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Brain activity-derived biomarkers for epileptogensis

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Abstract

Epilepsy is a neurological disorder associated with recurrent spontaneous seizures affected by synchronized neuron activity. A biomarker is a biological indicator of a disease that is used to diagnose or understand it. Biological markers that indicate the development of epilepsy can be beneficial in predicting its onset and tracking its progression. Further knowledge of the disease can also lead to new discoveries, including previously unknown mechanisms of its development, enabling more effective treatments to be developed. In this project, my goal is to combine established biomarkers and develop a program that can predict epilepsy within the first few days following a seizure. I investigated data from rats that were injected with pilocarpine to induce temporal lobe epilepsy and recorded for 12 days following their injection in order to establish this claim. This study's outcomes will be compared to a subsequent recording of the same rats taken three months later to identify which rats exhibit epilepsy.

Introduction

1. Epilepsy

Epilepsy is the most common chronic neurological disorder and it's associated with recurrent spontaneous seizures, affecting both children and adults, with variable manifestations, numerous etiologies, and diverse treatments. Epileptic seizures are caused by abnormal and hyper-synchronous neuronal discharges and can be diagnosed and characterized using an EEG - a non-invasive recording of the patient's brain waves. Many factors contribute to the etiology, including brain trauma, stroke, tumors, genetic factors, etc. Epilepsy can be divided into two main types: Focal epilepsy - seizures occur in a certain area of the brain. In this area there are neurons with poor function, and sometimes they can be removed by surgery (if certain standards are met). On the other hand, in the second type of epilepsy, the whole brain is affected, so there is no single focus. In both types, seizures may be very dangerous and may lead to death in severe cases, while epilepsy patients also face many challenges in their daily lives. These difficulties include anxiety or depression, a decreased quality of life and the inability to integrate into society. Epilepsy is an incurable disorder, but there are treatments available today to prevent the symptoms of seizures and reduce their frequency. Anti-epileptic drugs (AED) are designed to achieve a balance between excitation and inhibition, which will prevent the hyper-synchronization of neuronal activity. This can be accomplished through several mechanisms that are involved in the processes of synaptic transmission in the brain. The drug is selected according to the frequency and intensity of the patient's seizures, and can include side effects such as headaches, confusion, cognitive problems, and more. The problem is that there are patients who do not respond to drug treatment or the removal of the focus does not contribute to the control of their disease. Therefore, many studies are being conducted on the subject to seek additional solutions. [1]

1.1 Epileptogenesis

Epileptogenesis is the process of modifying a normal functioning neuronal network into an epileptic one. The process is complex and not fully understood, but it is thought to involve a combination of genetic, developmental, and environmental factors. It is a process that combines neurogenesis and cell loss that causes new synaptic organization, increased excitability and improves the synchronization of pathological neuronal discharges.[2]

1.2 Electroencephalogram (EEG)

The basis of the nervous system is the nerve cell, the neuron, which is responsible for transmitting information to and from the brain. The neuron consists of the cell body, soma, and dendrites that are responsible for transmitting electrical information between the neurons in the brain. EEG, or electroencephalography, is a technique used to measure the electrical activity of the brain. It involves the use of electrodes that are placed on the scalp to detect the collective electrical activity of millions of neurons

at a depth of several millimeters that produce a strong electric field. The electrical field in the brain is primarily produced by the flow of electrical currents that occur during the stimulation of the dendrites through excitatory postsynaptic potentials (EPSPs) at synapses. These signals allow a non-invasive way to understand the mechanisms and electrical processes that occur in the brain. An EEG signal's amplitude is determined by the level of synchrony of the neurons. Large amplitude signals are produced when a group of neurons are synchronously excited, while asynchronous excitation results in a low-amplitude irregular EEG signal. The frequency of the signal is affected by the thalamus, which has the ability to produce self-sustaining firing rates or by coordinated interactions between neurons in a certain area. States of wakefulness or dream sleep (REM) are characterized by high frequency and low amplitude, and in contrast, drowsiness or non-dream sleep is characterized by low frequency and high amplitude. EEG measurements can use two different types of electrodes: bipolar and unipolar. Bipolar electrodes consist of 2 electrodes located close to each other, one of them positive and one negative and they are used to measure the potential difference between them. In addition, unipolar electrodes are electrodes with a single point of contact with the scalp. The potentials are measured between them and a remote reference electrode in order to eliminate noise. Also, when measuring an EEG signal, the two types can record noises such as eye movement, electrode movement, heart or muscle activity. These noises must be filtered in a way that does not damage the desired signal. [3]

