Finding Improved Protocols for Using Deep-Brain Stimulation for Treating Epileptic Seizures in Rats

AUTHOR **Yusra Sarhan & Tiwalade Sobayo**
FACULTY ADVISOR **Professor David Mogul**

Abstract

Epilepsy, a neural disorder marked by seizures, affects millions of people and is a tremendous reason for distress in the lives of epileptic patients and their loved ones. There are many ways to treat epilepsy such as antiepileptic drugs (AEDs), surgery, and devices like the Vagus Nerve Stimulator. Regardless, the aforementioned methods of treatment are not efficient enough because they do not target the main source of the disorder. In order to target the main source, the natural dynamics of the brain that are effected by seizures need to be understood and this can be done by stimulating seizures in Sprague Dawley rats and analyzing their local field potentials during seizures and comparing the extracted data to real-time videos of the rats while having seizures for verification. Also, histological analysis will enhance this understanding. By doing this, the most effective frequency for the therapeutic stimulation can be found and this can be used to mitigate and, ideally, terminate seizures. This verification will be necessary to move on to the future steps which entail the integration of the edited videos with electroencephalogram (EEG). The extracted data and real-time videos should correspond with the EEG. This will aid in the preparation for the next group of rat subjects.

Introduction

Epilepsy, a disorder which is categorized by abnormal electrical activity in the brain, was coined the 'falling sickness' in the play "Julius Caesar" by Shakespeare because it elicits seizures. Interestingly enough, the ancient Romans used to tie

the falling sickness to supernatural forces. Seizures can result when there is an increased amount of excitatory neurotransmitter activity or when there is a decreased amount of inhibi tory neurotransmitter activity (Incidence and Prevalence). In the United States alone, about 3 million people are affected by epilepsy in some way whether it is relatively infrequent where a seizure is experienced once a month or it may be a dangerous state where seizures occur once every day or even every hour. A cure for epilepsy has not been found as it is not clear what triggers the abnormal brain activity and it is also unclear how the natural dynamics of the brain function to produce a seizure. For this reason, it is crucial to research the electrophysiological aspect of epilepsy in order to develop more efficient treatment for epileptic patients.

The current state of technology is treatment for epilepsy through numerous methods which include medicine, surgery, and the use of devices that can control, more or less, seizures. The medicine that is often used is called antiepileptic drugs (AEDs) and this medicine exists in many forms, some more efficient than others. Nonetheless, AEDs are the most widely used method of treatment because AED medicines are generally effective. Additionally, surgery is another option that is used to treat epilepsy but surgical treatment is still undergoing evaluation. On the other hand, there are devices that are used to mitigate the effects of seizures, prevent them from happening, and/or stop them completely. The most prevalent of these devices is called the Vagus Nerve Stimulator (VNS) which is named after the nerve that it sends electrical impulses to: the vagus nerve. This nerve is located in the back of the neck and when it receives an electrical impulse, it blocks other impulses in the brain that may cause a seizure (Current Epilepsy Treatment and New Medications). In regards to the mechanical aspect of the VNS device, this device is similar to a pacemaker in the sense that it sends a signal to an organ in order to inhibit that organ from reacting to irregularities, i.e. increased/decreased neurotransmitter activity. The future direction of research on epilepsy includes new devices that may be able to stop epileptic seizures before they start. All in all, the current state of technology needs to be improved.

Some organizations, such as the Epilepsy Foundation of America, and researchers, such as David Mogul, PhD, are currently working towards enhanced treatments by approaching the issue from different angles and utilizing different methods of research (Faculty Research Program).

