pulses showed significant differences among the different time periods, thereby, showing imbalances in

excitatory/inhibitory functions in epileptic groups, as the epileptic group of rats did not habituate to the repeated stimuli. This was the same with FXS syndrome, as Ethridge et al. showed that the percent decrease in N1 amplitude from the initial N1 peak at 2^{nd} and 3^{rd} tones was diminished in FXS patients, when compared to the control group, for habituation task, [24].

Chirp Modulation: The inter-trial coherence revealed an increase in neuronal synchrony (i.e. the signal from neurons resonating with respect to chirp sound pulse is same across the trials and across the subjects) at 80-100 Hz during one week, 20-55 Hz and 60-100 Hz during one month and 40-55 Hz and 60-100 Hz during two months after SE, when compared with before SE group for left hippocampal electrode. When ITC values were subjected to repeated measure ANOVA, it showed significant differences in high gamma band (35-60 Hz and 70-100 Hz) between the controls and epileptics, and we could detect the brains' ability to synchronize at high gamma ranges in epileptic subjects, using chirp modulated stimulation.

In this study, the ITC revealed an increase in phase locking 20-55 Hz and 60-100 Hz in epileptic groups. Some of the other studies showed that in Parkinson's disease model rats, Pérez-Alcázar et al. found that, when up-chirp was given, the frequency response in 40-60 Hz is reduced [118]. Poulsen et al. showed that the frequency of the peak response is increased in 25-55 Hz range for healthy adults [119]. In schizophrenia patients, the response amplitude was reduced in 30-50 Hz and 90-100 Hz range [120,121]. Ethridge et al. showed a decreased gamma phase-locking in the 30–58 Hz range in Fragile X syndrome mice due to decreased GABAergic function [24].

MMN Task: MMN ERP responses for odd and regular tones revealed a decrease in the area under the response curve from 250-350 ms post-stimulus as the rat progresses through epilepsy for left-hippocampus. This result indicates a loss of ability to distinguish tones, revealing a problem with their associative memory during epileptogenesis.

CHAPTER 6

FUTURE WORK

Our study can be extended in multiple ways in the near future. More subjects can be included in the future, and the method can be implemented on humans as it is a non-invasive and is cost effective. Along with the presented auditory tasks, one can employ other auditory tasks that differ in frequency, variations in stimulus intensity, length of interval times, number of trials etc. For the habituation task, by changing different the length of interval times, number of trials, one can look at where the inhibitory function breaks down and can get to know the neural system dysfunction. For the chirp task, one can change the order of the chirp stimulation, that is by giving down chirp instead of up-chirp signals, and can look at the neural frequency response of the rats to different frequencies. Also, one can employ other paradigms like visual evoked potentials to study cognition, behavioral and memory deficits. It is also possible to follow the patients longitudinally.

As an extension to the work presented in this dissertation, one can combine the ERP recordings with neurochemical sensor probes to track the dynamic behaviors/changes (monitored in real time) in neurotransmitters such as GABAergic inhibition and glutamatergic excitation. Using this approach, one can correlate the changes in the activity of local interactions between neurons and interneurons (elicited due to event-related responses using auditory ERPs) and track synchronous changes to neurotransmitter release,

thereby indicating loss of inhibitory and/or increase in excitatory neurotransmitters in epileptic groups. As these neurotransmitters are essential to mental and physical health, one can track those changes in their activity, as it is critical for medical treatment and clinical analysis. This tool can be a powerful for monitoring and treating patients with epilepsy.

Apart from the features employed in our analysis (such as ITC, ERSP), extraction of additional EEG time-frequency features. Wavelet transform (WT), ITLC-inter-trial linear coherence- weighs each epoch according to the amplitude. The avWT corresponds to time-frequency transformed evoked potential-induced activity, that is measuring everything that is not phase locked to the particular stimulus. Other methods include evoked response phase coherence and evoked response linear coherence. We can also perform Morris water navigation (MWN) test, novel object recognition (NOR) test, elevated plus maze (EPM) to assess the memory deficits in rodents and see the latency periods in recognizing a particular task/test. We can finally, combine and correlate the ERP responses/measures (obtained from auditory tasks) with other behavioral tests (like MWN, NOR, EPM), which provides more detailed information regarding the cognitive process that one wants to study. This may help us to understand the brain-behavior relationships that may lead to other innovative neurocognitive assessment techniques that could help in diagnosis of cognition, behavioral and memory deficits in epileptic patients.

After performing the auditory tasks, one can perform histology on the brain to see which regions of the brain are affected by lithium pilocarpine model of epilepsy and correlate the ERP results from the affected region of the brain. The results could give more detailed information regarding impaired brain ability to oscillate to different frequencies

of stimulation. Apart from the lithium pilocarpine animal model of epilepsy, one can implement the auditory tasks, on different models of epilepsy on rats and compare the results. By this, one could get more detailed information regarding impaired brains ability to respond to different stimuli and can tell whether the response is seen commonly in all animal models of epilepsy or is it model specific.

One can also look at the seizure frequency information for all the rats over the period of three months and correlate the seizure frequency to the ERP responses to see the effects of seizures on cognitive function such as memory, processing or attention difficulties, and mental health problems.

APPENDIX A

Habituation Feature Plots

Figures A-1 through A-4 show Normalized N70 ERP response of four repeated pulses for before SE and during epileptogenesis (one week, one month and two months after SE), for LH, F3, LT and RT electrodes.

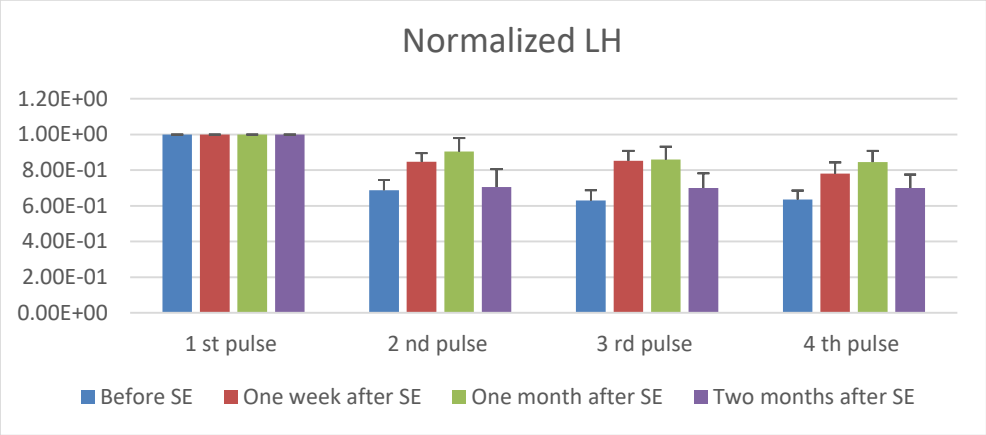


Figure A- 1: Normalized N70 ERP response of four repeated pulses for before SE and during epileptogenesis (that is one week, one month and two months after SE) for N=8, mean±SEM for left hippocampus.

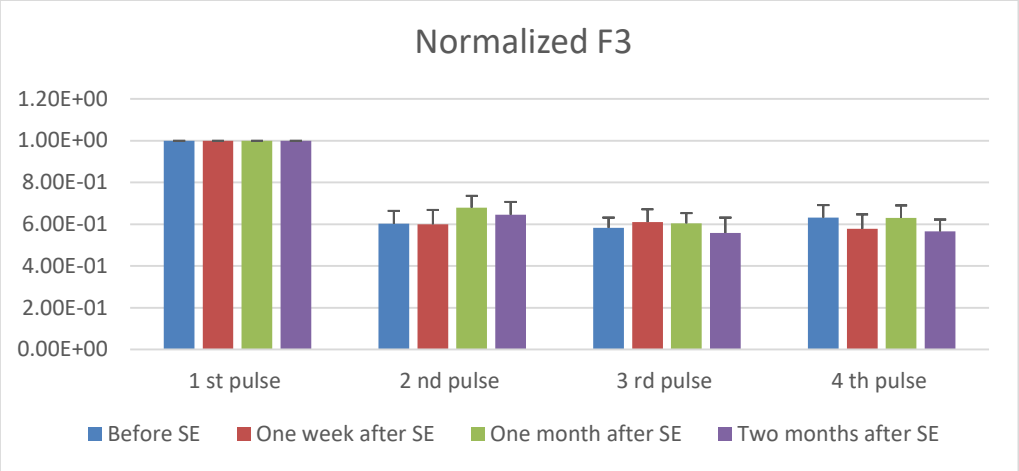


Figure A- 2: Normalized N70 ERP response of four repeated pulses for before SE and during epileptogenesis (that is one week, one month and two months after SE) for N=8, mean±SEM for left frontal F3.

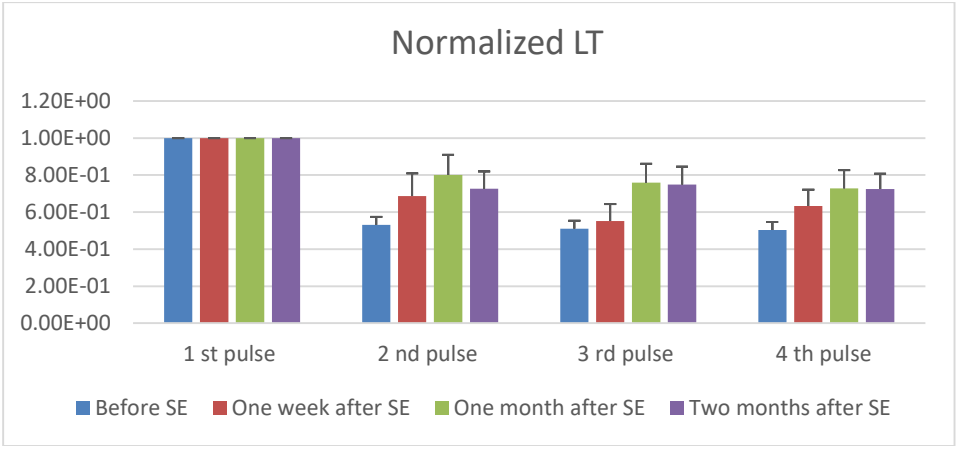


Figure A- 3: Normalized N70 ERP response of four repeated pulses for before SE and during epileptogenesis (that is one week, one month and two months after SE) for N=8, mean±SEM for left thalamus**.**

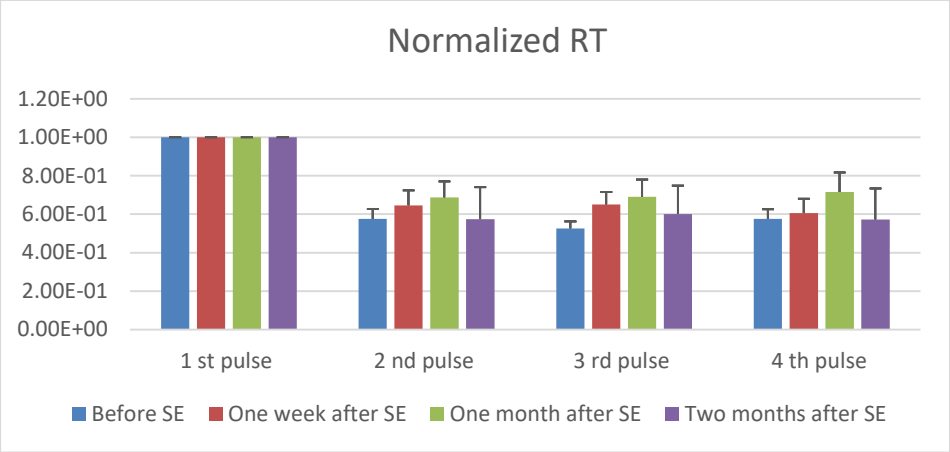


Figure A- 4: Normalized N70 ERP response of four repeated pulses for before SE and during epileptogenesis (that is one week, one month and two months after SE) for N=8, mean±SEM for right thalamus.