ECoG, or electrocorticography, is an invasive method used in research and clinical settings for measuring the electrical activity of the brain by placing electrodes directly on the surface of the brain. With ECoG, neural activity in specific brain areas can be recorded with high spatial resolution and sensitivity.

2. Epileptogenesis Biomarkers

A biomarker is an objective measure of a biological process that can be used to diagnose or better understand a disease. It can be measured through blood, urine, saliva, electrode monitoring and more. Finding biological markers for epileptogenesis can help in a number of areas including predicting the development of epilepsy and measuring the progress of the disease. A biomarker allows us to gain a deeper understanding of the disease and to allow researchers to discover things about it that are still unknown, such as mechanisms related to its development. This will make it possible to develop and treat the disease more effectively.[4] Finding a biomarker for epilepsy is particularly necessary in cases after the first seizure, when there is uncertainty about whether the person will develop epilepsy (only 30% of patients). Due to the low percentage, most patients do not receive treatment after the first seizure, and those who develop epilepsy may suffer serious consequences without early treatment. Using such a biomarker will enable diagnosis to be made in advance and more patients to be treated. A biomarker can also be helpful to prevent or help in cases like brain traumatic injury or stroke, in which there is no anti-epileptic intervention.[5] There are many studies trying to find a biomarker of epileptogenesis and I will focus on those monitored with EEG or ECoG. I intend to create a predictive model based on proven biomarkers and will expand on the most promising ones.

2.1 Seizures detection

Epileptic seizures are caused by abnormal and hyper-synchronized neuronal discharges and are detectable by EEG or ECoG recordings. In one sense, seizures do not necessarily indicate a chronic disorder, since epilepsy is not always the cause of seizures. However, not all seizures caused by epilepsy indicate the presence of a chronic illness.[5] Using epilepsy models on rats, we obtain status epilepticus (SE), a strong and prolonged seizure. It has been suggested in the literature that there is a 'latent period' following SE, characterized by periods without seizures, whereas animals can still suffer seizures during this time. Due to this, seizures and their characteristics during a 'latent period' may indicate the onset of epilepsy. As a result, this can serve as a reliable biomarker for the development of epilepsy in the future.

2.2 PSWE

PSWE- Paroxysmal slow wave event, is defined as a slow wave with frequency under 6 Hz for at least 5 consecutive seconds. According to Daniel Zelig et al. (2021), the predictive value for epilepsy with PSWE is 82% in patients monitored by EEG 72 hours following the first seizure. A comparison was made based on the percentage of time spent in PSWE from all the recording time and the mean number of electrodes that detect PSWE. This study also found that a low mean power frequency of PSWE and a long duration of PSWE are significantly associated with epilepsy. [6] Another study in epilepsy patients separated the PSWE into hPSWE (high) and lPSWE (low). Compared to the control group, 90% of the hPSWE was found in the epilepsy group. Additionally, they conducted research on rats that found PSWE to be significantly more frequent and longer in epileptic rats.[7]