Epileptic research is relevant to the Health aspect of IIT Engineering Themes project because the research that is to be conducted furthers the endeavors that have been made so far in an effort to better understand epilepsy. Although the cause of aberrant excitatory electrical brain activity is not entirely known, it is clear that there is synchrony in different sites (parts of the brain) during epileptic seizures. In fact, synchronies in different sites are a natural part of epileptic seizure terminations. For this reason, the research project entails the integration of the edited videos of rat seizures from the previous research project with an electroencephalogram (EEG), which is a device used to record electrical activity in the brain. This is to be done so that the EEG can be analyzed in real-time while watching the videos. The EEG should correspond with the videos because, after being integrated into real-time data, the EEG will reflect the data values that were extracted from the real-time videos. This is one of the final steps of the research project. Before reaching this step, the videos need to be edited to suit the purposes of the research project. The edited videos of the experimental rat seizures will also be compared with the videos of the control groups so that the effect of the frequencies of the therapeutic stimulations can be analyzed. Also, brain slices will be stained and histological methods will be implemented to better study the brains of former rat subjects. Moreover, additional rat subjects will be operated on and electrodes will be installed in their brains. Through an initial chemical induction, the subjects will be made susceptible to having seizures and will become epileptic. This will help prepare the next group of subjects for data collection and analysis.

Thus, this research project is a continuation of the previous PURE R&D research project which was comprised of the analysis of the local field potentials in the brains of Sprague Dawley rats at different times and sites. These measurements were analyzed to see when synchronizations occur. Also, stimulated seizures, along with spontaneous seizures, were utilized (and will continue to be utilized) to better understand the natural dynamics of the rat brain.

Methods

There were two main experimental techniques that were used for this research project: local field potential (LFP) data extraction and real-time video data extraction.

For the local field potential data extraction, data was extracted from the LFPs in the hippocampi and the thalamus while the rat subjects were having seizures to calculate the duration of the seizure induction and the therapeutic induction at a certain frequency. Then, induction start time was calculated from the extracted data. Later, the data that was collected from the LFPs was used to collect additional data from the real time videos of the rat subjects while having seizures. NeuroControl and Matlab were used to collect the brain signals and analyze them, respectively.

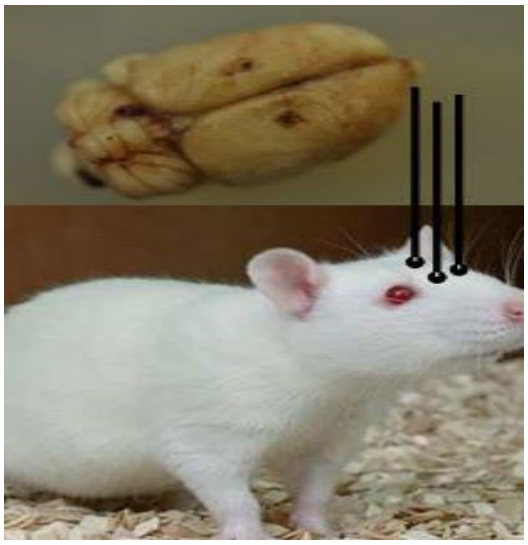

Figure 1. *This figure displays a rat brain with marks that represent where electrodes are placed.*

Calculations Used to Find Correct Induction Start, Therapeutic Start, Segment Start, and Segment End			
Correct Induction Start	**Therapeutic Start**	**Segment Start**	**Segment End**
Can be found by watching video in MovieMaker	Therapeutic Start (in seconds)-Induction Start (in seconds)+Correct Induction Start (TS-IS+Correct IS=Correct TS)	Add 30 seconds to the correct induction start (30 sec + Correct IS)	Add 30 seconds to the correct therapeutic start (30 sec + Correct TS)

Figure 2. *This figure displays the calculations used to find the therapeutic start, segment start, and segment end.*

The real-time video data extraction was done to verify that the data collected from the LFPs was accurate and corresponds to the real-time videos. The actual induction start time from the videos was recorded and the therapeutic start time was calculated. The therapeutic start time was then used to calculate the segment start time and segment end time for the video (calculations demonstrated in figure 2). The video was shortened to the segment start/end times and captioned to clearly illustrate the "Induction On" and "Stimulation On" periods. The video editing was completed by using MovieMaker. All data that was collected was stored in an Excel spreadsheet.