Table A-1 shows the number of subjects, its p-values for different electrode sites for second, third and fourth pulses.

Table A- 1: Repeated measure ANOVA test with repeated measures of 2^{nd}, 3^{rd}, 4^{th} pulses between the different groups (before SE, and one week after, one month after, two months after SE), for all electrodes. n/a- not applicable.

	n	2^{nd} pulse	3^{rd} pulse	4^{th} pulse
F3	8	0.544	0.8359	0.6862
F4	0	n/a	n/a	n/a
LT	8	0.1106	0.0258	0.0377
RT	5	0.6575	0.5659	0.8785
P3	3	0.8971	0.7138	0.6864
P4	2	0.8498	0.9649	0.9315
LH	8	0.0179	0.0505	0.0512
RH	8	0.0327	0.091	0.0246

Figures A-5 through A-8, show the grand average ITC and P-values for before SE and during epileptogenesis (one week, one month and two months after SE), for F3, LH electrodes.

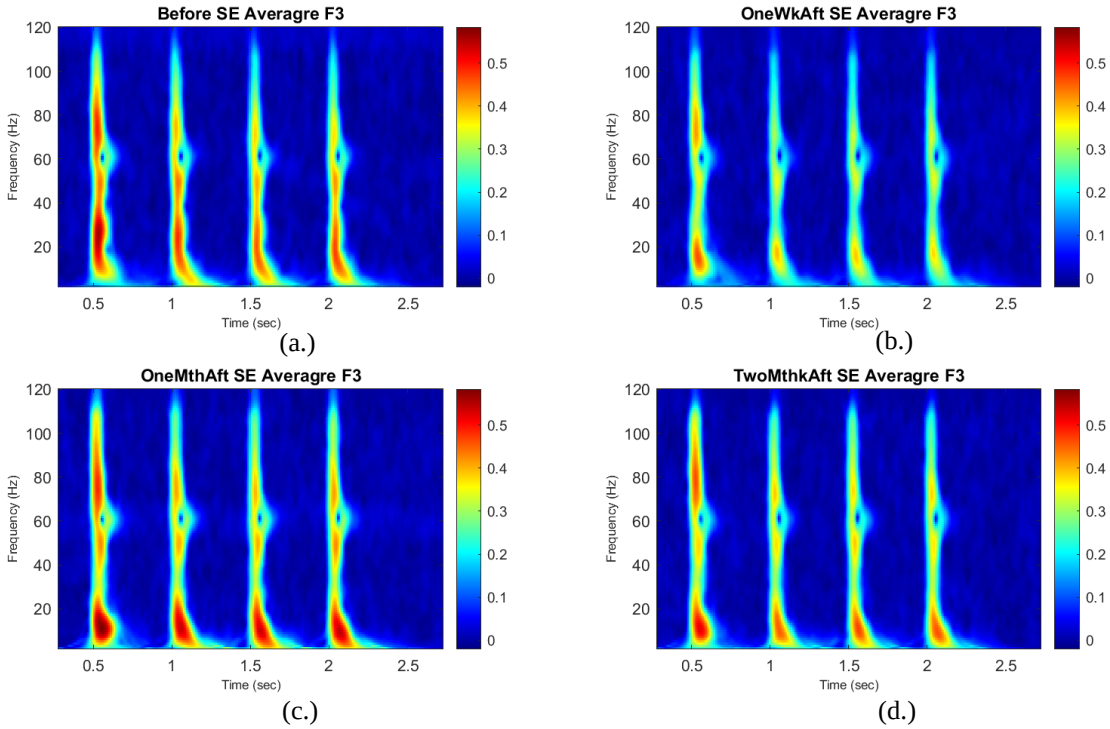
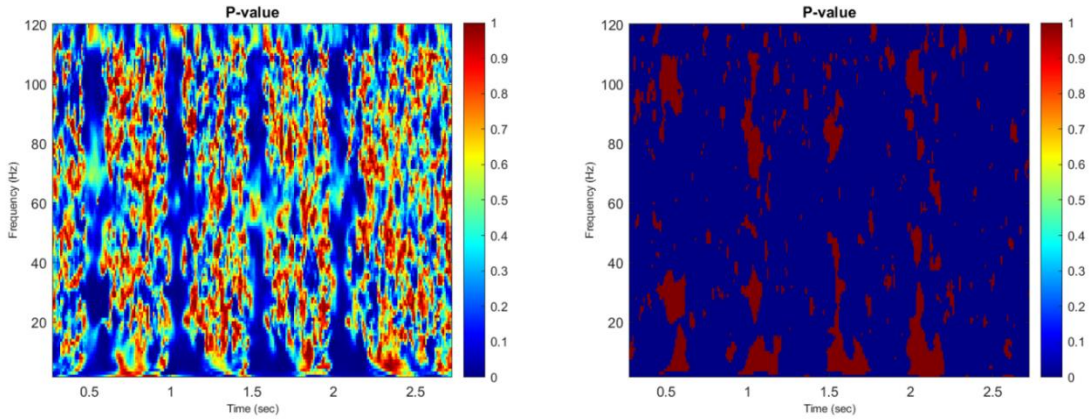


Figure A- 5: Averaged ITC values (from 500 trials, across all subjects N=8) of four repeated sound pulses at 0.5, 1.0, 1.5 and 2.0 secs for (a.) before SE and (b., c., d.) during epileptogenesis, for left frontal electrode.



(a) **(b)**

Figure A- 6: P-values using repeated measure ANOVA (left fig (a.)), and P-values < 0.05, where red color showing significant differences at delta, theta, alpha and low beta frequencies and blue color showing no significant differences at other frequencies (among control and epileptic groups) (right fig (b.)), when the four repeated sound pulses are played at 0.5, 1.0, 1.5 and 2.0 secs, for F3 electrode.

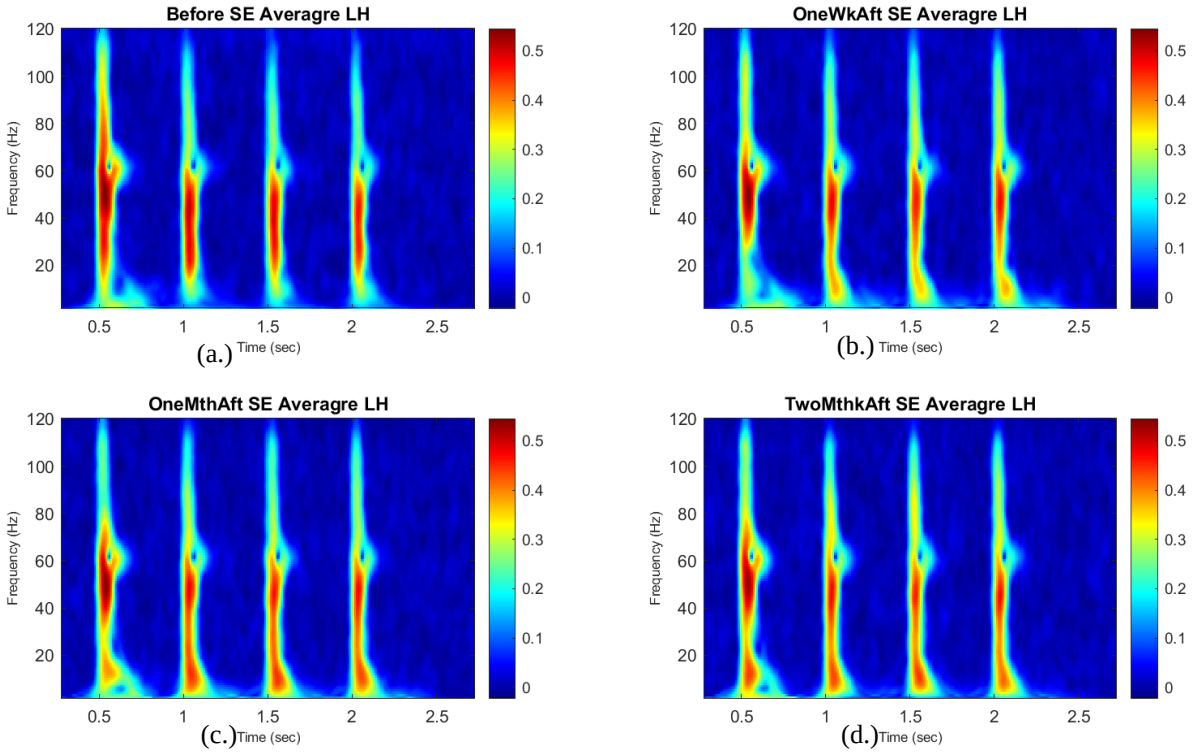
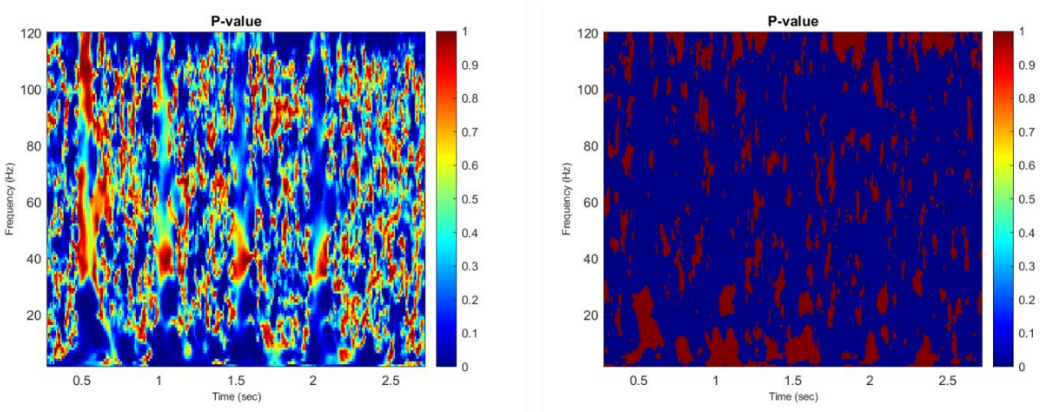


Figure A- 7: Averaged ITC values (from 500 trials, across all subjects N=8) of four repeated sound pulses at 0.5, 1.0, 1.5 and 2.0 secs for (a.) before SE and (b., c., d.) during epileptogenesis, for LH electrode.