2.3 Theta and gamma waves

Theta waves are brain rhythms occurring in the range of 4-7 Hz and are associated with drowsiness or certain stages of sleep. The high-gamma rhythm is in the range of more than 30 Hz and occurs when the person is actively processing information. [3] Temporal lobe epilepsy (TLE) is most often associated with cognitive deficits. Based on pilocarpine model research on rats, researchers found that a decrease in theta activity was correlated with cognitive impairment and suggest that this may be the explanation for the cognitive deficit in TLE as well as a reliable biomarker for epileptogenesis. Researchers also found that spatial memory was altered during the latent period, which corresponds to the period of the record that I am analyzing. [8] Dan Milikovsky et al. (2017) investigates 5 models of post-injury epilepsy. They discovered that a decrease in theta waves during epileptogenesis is a reliable predictor of epilepsy with high sensitivity and specificity (90%). Further, they found that early epilepsy development is associated with sharp declines in theta activity and there is also an inverse correlation between theta waves and high-gamma activity (30-100 Hz). [9]

2.4 pHFO

HFO- high frequency oscillations, are fast ripples in the frequency range of more than 100 Hz and probably have a role in sensory information processing. They appear in some brain regions with a frequency of 100-200 Hz such as the subiculum, the entorhinal cortex and some regions of the hippocampus (CA1,CA3). In rats and humans neocortical they appear at 200-600 Hz.[10] pHFOs, or pathological HFOs, are occurring in abnormal places at a frequency of 200-600 Hz, such as the hippocampus (normal hippocampus HFO is 80-100 Hz). However, it is pertinent to note that there is currently no simple way to distinguish between normal and pathological activity. pHFOs are the result of the combined electrical activity of neurons that fire together in synchronization and they are believed to be a biomarker of epileptogensis .[5] It has been shown to be a useful biomarker to detect epilepsy focal, the development of the disease, and treatment response. Researchers discovered that soon appearance of pHFO is correlated with soon appearance of the first spontaneous seizure. HFO is found in brain areas capable of generating spontaneous seizures.[10] Also found that there is a connection between early detection of pHFO and an increased rate of spontaneous seizures. [11] Anatol Bragin et al. (2016), used a traumatic brain injury (TBI) model of epilepsy and found that pHFO was observed in 58% of the TBI group within or near the trauma focal, but not in the control group. [12] It is important to note that the pHFO frequency ranges from 200 to 600 Hz, and my sampling rate is 200 Hz. Thus, it is not possible for me to use this biomarker, even though it is a reliable one.

2.5 Asymmetry

The term asymmetry refers to a recording period during which there is a change of more than 50% in the amplitude of the signal or a change in frequency of more than 1 HZ between the two hemispheres. [13] Carla Benets et al. (2018) demonstrates that asymmetry in the background activity of EEG recordings during the year following a stroke is a reliable biomarker that can predict epilepsy. [14]

2.6 Sleep stages

Pedro Andrade et al. (2017) discovered that 92% of spontaneous generalized seizures occur during the transition from sleep stage 3 to REM in post-traumatic epilepsy. Also, the researchers discovered that a reduction in spindle duration and dominant frequency during this transition between sleep stages is a reliable predictor of this type of epilepsy with high sensitivity and specificity. Stage 3 was defined by high-amplitude and slow-frequency synchronous activity, while REM was defined by low-amplitude synchronous activity in the theta band. The transition between these stages contains a cluster of spindles.[15]

3. Research data

In my research, I used data obtained from the "Institute of Pharmacology" in Milan. They used pilocarpine to induce temporal lobe epilepsy (TLE) in rats. A TLE is characterized by seizures that originate in the limbic system, an "initial precipitating" injury, a 'latent period' where seizures do not occur, and hippocampus sclerosis causing neural network changes. With the injection of pilocarpine, we get an imbalance between inhibition and excitation, which causes status epilepticus (SE) - a severe and prolonged seizure. In the literature, there is a supposed 'latent period' after SE, even though animals can still suffer seizures during this time. Around 12 days later, epilepsy animals develop recurrent spontaneous seizures. The data in my research include recordings started immediately after injection for 12 consecutive days, 24/7, for 14 rats. Approximately three months after injection, additional data were collected to determine which rats developed epilepsy, allowing a comparison to be made with the research findings. The recording shows that 9 rats aren't epileptic while 5 rats are. Two epidural screw electrodes were used bilaterally (2 channels each) for ECoG recordings (placing electrodes directly on the surface of the brain) with a sampling rate of 200 Hz. Ground electrode was implanted above the sinus nasali, and reference electrode was implanted above the cerebellum. Using feature extraction and biomarkers proven in the literature, I aim to predict which animals will develop epilepsy. This can have a meaningful effect because epilepsy can be treated more effectively by predicting it in advance. [16]