Additionally, there were supplementary histological methods that were implemented after the rat brains were extracted. The extracted brains were

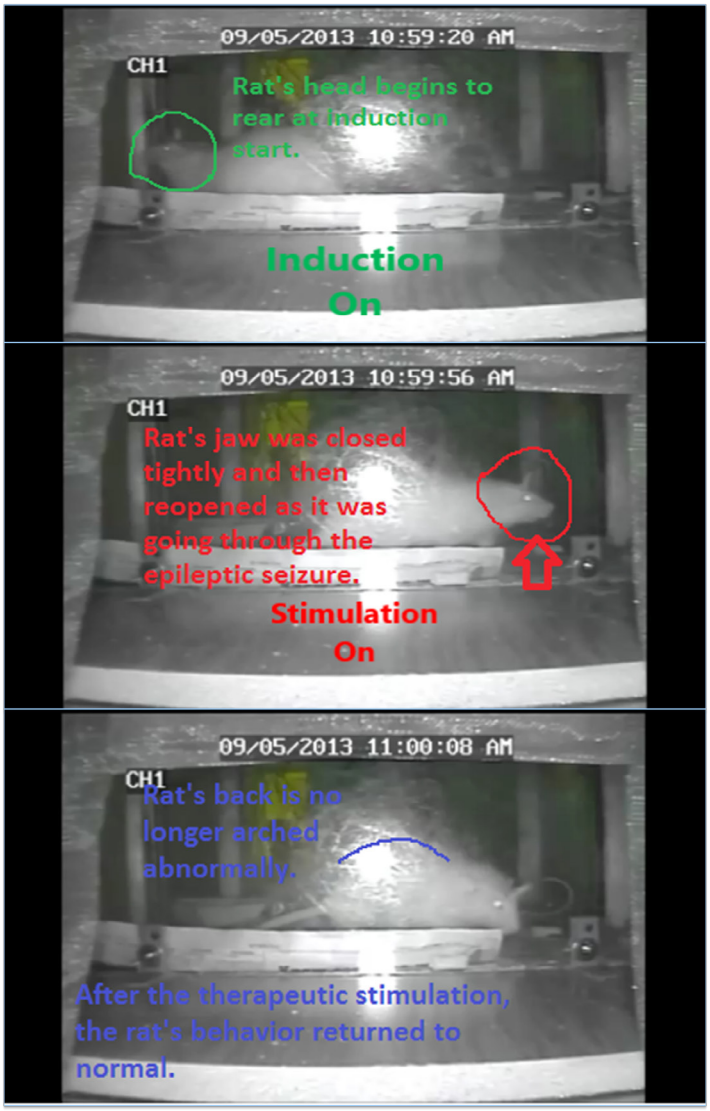

Figure 4. *This figure displays the corresponding real-time video of the rat during the seizure.*

sectioned using a Vibratome and then stained with a cresyl violet dye to highlight the hippocampus and the thalamus. This was done to verify that the electrodes were placed in the correct area. After completing the cresyl violet staining, the brain sections were placed on microscope slides and cover slipped in order to be analyzed under a confocal microscope. Spaces or tears in the hippocampi and the thalamus were searched for while using the microscope.

Results

Electrodes were placed in the hippocampi and the thalamus in order to collect data. The following results represent the data that was collected from the right hippocampus.

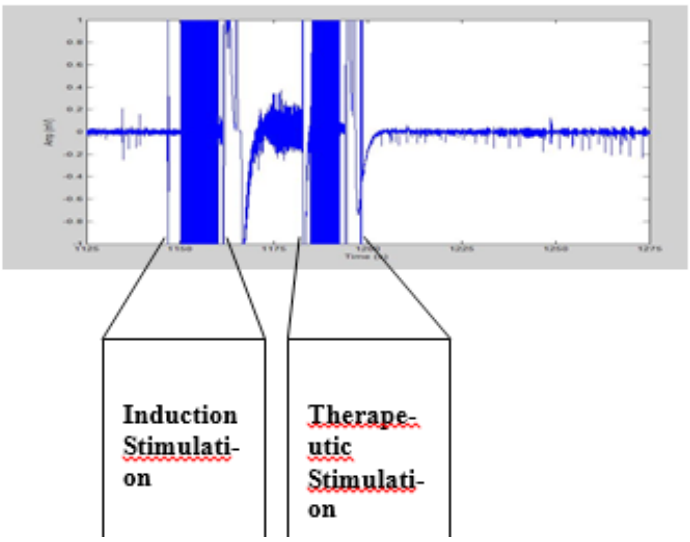

Figure 3. *This figure displays the plot of the LFP from the right hippocampus during seizure. The frequency used in the therapeutic stimulation was 15 Hertz .*