(a) **(b)**

Figure A- 8: P-values using repeated measure ANOVA (left fig (a.)), and P-values < 0.05, where red color showing significant differences at delta, theta, alpha and low beta frequencies and blue color showing no significant differences at other frequencies (among control and epileptic groups) (right fig (b.)), when the four repeated sound pulses are played at 0.5, 1.0, 1.5 and 2.0 secs, for LH electrode.

Figures A-9 through A-12, show the grand average ERSP and P-values for before SE and during epileptogenesis (one week, one month and two months after SE), for F3, LH electrodes.

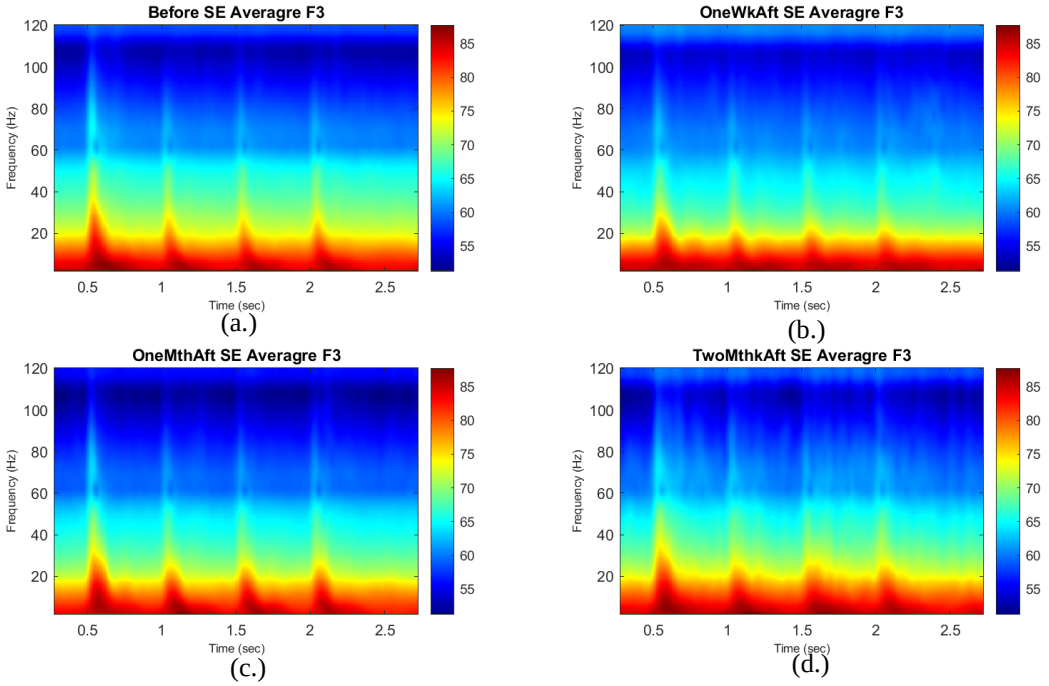
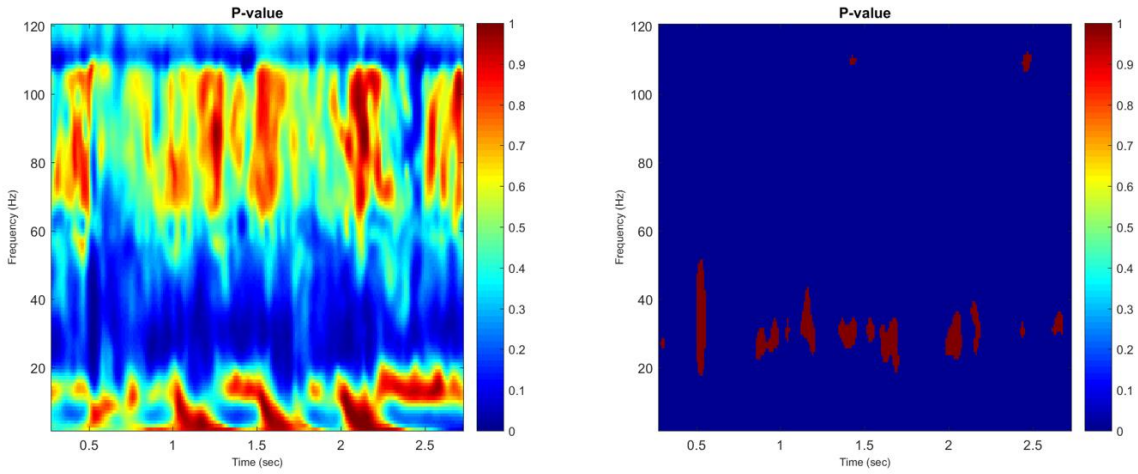


Figure A- 9: Averaged ERSP values (from 500 trials, across all subjects N=8) of four repeated sound pulses played at 0.5, 1.0, 1.5 and 2.0 secs for (a.) before SE and (b., c., d.) during epileptogenesis, for F3 electrode.



(a) **(b)**

Figure A- 10: P-values using repeated measure ANOVA (left fig (a.)), and P-values < 0.05, where red color showing significant differences at 20-30 Hz frequencies and blue color showing no significant differences at other frequencies (among control and epileptic groups) (right fig (b.)), when the four repeated sound pulses are played at 0.5, 1.0, 1.5 and 2.0 secs, for F3 electrode.

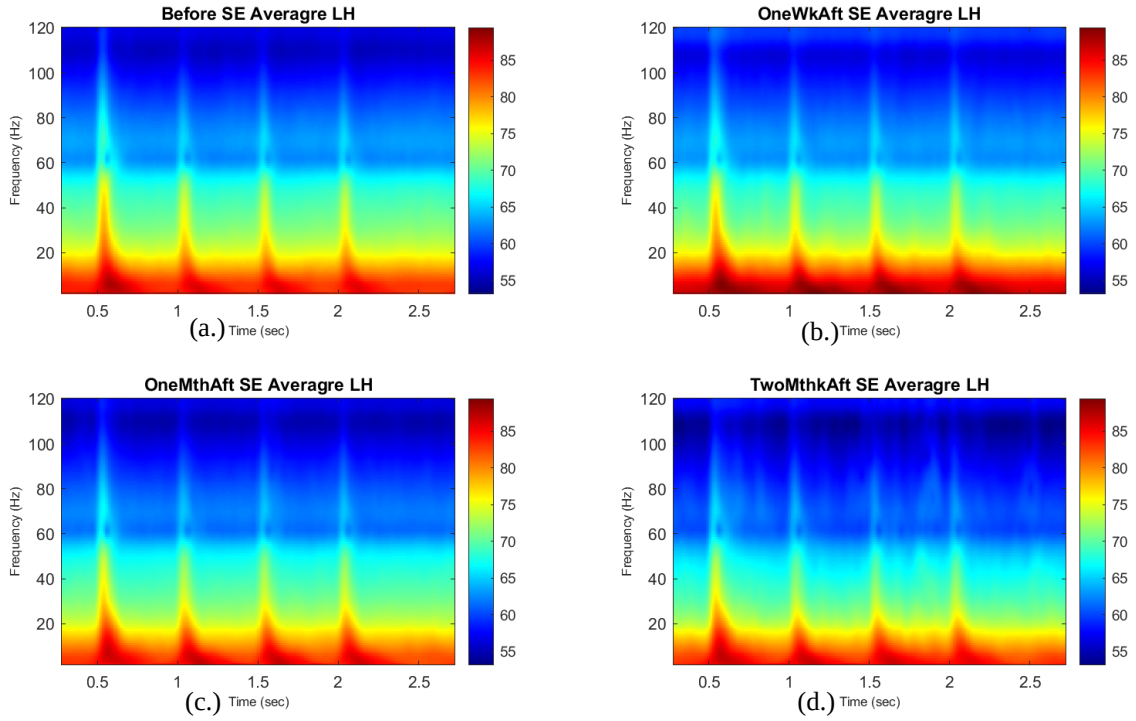
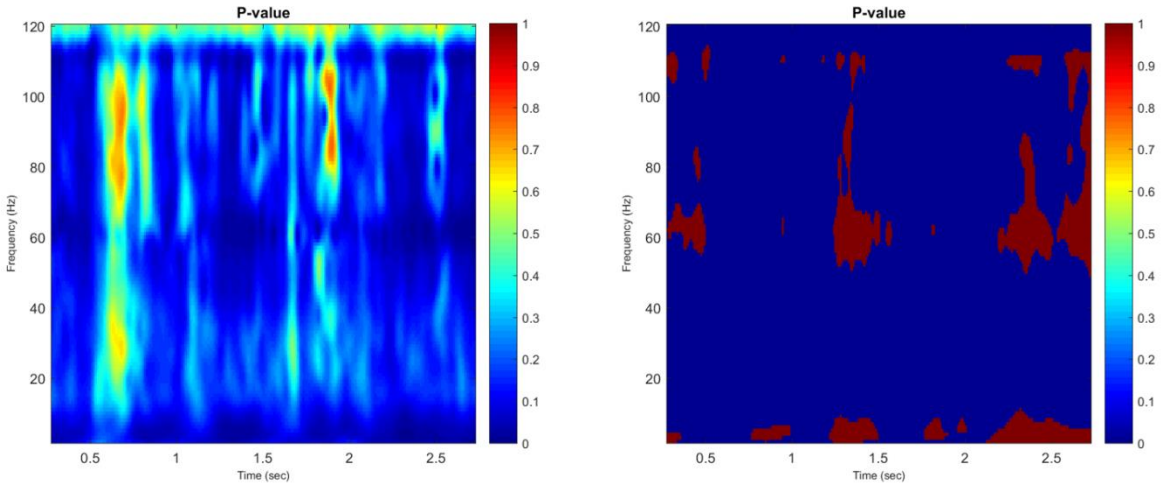


Figure A- 11: Averaged ERSP values (from 500 trials, across all subjects N=8) of four repeated sound pulses played at 0.5, 1.0, 1.5 and 2.0 secs for (a.) before SE and (b., c., d.) during epileptogenesis, for LH electrode.



(a) (**b**)

Figure A- 12: P-values using repeated measure ANOVA (left fig (a.)), and P-values < 0.05, where red color showing significant differences at 0.5-10 Hz and 55-75 Hz frequencies and blue color showing no significant differences at other frequencies (among control and epileptic groups) (right fig (b.)), when the four repeated sound pulses are played at 0.5, 1.0, 1.5 and 2.0 secs, for LH electrode.

Figures A-13 through A-16, show the grand average baseline normalized ERSP and P-values for before SE and during epileptogenesis (one week, one month and two months after SE), for F3, LH electrodes.