Goals

This project aims to diagnose epileptogenesis using a reliable and proven biomarker that can be used to identify or predict epileptogenesis.

The main goal will be achieved through:

- Find the most distinct features that distinguish those with epilepsy from those without epilepsy during epileptogenesis - calculating the proven features from the literature and determining whether they contribute to the data.
- Build a model that simulates separation with high sensitivity and specificity – using the features from the previous goal. Also, generalize the predictive model by using other epilepsy models (such as aging or genetic modification). This will lead to a more accurate and generalized model.

Methods

This chapter includes methods for calculating characteristics and building the model. Since the data sampling rate is 200 Hz, I will use only features relevant to this rate. I decided to start with seizure detection. I will then use all the features calculated by Nvision program to search for a suitable separation based on these features. In this project I encountered a problem of imbalanced data because only 5 animals out of 14 had epilepsy. The solution to this problem could be making a new separation according to the amount of seizures the animal developed in the last days of the recording. This solution is effective because epilepsy is defined by the development of repeated spontaneous seizures. Furthermore, I intend to generalize the model using other epilepsy models. The addition of animals will also help me avoid imbalances in the data

and prevent overfitting as a result of a small sample size. With more information, the model is able to find even more differences between the data, resulting in a much more accurate and generalized model, which is not restricted by the data entered.

1. **Seizure detection**

Seizure detection is done by the Nvision GUI written by Lyn Kamintsky. This program segments the data into epochs of 2 sec (according to the sampling rate) with a 1 sec overlap. The size was chosen so that it would allow local stationary assumptions and satisfactory temporal and frequency resolution. For each epoch, 22 features are calculated, including energy, maximum amplitude, amplitude distribution features, skewness, kurtosis, zero crossing, Fourier transform features, etc. To ensure that there is no interference between the animals and between the electrode locations, all features have been normalized. The artificial neural network (ANN) algorithm and forward sequential selection (FSS) are used to determine the most effective separation. ANN output is buffered into 6 second intervals with 5 second overlaps. The mean was calculated for every 6 outputs and compared with the threshold using sliding windows. [17] The features that were chosen by the FSS were:

1. Energy: assessment of signal strength. Due to synchronized activity, this measurement is supposed to be higher in epileptic seizures. Calculated by $E = \frac{1}{N}\sum_{n=1}^{N}(x_n)^2$
2. Curve length: this measure is sensitive to changes in amplitude and frequency and such changes occur during seizures. Calculated by $CL = \sum_{n=2}^{N}|x_n - x_{n-1}|$
3. Standard deviation: a measure of the difference between the values in relation to the average. Calculated by $\sigma = \sqrt{\frac{1}{N}\sum_{n=1}^{N}(x_n - \bar{x})^2}$
4. Relative power in the low gamma frequency band (20-40 Hz), calculated by: $Low\ \gamma = \frac{1}{F_T}\sum_{j=20}^{40} f_j$
5. Relative power in the beta frequency band (12-20 Hz), calculated by: $Low\ \beta = \frac{1}{F_T}\sum_{j=12}^{20} f_j$

When f_j is the Fourier transform and $F_T = \sum_{j=1}^{100} f_j$ for normalized. [17]