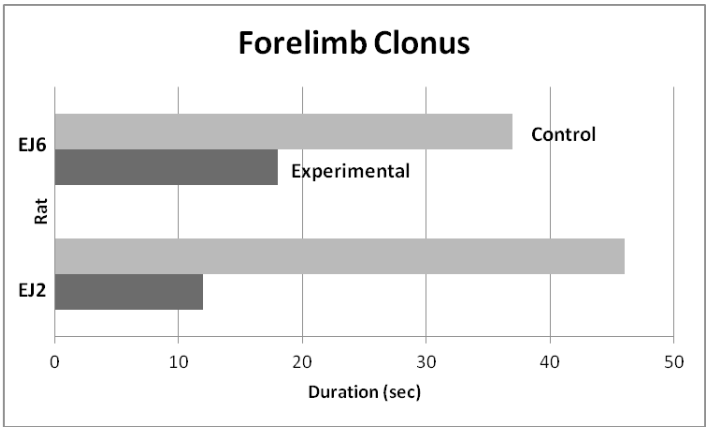

Figure 6. *This bar graph compares the duration of the seizure for the animals who have no therapeutic stimulation (control) or 10 seconds of the frequency (experimental) for two different rats, EJ6 and EJ2.*

Based on the LFP plot, the brain activity is much less active after the therapeutic stimulation is sent to the right hippocampus.

Discussion

Determining which frequency is most effective in the termination of seizures is significant because it leads to a better understanding of the dynamics in the rat brain. This is supported in A Study of Multi-Site Brain Dynamics during Limbic Seizures as Sobayo expressed "Understanding the nature of this synchrony and the dynamics of neuronal oscillators in the brain is a critical component towards decoding such complex behaviors" (Sobayo, et al). Based on the results for the experiment shown above, it was found that 15 Hertz is the most effective frequency that was utilized in the experiment shown. As is seen in figure 3, the seizure, which was represented by the induction stimulation, was followed by a

Group	Rat	Behavior	Duration (sec)
Experimental	**EJ6**	Mouth and Facial Movement	NA
		Head Nodding	37
		Tail Movement	NA
		Arched Back	NA
		Forelimb Clonus	18
		Rearing	34
		Rearing and Falling	NA
Control	**EJ6**	Mouth and Facial Movement	18
		Head Nodding	NA
		Tail Movement	34
		Arched Back	33
		Forelimb Clonus	37
		Rearing	35
		Rearing and Falling	32

Figure 5. *This figure is a table of the behavior observations of the rat during an experimental and controlled seizure (NA=Not Applicable).*

therapeutic stimulation with a frequency of 15 Hertz. Shortly after the therapeutic stimulation ended, the hyperactivity in the local field potential ended. This marked the end of the seizure in the right hippocampus. Moreover, the real-time video in figure 4 corresponded with the local field potential and this was made clear by the rat's epileptic behavior. In the real-time video which is displayed in figure 4 (a screen shot of the video is shown), the rat's head began to rear at 10:59:20 AM. This marked the beginning of the seizure. In the screen shot, the therapeutic stimulation was being sent at 10:59:56 AM. The rat was still experiencing the epileptic seizure but, according to the Racine scale (a scale used to measure the severity of rat seizures based on the rat's movements and posture) the seizure had decreased in severity because the head was no longer rearing (D'Ambrosio, R., et al). Then, the rat returned to normal behavior and its back was no longer arched abnormally. This occurred after the therapeutic stimulation is sent. This indicated the end of the rat seizure and was supported by the Racine scale because normal behavior and normal back arching are signs that there is no seizure occurring (D'Ambrosio, R., et al).