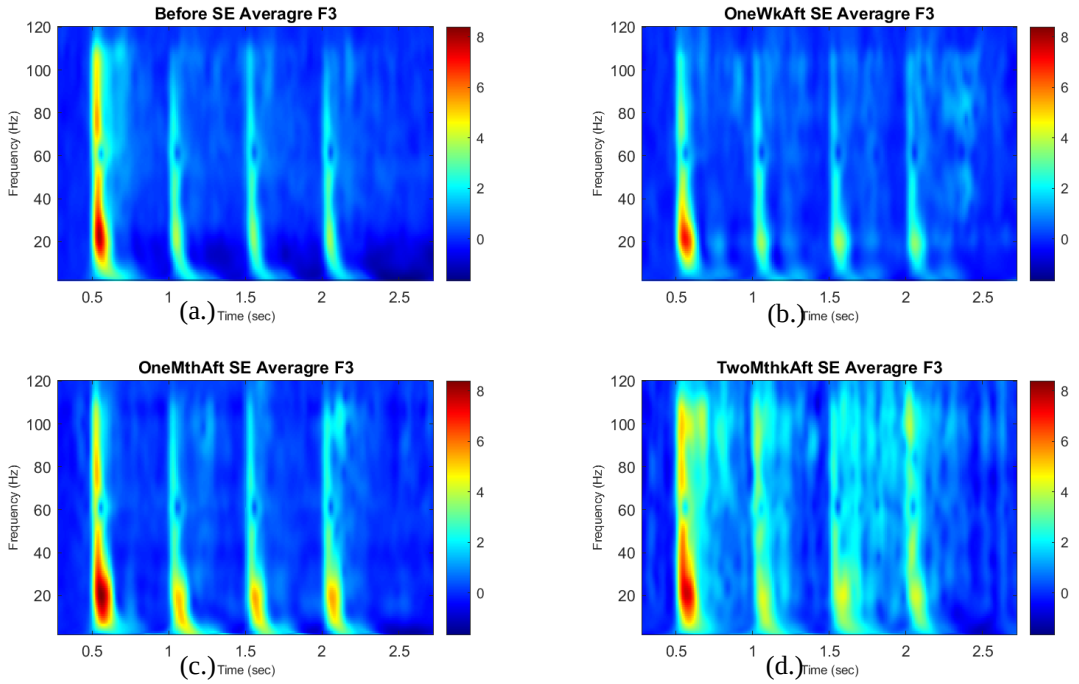


Figure A- 13: Baseline corrected averaged ERSP values (from 500 trials, across all subjects N=8) of four repeated sound pulses played at 0.5, 1.0, 1.5 and 2.0 secs for (a.) before SE and (b., c., d.) during epileptogenesis, for F3 electrode.

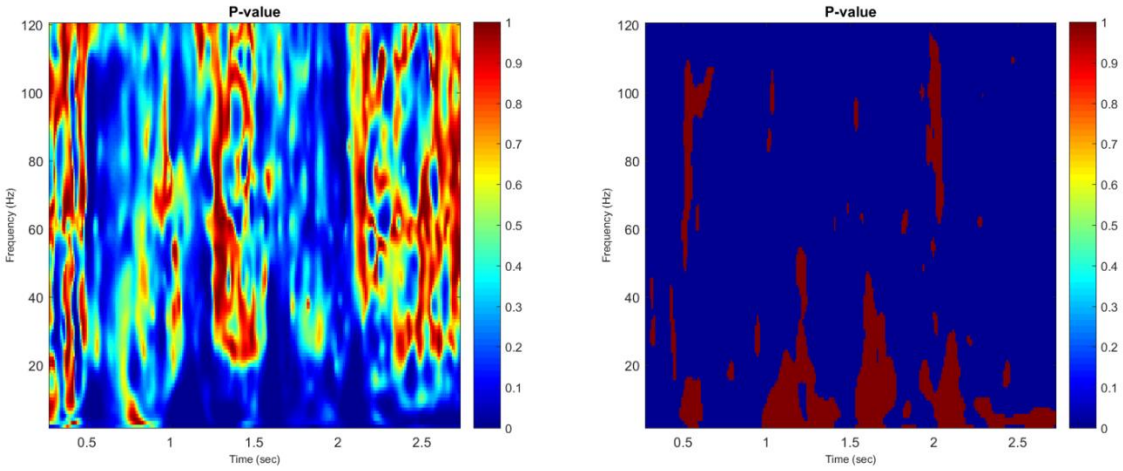


Figure A- 14: P-values using repeated measure ANOVA (left fig (a.)), and P-values < 0.05, where red color showing significant differences at (0.5-35 Hz) and blue color showing no significant differences (among control and epileptic groups) (right fig (b.)), when the four repeated sound pulses are played at 0.5, 1.0, 1.5 and 2.0 secs, for F3 electrode.

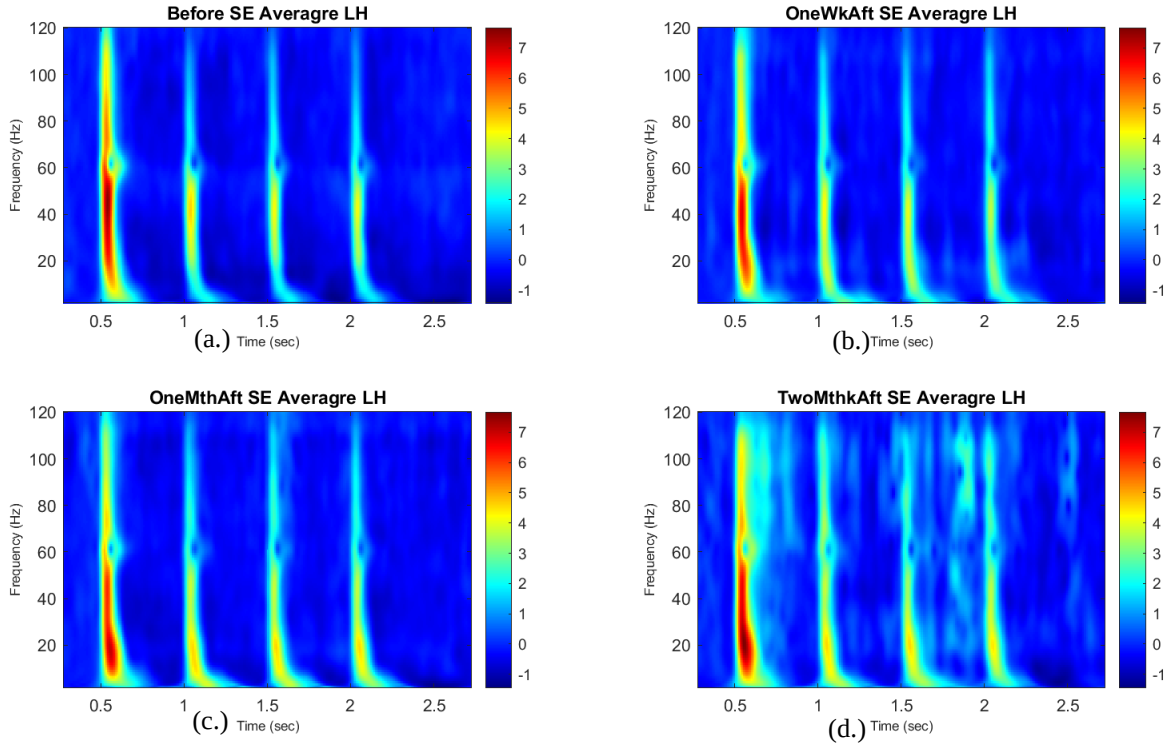
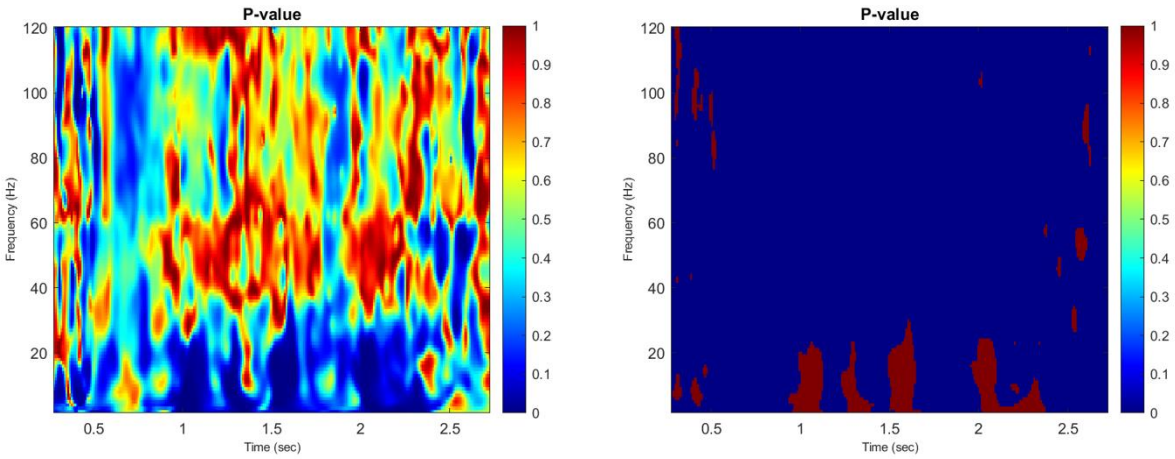


Figure A- 15: Baseline corrected averaged ERSP values (from 500 trials, across all subjects N=8) of four repeated sound pulses played at 0.5, 1.0, 1.5 and 2.0 secs for (a.) before SE and (b., c., d.) during epileptogenesis, for LH electrode.



(a) **(b)**

Figure A- 16: P-values using repeated measure ANOVA (left fig (a.)), and P-values < 0.05, where red color showing significant differences at (0.5-25 Hz) and blue color showing no significant differences (among control and epileptic groups) (right fig (b.)), when the four repeated sound pulses are played at 0.5, 1.0, 1.5 and 2.0 secs, for LH electrode.

APPENDIX B

Chirp Feature Plots

Figures B-1 through B-4, show the grand average ITC and P-values for before SE and during epileptogenesis (one week, one month and two months after SE), for RH and F3 electrodes.