2. **Dynamic and stationary changes**

Based on Dan's Milikovsky research, I will examine the 22 features mentioned above, specifically the relative power in 5 frequency bands, including theta and gamma waves. Using linear regression, I plan to calculate the slope of this band and examine changes in epileptogenesis. For the purpose of evaluating the predictive ability of each feature, I will employ statistical tools such as p-values to determine whether the results are statistically significant. ROC analysis will also be used to determine the sensitivity and specificity of the measurements. [9]

3. **Separation methods**

I intended to use supervised learning techniques to separate epileptic from non-epileptic animals. Models are trained using tagged databases in this type of learning. For each error of the model, a "penalty" is given which should encourage the model to produce results more similar to the real labeling. An example of a supervised model is a regression model. The goal of this type of model is to produce a continuous function that matches each input and output. This function can be, for example, linear or polynomial. Another type is decision trees, a model that uses a tree-like structure to model the relationship between the input features and the target variable. Support vector machine (SVM) is also a supervised method that finds the most appropriate boundary that separates the different classes in the data. My choice will be based on the model that performs the best separation between the data sets after checking various options.

Preliminary results

Using Nvision I noticed two different types of seizures. Figure 1 shows a base line and the two types of seizures I found. Graph (C) illustrates an example of 'Seizure like events' (SLE), i.e. a seizure with high amplitude, long duration, and high frequency components. The slow oscillation that is shown in graph (B) is a shorter seizure with a lower amplitude and frequency. Typically, the peak frequency of these seizures is between 5 and 6 Hz, whereas the peak frequency of the SLEs is between 8 and 10 Hz. While SLEs were found to be a reliable biomarker, slow oscillation did not show a significant difference between the two groups. A summary of the most significant results from seizure detection is shown in Figure 2. SLEs were observed in all epileptic animals (100%, n=4) on days 9-13, whereas only 2 non-epileptic animals (22.2%, n=9) developed SLEs during those days (Figure 2A). During day 10, there is significant separation between the groups in terms of number of seizures (p-value=0.014, t-test) and time spent in SLEs in a day (p-value=0.014, t-test). The mean time in SLEs in week 2 differed significantly between the two groups (p-value=0.0014, t-test, Figure 2C). Therefore, I examined the data more deeply by separating the groups at four-day intervals. The results revealed that the majority of epileptic rats (75%, n=4) experienced increases in seizures between days 6-9 and 10-13, whereas only one of the non-epileptic rats (11.11%, n=9) experienced such increases (Figure 2D).