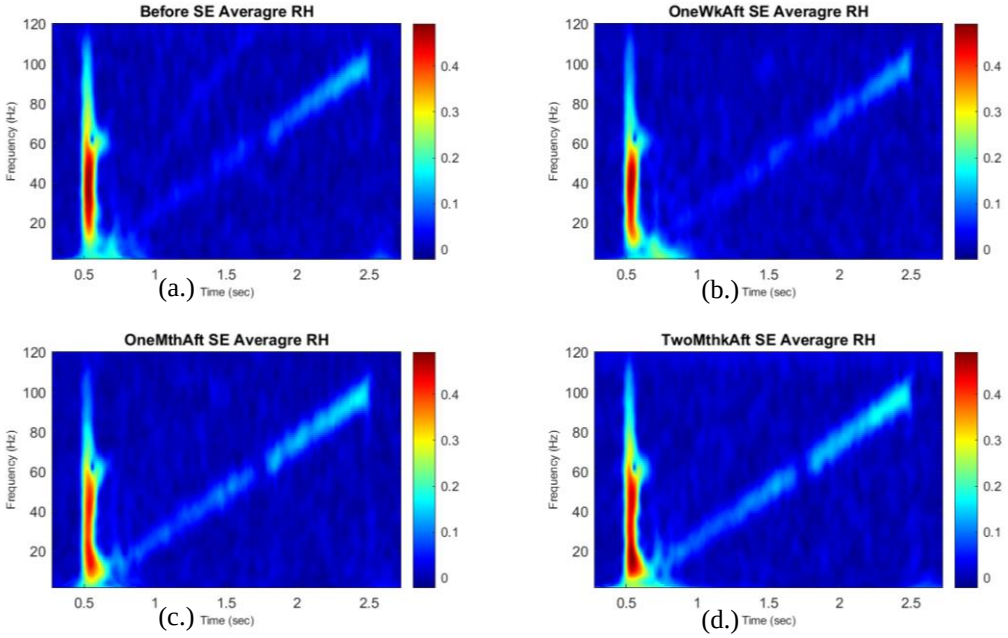
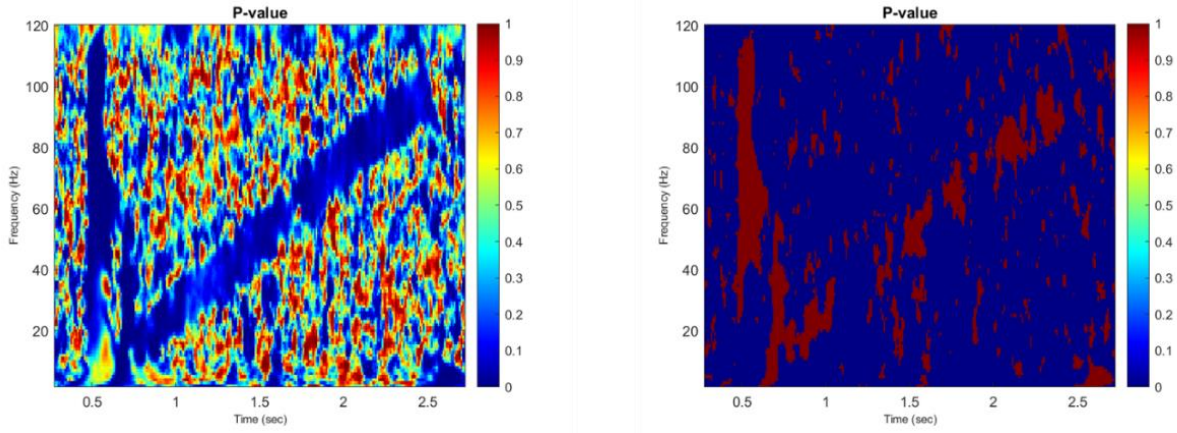
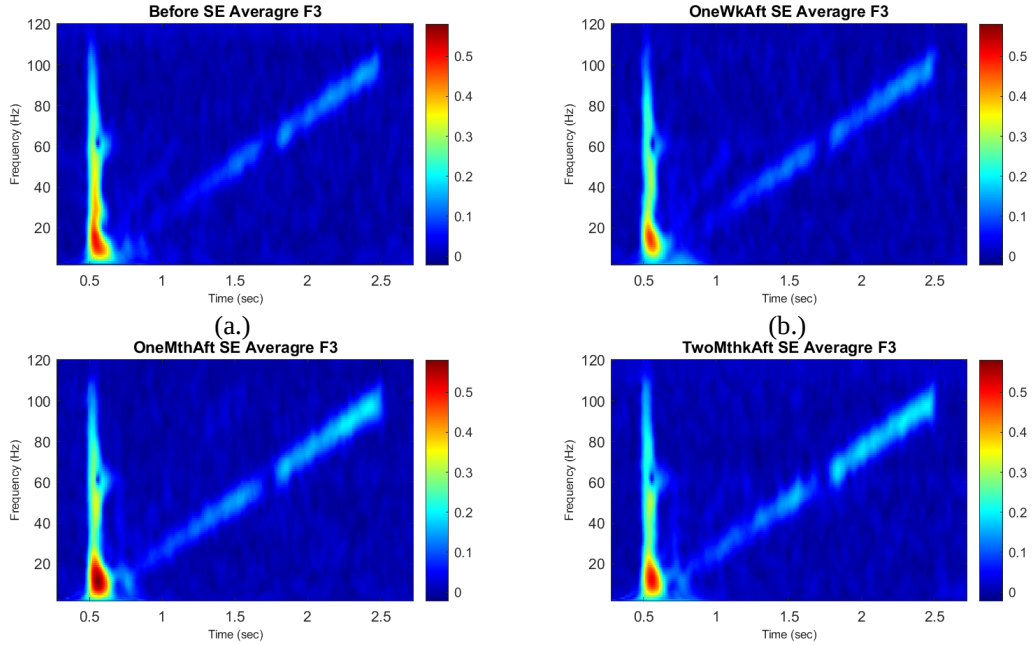


Figure B- 1: Averaged ITC values (from 500 trials, across all subjects N=8) of chirp stimulus starting at 0.5 secs to 2.5 secs for (a.) before SE and (b., c., d.) during epileptogenesis, for RH electrode.



(**a**) (**b**)

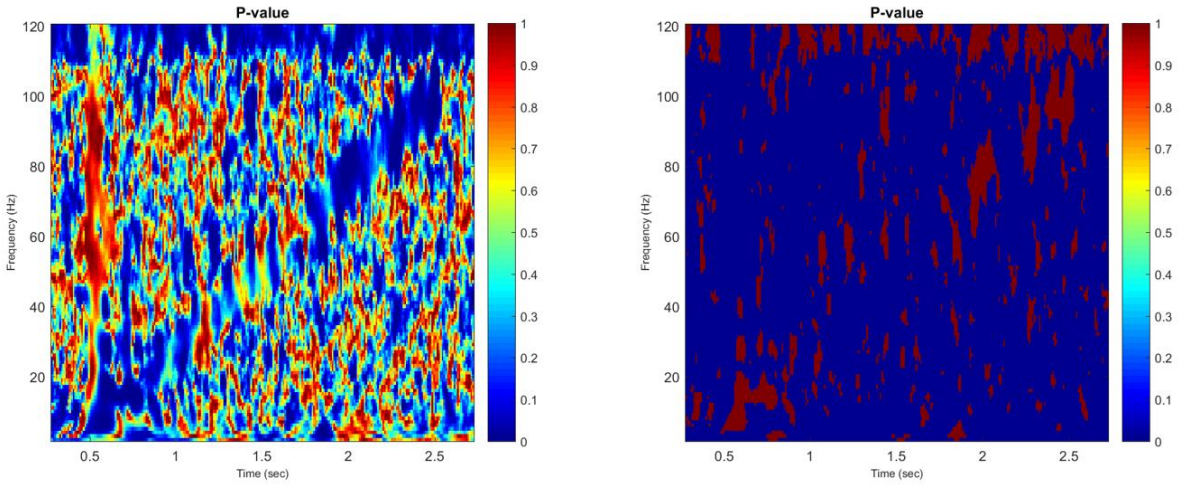
Figure B- 2: a.) P-values using repeated measure ANOVA (left figure (a.)), and P-values < 0.05, where red color showing significant differences at 10-40, 45-60, and 70-100 Hz frequencies and blue color showing no significant differences (among control and epileptic groups) (right figure (b.)), for RH electrode.



(a.) (b.)

(c.) (d.)

Figure B- 3: Averaged ITC values (from 500 trials, across all subjects N=8) of chirp stimulus starting at 0.5 secs to 2.5 secs for (a.) before SE and (b., c., d.) during epileptogenesis, for F3 electrode.



(**a**) (**b**)

Figure B- 4: a.) P-values using repeated measure ANOVA (left figure (a.)), and P-values < 0.05, where red color showing significant differences at (5-20, 60-80, 90-100 Hz) and blue color showing no significant differences (among control and epileptic groups) (right figure (B.)), for F3 electrode.

APPENDIX C

Odd-ball Feature Plots

Figures C-1 and C-2 show area under the curve of P300 (around 250-350 ms) for before SE and during epileptogenesis (one week, one month and two months after SE), for RH and F3.

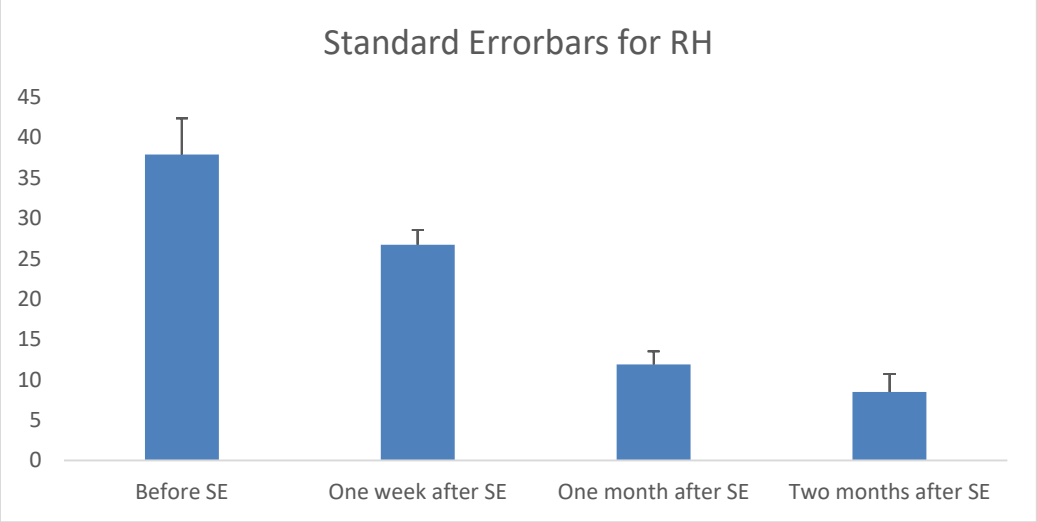


Figure C- 1: MMN AUC around P300 peak (250-350 ms) after the onset of the odd tone during epileptogenesis (N=5, mean±SEM), for RH electrode.

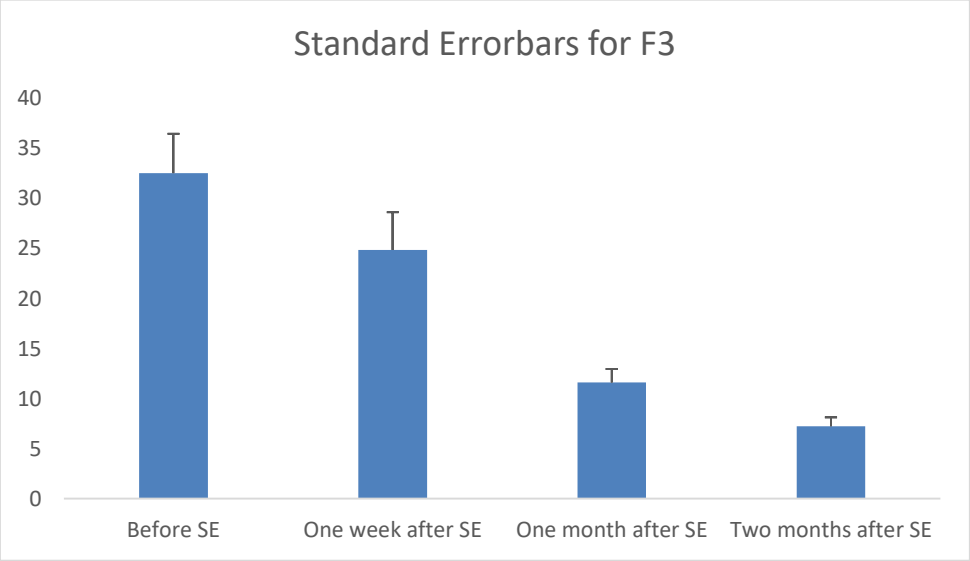


Figure C- 2: MMN AUC around P300 peak (250-350 ms) after the onset of the odd tone during epileptogenesis (N=5, mean±SEM), for F3 electrode.