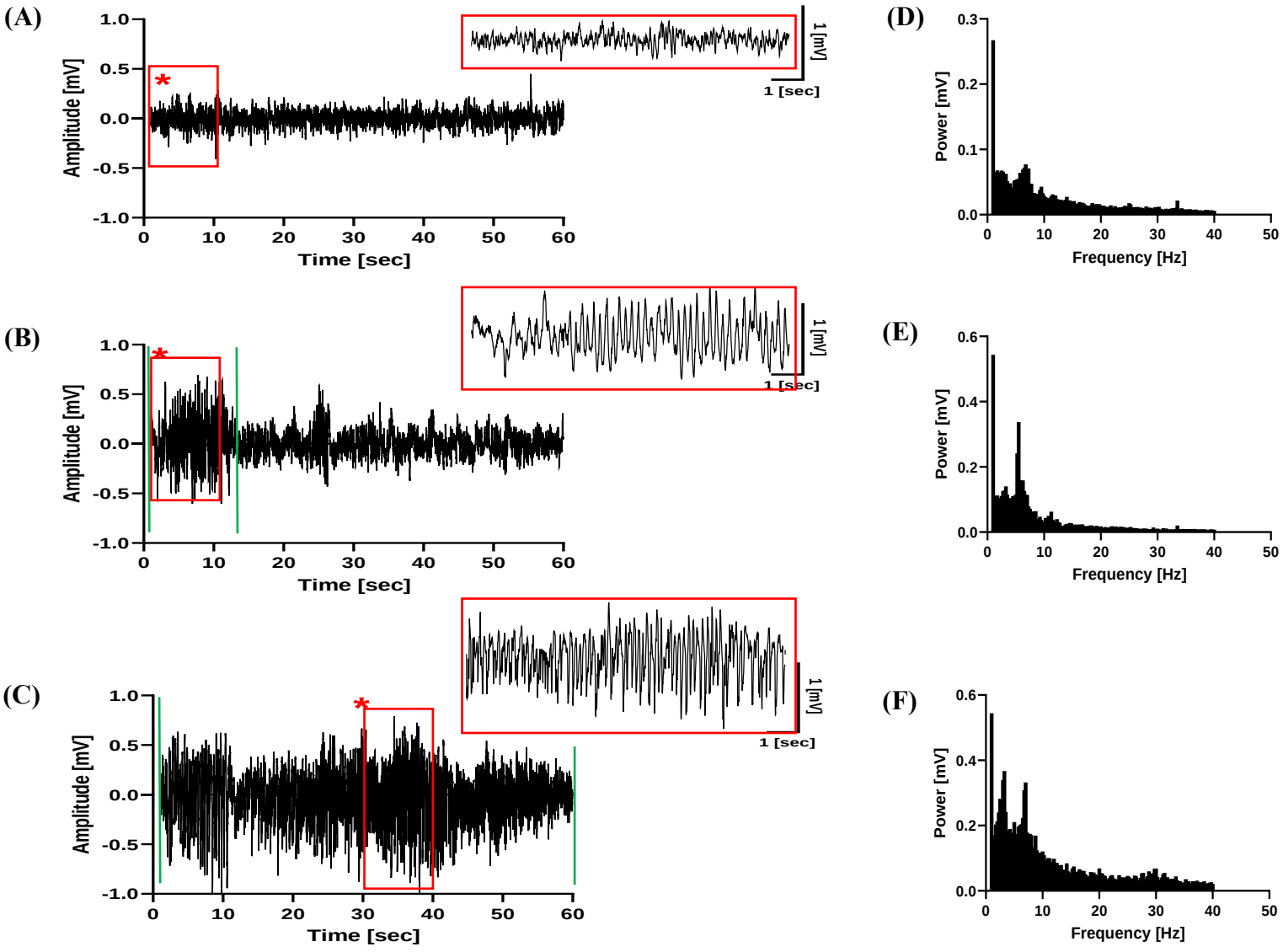

Figure 1: The figure represents a base line signal and two types of seizures: "seizures-like events" (SLE) and "slow oscillations". The green line represents the range of seizures. (A) An example of a base line. (B) 'Slow oscillation' - short duration and low amplitude. (C) 'Seizure like event' - long duration and high amplitude. (D) A graph representing the power per frequency for regular signals, characterized by low power at all frequencies. (E) 'Slow oscillation' - contains frequencies mainly below 6 Hz. (F) Power per frequency for the 'seizure-like event' - this signal has higher frequency components. This can be seen by the fact that the power of frequencies higher than 10 Hz is significantly higher than the other two types of graphs.

Due to my belief that the seizure properties within the days following the injection may be useful in predicting later epilepsy, I intended to develop a filter that would automatically distinguish between 'seizure like events' and 'slow oscillations'.