Table C-1 shows the number of subjects, its p-values for different electrode sites for the area under the curve around P300.

Table C- 1: Repeated measure ANOVA test, between the different groups (before SE, and one week after, one month after, two months after SE), for all electrodes. NaN= Not a number

	n	P-value
F3	5	0.00971
F4	0	NaN
LT	4	0.01921
RT	2	NaN
P3	3	0.038209
P4	2	NaN
LH	5	0.0002
RH	5	0.00032252

REFERENCES

[1] C. E. Stafstrom and L. Carmant, “Seizures and Epilepsy: An Overview for Neuroscientists”, Cold Spring Harb Perspect Med. 2015 Jun; 5(6): a022426.

[2] S. D. Shorvon, Epilepsy, 1st ed. Oxford University Press, 2009.

[3] M. J. England, C. T. Liverman, A. M. Schultz, and L. M. Strawbridge, Epilepsy across the spectrum: Promoting Health and Understanding. 2012.

[4] L. Burghaus, C. Dohmen, and M. Malter, “Status epilepticus,” Fortschritte der Neurology and Psychiatry, 2018vol. 86, no. 5, pp. 279–286.

[5] S. Sharma, S. Puttachary, A. Thippeswamy, A. G. Kanthasamy, and T. Thippeswamy, “Status Epilepticus: Behavioral and Electroencephalography Seizure Correlates in Kainate Experimental Models,” Front Neurol. 2018; 9: 7

[6] G. L. Holmes, “Role of glutamate and GABA in the pathophysiology of epilepsy,” Mental Retardation and Developmental Disabilities Research Reviews, 1995; pp. 208-219.

[7] C. Helmstaedter and J.A. Witt,” Epilepsy and cognition – A bidirectional relationship?” Seizure, Volume 49, July 2017, Pages 83-89.

[8] P. Kwan and M. J. Brodi, “Neuropsychological effects of epilepsy and antiepileptic drugs,” The Lancet, 2001 Jan, Vol 357.

[9] X. Wang, Y. Lv, W. Zhang, and H. Meng, “Cognitive Impairment and Personality Traits in Epilepsy Characterization and Risk Factor Analysis,” The Journal of Nervous and Mental Disease 2018 October; Volume 206-10.

[10] C. S. Jacobs, K. C. Willment, & R. A. Sarkis, “Non-invasive Cognitive Enhancement in Epilepsy”. Frontiers in neurology, 10, 167, 2019. https://doi.org/10.3389/fneur.2019.00167.

[11] W.Z. Wang, J.Z. Wu, G.Y. Ma, X.Y. Dai, B. Yang, T.P. Wang, C.L. Yuan, Z. Hong, G.S. Bell, L. Prilipko, H.M. de Boer, J.W. Sander, “Efficacy assessment of phenobarbital in epilepsy: a large community-based intervention trial in rural China”. Lancet Neurology, 5: 46–52.

[12] M. E. Clare, E.R. Hugh & E.C. Andrea, “The cognitive impact of antiepileptic drugs”, Therapeutic Advances in Neurological Disorders, 2011 Nov; 4(6): 385–407, doi: 10.1177/1756285611417920.

[13] E.P. Calandre, R. Dominguez-Granados, M. Gomez-Rubio, J.A. Molina-Font, “Cognitive effects of long-term treatment with phenobarbital and valproic acid in school children”, Acta neurologica Scandinavica, 81: 504–506.

[14] D. Millett, “Hans Berger: From Psychic Energy to the EEG,” Perspectives in Biology and Medicine, vol. 44, no. 4, pp. 522–542, 2001.

[15] I. Rektor, M. Brázdil, I. Nestrasil, M. Bares and P. Daniel,” Modifications of cognitive and motor tasks affect the occurrence of event-related potentials in the human cortex. European Journal of Neuroscience 2007;26(5):1371-80.

[16] B. W. Müller, M. Specka, N. Steinchen, D. Zerbin, E. Lodemann and T. Finkbeiner,” Auditory target processing in methadone substituted opiate addicts: The effect of nicotine in controls,” BMC Psychiatry 2007;7:63.

[17] C. Beste, C. Saft, O. Güntürkün and M. Falkenstein,” Increased cognitive functioning in symptomatic Huntington’s disease as revealed by behavioral and event-related potential indices of auditory sensory memory and attention,” Journal of Neuroscience 2008;28(45):11695-702.

[18] M. Zgorzalewicz,” Long latency auditory evoked potentials in school children and adolescents with epilepsy,” Przeglad Lekarski, 2006;63(Suppl 1):8-13.

[19] O. A. Ozmenek, B. Nazliel, A. Leventog˘lu and E. Bilir,” The role of event related potentials in evaluation of subclinical cognitive dysfunction in epileptic patients,” Acta neurol. belg., 2008, 108, 58-63.

[20] J. G. Webster,” Event-related potentials,” Encyclopedia of Medical Devices and Instrumentation, 2nd Edition, 2006.

[21] M. Titlic, M. Mihalj, A. B. Petrović and E. Suljic,” P300 as an auxiliary method in clinical practice: A review of literature,” Journal of Health Sciences 2016;6(3):143-148.

[22] Polich j., Ladish c., Bloom E. F. P300 assessment of early Alzheimer’s disease. Electroencephalography and clinical Neurophysiology, 1990, 77: 179-189.

[23] N. I. Triantafllou, I. Zalonis, P. Kokotis, M. Anthracopoulos, A. Siafacas, S. Malliara, H. L. Hamburger, C. Papageriou,” Cognition in epilepsy: a multichannel event related potential (P300) study,” Acta Neurol. Scan., 1992, 86: 462- 465.

[24] G. Pfurtschellera, F.H. Lopes da Silvab,” Event-related EEG/MEG synchronization and desynchronization: basic principles,” Clinical Neurophysiology 110 (1999), 1842-1857.

[25] H. Berger," About the human electroencephalogram,". Archive for Psychiatry Nerve Crisis, 1929, 87, 527–570. https://doi.org/10.1007/BF01797193.

[26] R.B. Paranjape, J. Mahovsky, L. Benedicenti, Z. Koles, "The Electroencephalogram as a Biometric," Canadian Conference on Electrical and Computer Engineering, 2001, 2, 1363-1366.

[27] A.F. Jackson, D.J. Bolger," The neurophysiological bases of EEG and EEG measurement, "A review for the rest of us. Psychophysiology, 2014, 51, 1061–1071. https://doi.org/10.1111/psyp.12283

[28] S. Murakami, Y. Okada, "Contributions of principal neocortical neurons to magnetoencephalography and electroencephalography signals," The Journal of Physiology, 2006, 575, 925–36. https://doi.org/10.1113/jphysiol.2006.105379

[29] G. Buzsáki, C. A. Anastassiou, and C. Koch, "The origin of extracellular fields and currents-EEG, ECoG, LFP and spikes," *Nat. Rev. Neurosci.*, vol. 13, no. 6, pp. 407–420, 2012.

[30] A. V. Medvedev, "Epileptiform spikes desynchronize and diminish fast (gamma) activity of the brain: An 'anti-binding' mechanism?," *Brain Res. Bull.*, vol. 58, no. 1, pp. 115–128, 2002.

[31] T. V. Ness, M. W. H. Remme, and G. T. Einevoll, "Active subthreshold dendritic conductances shape the local field potential," *J. Physiol.*, vol. 594, no. 13, pp. 3809–3825, 2016.

[32] M. W. Reimann, C. A. Anastassiou, R. Perin, S. L. Hill, H. Markram, and C. Koch, "A biophysically detailed model of neocortical local field potentials predicts the critical role of active membrane currents," *Neuron*, vol. 79, no. 2, pp. 375–390, 2013.

[33] F. G. Pike, R. S. Goddard, J. M. Suckling, P. Ganter, N. Kasthuri, and O. Paulsen, "Distinct frequency preferences of different types of rat hippocampal neurones in response to oscillatory input currents," *J. Physiol.*, vol. 529, no. 1, pp. 205–213, 2000

[34] A. Alonso and R. R. Llinás, "Subthreshold Na+-dependent theta-like rhythmicity in stellate cells of entorhinal cortex layer II," *Nature*, 1989.

[35] T. Dugladze *et al.*, "Impaired hippocampal rhythmogenesis in a mouse model of mesial temporal lobe epilepsy," *Proc. Natl. Acad. Sci. U. S. A.*, 2007.

[36] G. Gonzalez-Burgos, T. Hashimoto, and D. A. Lewis, "Alterations of cortical GABA neurons and network oscillations in schizophrenia," *Current Psychiatry Reports*. 2010.

[37] B. Lega, S. Dionisio, W. Bingaman, I. Najm, and J. Gonzalez-Martinez, “The gamma band effect for episodic memory encoding is absent in epileptogenic hippocampi,” *Clin. Neurophysiol.*, 2015.

[38] D. H. Mathalon and V. S. Sohal, “Neural oscillations and synchrony in brain dysfunction and neuropsychiatric disorders it’s about time,” *JAMA Psychiatry*. 2015.

[39] A. J. Mably and L. L. Colgin, “Gamma oscillations in cognitive disorders,” *Current Opinion in Neurobiology*, vol. 52. Elsevier Ltd, pp. 182–187, 01-Oct-2018.

[40] J. A. Cardin *et al.*, “Driving fast-spiking cells induces gamma rhythm and controls sensory responses,” *Nature*, vol. 459, no. 7247, pp. 663–667, Jun. 2009.

[41] M. A. Whittington, R. D. Traub, and J. G. R. Jefferys, “Synchronized oscillations in interneuron networks driven by metabotropic glutamate receptor activation,” *Nature*, 1995.

[42] A. Bragin, G. Jandó, Z. Nádasdy, J. Hetke, K. Wise, and G. Buzsáki, “Gamma (40-100 Hz) oscillation in the hippocampus of the behaving rat,” *J. Neurosci.*, 1995.

[43] A. Fisahn, F. G. Pike, E. H. Buhl, and O. Paulsen, “Cholinergic induction of network oscillations at 40 Hz in the hippocampus in vitro,” *Nature*, 1998.

[44] J. C. Athilingam, R. Ben-Shalom, C. M. Keeshen, V. S. Sohal, and K. J. Bender, “Serotonin enhances excitability and gamma frequency temporal integration in mouse prefrontal fast-spiking interneurons,” *Elife*, vol. 6, pp. 1–22, 2017.

[45] M. Zijlmans, P. Jiruska, R. Zelmann, F. S. S. Leijten, J. G. R. Jefferys, and J. Gotman, “High-frequency oscillations as a new biomarker in epilepsy,” *Annals of Neurology*, 2012, vol. 71, no. 2, pp. 169–178, Feb.

[46] B.K.R. Karumuri,” Seizure predictability and study of sudden unexpected death in epilepsy using machine learning and association measures from eeg and ecg network analysis,” College of Engineering and Science, Louisiana Tech University, 2017

[47] D.A. Prince, B.W. Connors and L.S. Benardo, “Mechanisms underlying interictalictal transitions”, Advances in Neurology, 1983, 34: 177–187.

[48] R.D. Traub, R.K. Wong, “Cellular mechanism of neuronal synchronization in epilepsy,” Science, 1982 216: 745–747.

[49] E.D. Adrian, K. Yamagiwa, “The origin of the Berger rhythm. Brain.”, 1935, 58:323–351.

[50] H.H. Jasper, “Electrical signs of cortical activity, “Psychological Bulletin. 1937, 34:411–481.

[51] HH. Jasper, “Charting the Sea of Brain Waves,” Science,1948, 108(2805):343-7.

[52] P.A. Davis, “Effects of acoustic stimuli on the waking human brain”, Journal of Neurophysiology, 1939, 2:494–499.

[53] W.G. Walter, “Critical review: the technique and application of electro-encephalography”, Journal of Neurology and Psychiatry, 1938, (4):359-85.

[54] R. Cooper, A.L. Winter, H.J. Crow, W.G. Walter,” Comparison of subcortical, cortical and scalp activity using chronically indwelling electrodes in man”, Electroencephalography and Clinical Neurophysiology, 1965, 18:217-28.

[55] H. Davis,” Enhancement of evoked cortical potentials in humans related to a task requiring a decision,” Science, 1964, 10; 145(3628):182-3.

[56] E. Donchin, “Average Evoked Potentials, Methods, Results, and Evaluations,” U.S. Government Printing Office; Washington, D.C.: 1969.

[57] E. Donchin, L. Cohen, “Averaged evoked potentials and intramodality selective attention., “Electroencephalography and Clinical Neurophysiology, 1967; 22(6):537-46.

[58] W.G. Walter, R. Cooper, V.J. Aldridge W.C. Mccallum, A.L. Winter, “Contingent negative variation: an electric sign of sensorimotor association and expectancy in the human brain”, Nature. 1964, 25; 203():380-4.

[59] S.J. Luck, “An introduction to the event-related potential technique, “MIT Press; Cambridge, MA, 2005.

[60] T.C. Handy, “Event-Related Potentials: A Methods Hand book. MIT Press; Cambridge, MA, 2005.

[61] D. Regan, “Human Brain Electrophysiology: Evoked Potentials and Evoked Magnetic Fields in Science and Medicine, “Elsevier, 1989.

[62] P.L. Nunez, R. Srinivasan, “Electric fields of the brain: The neurophysics of EEG, “Oxford University Press, Inc., 2nd ed.; Oxford: 2006.

[63] N.K. Logothetis, J. Pauls, M. Augath, T. Trinath, A. Oeltermann, “Neurophysiological investigation of the basis of the fMRI signal, “Nature, 2001 Jul 12; 412(6843):150-7.

[64] J.S. Ebersole, “Defining epileptogenic foci: past, present, future, “Journal of Clinical Neurophysiology, 1997 Nov; 14(6):470-83.

[65] H.H., Kornhuber, L. Deecke, “Changes in the brain potential in voluntary movements and passive movements in man: readiness potential and reafferent potentials. Pflügers Archiv: European Journal of Physiology, 1965 May 10; 284():1-17.

[66] G. D. DAWSON, “A summation technique for the detection of small evoked potentials, “Electroencephalography and Clinical Neurophysiology, 1954 Feb; 6(1):65-84.

[67] E. Donchin, E. Heffley, “Minicomputers in the signal-averaging laboratory, “American Psychologist, 1975 Mar; 30(3):299-312.

[68] R. Galambos, G.C. Sheatz, “An electroencephalographic study of classical conditioning, “Paper presented at the American Journal of Physiology; 1962.

[69] H.H. Jasper, “The ten-twenty electrode system of the International Federation, “Electroencephalography & Clinical Neurophysiology, 1958;10:371–375.

[70] J.R. Boston,” Spectra of Auditory Brainstem Responses and Spontaneous EEG, “IEEE Transactions on Biomedical Engineering, BME-28, 1981 334–341. https://doi.org/10.1109/TBME.1981.324801.

[71] F. Musiek, S. Nagle, “The Middle Latency Response: A Review of Findings in Various Central Nervous System Lesions, “Journal of the American Academy of Audiology, 2018, 29, 855–867. https://doi.org/10.3766/jaaa.16141.

[72] R.S. Bruno, S.J. Oppitz, M.V. Garcia, E.P.V. Biaggio, “Potencial evocado auditivo de longa latência: diferenças na forma de contagem do estímulo raro, “Rev. CEFAC, 2016, 18, 14–26. https://doi.org/10.1590/1982-021620161816415

[73] M.P. Paulraj, K. Subramaniam, S. Bin Yaccob, A.H. Bin Adom, C.R. Hema,” Auditory evoked potential response and hearing loss: a review, “Open Biomedical Engineering Journal, 2015, 9, 17–24. https://doi.org/10.2174/1874120701509010017

[74] G.F. Woodman, “A brief introduction to the use of event-related potentials in studies of perception and attention, “Attention, perception & psychophysics, 2010, 72(8), 2031–2046. https://doi.org/10.3758/APP.72.8.2031

[75] P. A. F. Key, G. O. Dove, & M. J. Maguire, “Linking brainwaves to the brain: an erp primer, “Psychological and Brain Science.

[76] M. Waldo, G. Gerhardt, N. Baker, C. Drebing, L. Adler, & R. Freedman, “Auditory sensory gating and catecholamine metabolism in schizophrenic and normal subjects, “Psychiatry Research, 1992, 44, 21-32.

[77] N.K. Squires, & C. Ollo, “Human evoked potentials techniques, “Possible applications to Neuropsychology. In J.H. Hannay (Ed.), Experimental techniques in human neuropsychology, 1986, (pp.386-418). New York: Oxford University Press.

[78] S.A. Hillyard, R.F. Hink, V.L. Schwent, & T.W. Picton, “Electrical signs of selective attention in the human brain,” Science, 1973, 182, 177-180.

[79] R. Hari, K. Kaila, T. Katila, T. Tuomisto, & T. Varpula, “Interstimulus interval dependence of the auditory vertex response and its magnetic counterpart: implications for their neural generation,” Electroencephalography and Clinical Neurophysiology, 1982, 54, 561- 569.

[80] H.G. Vaughan Jr., & W. Ritter, “The sources of auditory evoked responses recorded from the human scalp, “Electroencephalography and Clinical Neurophysiology, 1970, 28, 360-367.

[81] R. Johnson, “Developmental evidence for modality dependent P300 generators: A normative study,” Psychophysiology, 1989, 26, 651-667

[82] E.J. Golob, & A. Starr, “Age-related qualitative differences in auditory cortical responses during short-term memory, “Clinical Neurophysiology, 2000, 111, 2234-2244.

[83] C. Sandman, & J. Patterson, “The auditory event-related potential is a stable and reliable measure in elderly subjects over a 3-year period,” Clinical Neurophysiology, 2000, 111, 1427-1437.

[84] K. Crowley, & I. Colrain,” A review of the evidence for P2 being an independent component process: Age, sleep, and modality, “Clinical Neurophysiology, 2004, 115, 732-744

[85] P.J. Holcomb, P.T. Ackerman, & R.A. Dykman, “Auditory event-related potentials in attention and reading disabled boys, “International Journal of Psychophysiology, 1986, 3, 263- 273.

[86] J. Polich, & T. Bondurant, “P300 sequence effects, probability, and interstimulus interval, “Physiology and Behavior, 1997, 61, 843-849.

[87] J. Polich, S. Eischen & G. Collins, G. (1994). P300 from a single auditory stimulus. Electroencephalography & Clinical Neurophysiology, 1994, 92, 253-261.

[88] M. Fabiani, G. Gratton, & M. Coles, M. G. H, "Event-related brain potentials: Methods, theory, and applications, "In J. T. Cacioppo, L. G., Tassinary, & G. G., Berntson (Eds.), Handbook of psychophysiology, 2nd edition, 2000, (pp. 53-84). Cambridge, UK: Cambridge University Press.

[89] T. Rosburg, "Left hemispheric dipole locations of the neuromagnetic mismatch negativity to frequency, intensity and duration deviants, "Cognitive Brain Research, 2003, 16, 83-990.

[90] E. Donchin, & M. Coles, "Is the P300 component a manifestation of context updating? "Behavioral & Brain Sciences, 1998, 11, 357-427.

[91] R. Emmerson, R. Dustman, D. Shearer & C. Turner, "P3 latency and symbol digit performance correlations in aging, "Experimental Aging Research, 1990, 15, 151-159.

[92] J. Polich, & S. Martin, "P300, cognitive capability, and personality: A correlational study of university undergraduates, "Personality and Individual Differences, 1992, 13, 533-543.

[93] B. O'Donnel, R. Cohen, H. Hokama, B. Cuffin, C. Lippa, M. Shelton, & D. Drachman," Electrical source analysis of auditory ERPs in medial temporal lobe amnestic syndrome, "Electroencephalography and Clinical Neurophysiology, 1993, 87, 394-402.

[94] K.A. Paller, G. McCarthy, E. Roessler, T. Alllison, & C. Wood," Potentials evoked in human and monkey medial temporal lobe during auditory and visual oddball paradigms, "Electroencephalography and Clinical Neurophysiology, 1992, 84, 269-279.

[95] Y. Katayama, T. Tsukiyama, & T. Tsubokawa, "Thalamic negativity associated with the endogenous late positivity component of cerebral evoked potential (P300): recordings using discriminative aversive conditioning in humans and cats", Brain Research Bulletin, 1985, 14, 223-226.

[96] M. Kutas, & S.A. Hillyard, "Event-related brain potentials to grammatical errors and semantic anomalies, "Memory and Cognition, 1983, 11, 539-550.

[97] R.A. Atchley, & K.M. Kwasny, "Using event-related potentials to examine hemisphere differences in semantic processing, "Brain and Cognition, 2003, 53, 133-138.

[98] T. Curran, & A.M. Cleary, “Using ERPs to dissociated recollection from familiarity in picture recognition, Cognitive Brain Research, 2003,15, 191-205.

[99] K. Allan, E.I. Wilding, & M.D. Rugg, “Electrophysiological evidence for dissociable processes contributing to recollection, “Acta Psychologica, 1998, 998, 231-252

[100] F. Guillem, B. N’Kaoua, A. Rougier, & B. Claverie, “Intracranial topography of event related potentials (N400/P600) elicited during a continuous recognition memory task,” Psychophysiology, 1995, 32, 382-392.