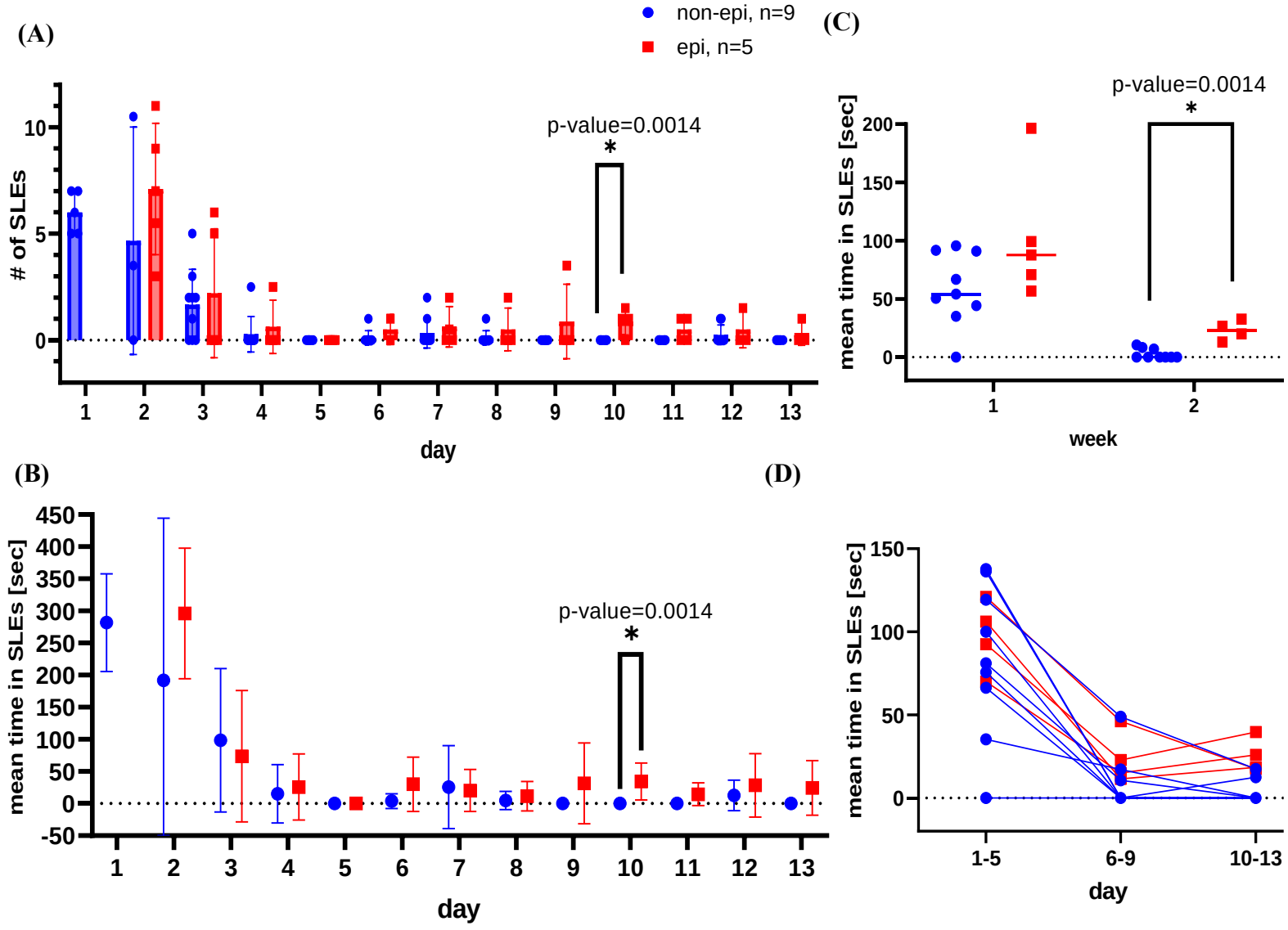

Figure 2: Primary results from seizures detection. (A) Number of SLE per day for each group. (B) Mean time in SLE per day for each group. (C) Separation for weeks - significant differences between groups in week 2. (D) Separation for 4 days - all rats with an increase between days 6-9 to days 10-13 are epileptic (75 % of the epileptic rats who had data on those days). (* = 0.01<p-value<0.05, ** = 0.001<p-value<0.01)

Schedule להוסיף תאריכים

Table 1: schedule

Literature review	done
Feature extraction	done
Seizures detection	done
Using features to detect dynamic changes (including theta and gamma)	
Detect and identify PSWE	
Build a model	
Develop a filter that separates between 'seizures like events' and 'slow oscillation' automatically	
Generalize the model using other models of epilepsy - aging, genetic modifications	

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