[101] A. S. Gevins, A. Rémond,” Methods of analysis of brain electrical and magnetic signals, “Handbook of Electroencephalography and Clinical Neurophysiology, Elsevier, Amsterdam.

[102] M. Teplan, “Fundamentals of EEG Measurement: Measurement Science review, 2002, Volume 2, Section 2.

[103] D. Regan, D, “Human brain electrophysiology: evoked potentials and evoked magnetic fields in science and medicine. Elsevier., 1989.

[104] T.W. Picton, M.S. John, A. Dimitrijevic, & D. Purcell, D, “Human auditory steady-state responses: Respuestas auditivas de estado estable en humanos, “International Journal of Audiology, 2003a, 42, 177–219. https://doi.org/10.3109/14992020309101316

[105] A. Compston, “The Berger rhythm: potential changes from the occipital lobes in man, by E.D. Adrian and B.H.C. Matthews, “From the Physiological Laboratory, Cambridge, Brain, 1934: 57; 355-385. Brain 133, 3–6. https://doi.org/10.1093/brain/awp324

[106] R. Galambos, S. Makeig, P.J. Talmachoff, “A 40-Hz auditory potential recorded from the human scalp, “Proceedings of the National Academy of Sciences of the United States of America, 1981, 78, 2643–7.

[107] Makeig, S., Galambos, R., “The CERP: Event-Related Perturbations in Steady-State Responses”, Springer, Berlin, Heidelberg, 1989, pp. 375–400. https://doi.org/10.1007/978-3-642-74557-7_30

[108] R.D. Lindens, K.B. Campbell, G. Hamel, T.W. Picton, T.W, “Human auditory steady state evoked potentials during sleep, “Ear and Hearing, “1985, 6, 167–174. https://doi.org/10.1097/00003446-198505000-00008

[109] B. Ross, T.W. Picton, C. Pantev, C., "Temporal integration in the human auditory cortex as represented by the development of the steady-state 80 magnetic field, "Hearing Research, 2002, 165, 68–84. https://doi.org/10.1016/S0378- 5955(02)00285-X.

[110] B. Ross, A.T. Herdman, C. Pantev, C., "Stimulus Induced Desynchronization of Human Auditory 40-Hz Steady-State Responses, "Journal of Neurophysiology, 2005. 94, 4082–4093. https://doi.org/10.1152/jn.00469.2005

[111] G. Pfurtscheller," EEG event-related desynchronization (ERD) and synchronization (ERS)," Electroencephalography and Clinical Neurophysiology, 1997, 103-26.

[112] D.L. Beck, D.P. Speidel, M. Petrak, "Auditory Steady-State Response (ASSR): A Beginner's Guide - Hearing Review, "Hearing Review, 2007, 14, 34–37.

[113] P. Korczak, J. Smart, R. M. Delgado, T. Strobel, C. Bradford, "Auditory Steady State Responses, "Journal of the American Academy of Audiology, 2012, 23, 146–170. https://doi.org/10.3766/jaaa.23.3.3

[114] M.X. Cohen, "A better way to define and describe Morlet wavelets for time-frequency analysis, "Neuroimage, 2019, 199, 81–86. https://doi.org/10.1016/J.NEUROIMAGE.2019.05.048

[115] M.X. Cohen, "Analyzing Neural Time Series Data: Theory and Practice, 2014.

[116] J. Artieda, M. Valencia, M. Alegre, O. Olaziregi, E. Urrestarazu, J. Iriarte, "Potentials evoked by chirp-modulated tones: a new technique to evaluate oscillatory activity in the auditory pathway, "Clinical Neurophysiology, 2004, 115, 699–709. https://doi.org/10.1016/j.clinph.2003.10.021

[117] K. Lehongre, F. Ramus, N. Villiermet, D. Schwartz, A.L. Giraud, "Altered Low-Gamma Sampling in Auditory Cortex Accounts for the Three Main Facets of Dyslexia, "Neuron, 2011 72, 1080–1090. https://doi.org/10.1016/j.neuron.2011.11.002

[118] M. Pérez-Alcázar, M.J. Nicolás, M. Valencia, M. Alegre, J. López-Azcárate, J. Iriarte, J. Artieda, "Cortical oscillations scan using chirp evoked potentials in 6-hydroxydopamine rat model of Parkinson's disease, "Brain Research, 2010, 1310, 58–67. https://doi.org/10.1016/J.BRAINRES.2009.11.031.

[119] C. Poulsen, T.W. Picton, T. Paus, "Age-Related Changes in Transient and Oscillatory Brain Responses to Auditory Stimulation in Healthy Adults 19-45 Years," Old Cerebral Cortex, 2007,17, 1454–1467. https://doi.org/10.1093/cercor/bhl056

[120] M. Alegre, P. Molero, M. Valencia, G, Mayner, F. Ortuño, J. Artieda, "Atypical antipsychotics normalize low-gamma evoked oscillations in patients with schizophrenia," Psychiatry Research, 2017, 247, 214– 221. https://doi.org/10.1016/j.psychres.2016.11.030

[121] E. Pippins," Evaluation of electrical brain responses to linear chirp-modulated tones: effect of task and changes in neuropsychiatric disorders", Vilnius University, 2020, https://orcid.org/0000-0003-4045-8203.

[122] M. A. Rigoulot, E. Koning, A. Ferrandon, and A. Nehlig," Neuroprotective Properties of Topiramate in the Lithium-Pilocarpine Model of Epilepsy," The journal of pharmacology and experimental therapeutics, Vol. 308(2004):787–795.

[123] R. F. Thompson & W. A. Spencer," Habituation: A model phenomenon for the study of Neuronal substrates of behavior," Psychological Review, Vol. 73, No. 1, 16-43, 1966.

[124] C. H. Rankin, T. Abrams, R. J. Barry, S. Bhatnagar, D. Clayton, J. Colombo, G. Coppola, M. A. Geyer, D. L. Glanzman, S. Marsland, F. McSweeney, D. A. Wilson, C. F. Wu, & R. F. Thompson, "Habituation Revisited: An Updated and Revised Description of the Behavioral Characteristics of Habituation," Neurobiology of Learning and Memory, Sep; 92(2): 135–138, 2009.

[125] J. W. Lovelace, I. M. Ethell, D. K. Binder & K. A. Razaka," Translation-relevant EEG phenotypes in a mouse model of Fragile X Syndrome," Neurobiology of Disease, 115, 39–48, 2018.

[126] D.W Purcell, S.M. John, B.A. Schneider & T.W. Picton," Human temporal auditory acuity as assessed by envelope following responses," Journal of the Acoustical Society of America, 116, 3581–3593, 2004.

[127] E. Wronka, J. Kaiser, & A. M.L. Coenen," Neural generators of the auditory evoked potential components P3a and P3b," Acta Neurobiologiae Experimentalis, 72: 51–64, 2012.

[128] D. Umbrichta & S. Krljesb," Mismatch negativity in schizophrenia: a meta-analysis," Schizophrenia Research, 76, 1–23, 2005.

[129] L. Harms, W. R. Fulham, J. Todd, T. W. Budd, M. Hunter, C. Meehan, M. Penttonen, U. Schall, K. Zavitsanou, D. M. Hodgson & P. T. Michie," Mismatch Negativity (MMN) in Freely-Moving Rats with Several Experimental Controls," PLOS One, 9, 10, 2014.

[130] T.J. McGee, C. King, K. Tremblay, T.G. Nicol, J. Cunningham, & N. Kraus, "Longterm habituation of the speech-elicited mismatch negativity," Psychophysiology, 2001, 38, 653- 658.

[131] S. J. Luck and E. S. Kappenman, "The Oxford Handbook of Event-Related Potential Components," The Oxford library of Psychology, 2011.

[132] N. K. Amy and A. Sharma," Inter-trial coherence as a marker of cortical phase synchrony in children with sensorineural hearing loss and auditory neuropathy spectrum disorder fitted with hearing aids and cochlear implants," Clinical Neurophysiology, Jul; 125(7): 1459–1470, 2014.

[133] A. Delorme. and S. Makeig., "EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis," Journal for Neuroscience Methods, 134, 9-21, 2004.

[134] G. Buzsaki A. Draguhn," Neuronal oscillations in cortical networks," Science, 304, 1926-9, 2004.

[135] P. Fries, "A mechanism for cognitive dynamics: neuronal communication through neuronal coherence," Trends Cognitive Science, 9, 474-80, 2005.

[136] W. J. Freeman & L. J. Rogers," Fine temporal resolution of analytic phase reveals episodic synchronization by state transitions in gamma EEGs," Journal of Neurophysiology, 87, 937-45, 2002.

[137] C. Tallon-Baudry, O. Bertrand, C. Delpuech & J. Pernier, "Stimulus specificity of phase-locked and non-phase-locked 40 Hz visual responses in human," Journal of Neuroscience, 16, 4240-9, 1996.

[138] R. Grandchamp & A. Delorme, "Single-trial normalization for event-related spectral decomposition reduces sensitivity to noisy trials," Frontiers in Psychology, 2011.

[139] J. B. Roach, D. H. Mathalon, " Event-Related EEG Time-Frequency Analysis: An Overview of Measures and An Analysis of Early Gamma Band Phase Locking in Schizophrenia," Schizophrenia Bulletin, 2008, Volume 34, Issue 5, Pages 907–926, https://doi.org/10.1093/schbul/sbn093.

[140] S. A. Hillyard and M. Kutas, "Electrophysiology of cognitive processing," Annual Review of Psychology, 1983. 34:33-61.

[141] D. D Didoné, M. V. Garcia, S. J. Oppitz, T. F. Silva, S. N Santos, R. S. Bruno, V. A. Filha, and P. L. Cóser, "Auditory evoked potential P300 in adults: reference values. Einstein (Sao Paulo, Brazil)," 2016, 14(2), 208–212. https://doi.org/10.1590/S1679-45082016AO3586.

[142] N. Kraus and T. McGee," Auditory event-related potentials. In: Hand book of clinical audiology," Baltimore: Williams and Wilkins; 1994, pp. 406–426.

[143] W. Karniski, R.C. Blair, "Topographical and temporal stability of the P300," Electroencephalography and Clinical Neurophysiology. 1989 May; 72(5):373-83.

[144] D.L. McPherson, "Late potentials of the auditory system", San Diego: Singular Publishing Group; 1996.

[145] 1C. S. Daivs, "Statistical Methods for the Analysis of Repeated Measurements", Springer, 2002.

[146] 2S. K. Pajouh, O. Renaud," A general permutation approach for analyzing repeated measures ANOVA and mixed-model designs", Stat Papers (2015) 56:947–967.

[147] N. Alexeyeva, "Dual balance correction in repeated measures ANOVA with missing data", Electronic Journal of Applied Statistical Analysis Vol. 10, Issue 01, April 2017, 146-159.

[148] H.J. Keselman, & J.C. Keselman, "Comparing repeated measures means in factorial designs. Psychophysiology," 1988, 25, 612-618

[149] Salkind, Neil J. "Repeated Measures Design". SAGE Research Methods. SAGE. Retrieved 8 January 2019.