As is seen in figure 5, the behavioral observations that were recorded indicate the severity of the seizure. There are five levels of severity which can be used to categorize epileptic seizures. As the level of the seizure increases, the severity of the seizure worsens (D'Ambrosio, R., et al). Most of the observable behaviors which pertain to the different levels of severity are summarized in figure 5. The observable behaviors are listed in the order of increasing severity in figure 5. It is important to note that the durations of the observable behaviors for the experimental seizure are significantly less than the durations of the observable behaviors for the control seizure. Additionally, in figure 6, a specific observable behavior is used for comparison: forelimb clonus-the loss of control of the arms. In both rats, rats EJ2 and EJ6, it is made clear that the observed behavior, forelimb clonus, lasted longer in the control seizures. Forelimb clonus, which indicates a level 3 seizure, was experienced for 12 seconds in the experimental seizure for rat EJ2 and 46 seconds in the control seizure. Similarly, for rat EJ6, forelimb clonus was experienced for 18 seconds in the experimental seizure and 37 seconds in the control seizure. This is a clear indication that control seizures last longer and are more severe than the experimental seizures. In turn, the experimental frequencies that were tested were effective in the termination of the experimental seizures.

Moreover, the histological methods that were conducted verified that the electrodes were, in fact, placed correctly in the subjects' brains. Verifying that the electrodes were placed in the correct areas reassures that the lab team is conducting the experiment correctly. This is better illustrated in figures 7 and 8.

As is seen in these figures, the white space/opening in the brain sections are the holes that were made by the electrodes. It is important to note that the procedure that was used was not exactly the same as the procedure that was suggested for cresyl violet staining in The Rat Brain atlas (Paxinos, et al). For instance, the suggested procedure called for the immersion of the slides in xylene for at least 10 minutes. However, after trying this, the lab team found that this was not efficient be-

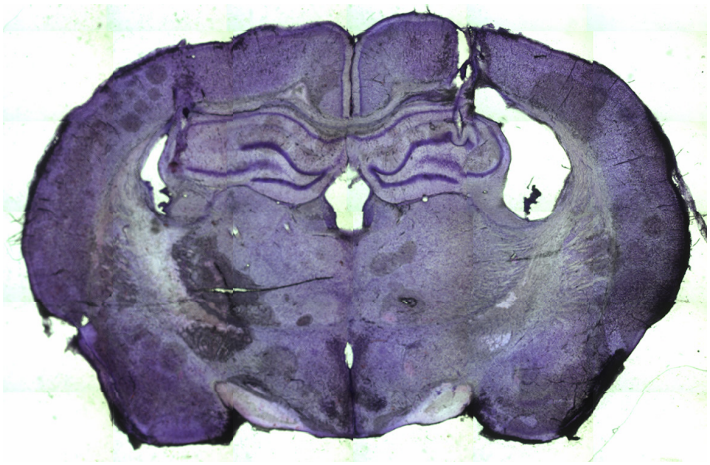

Figure 7. *This image displays the hippocampi and the space at the top is where the electrode*

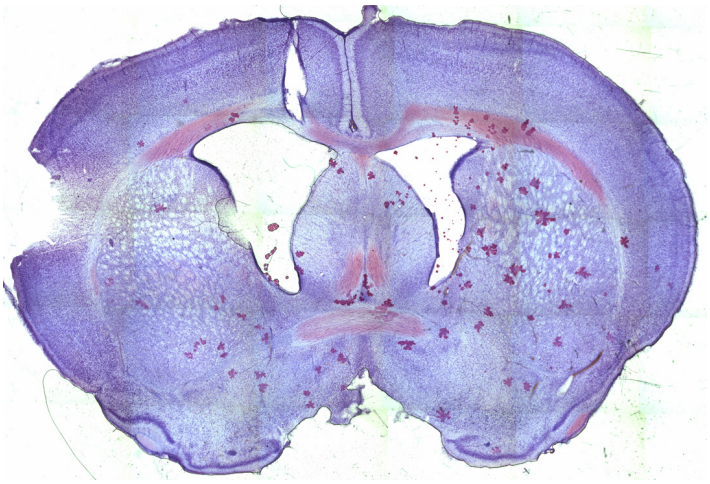

Figure 8. *This image displays the thalamus and the space at the top is where the electrode was placed.*

cause the sticky consistency of xylene caused the brain sections to stick to the bottom of the petri dishes. After experimenting with the suggested procedure, the lab team found the most efficient histological methods and stained the brain sections according to the newly found methods.

In order to extract data from the local field potentials, it was necessary to approximate when the induction stimulation and therapeutic stimulation started and ended. However, this was manually determined. For this reason, there was a higher chance of making errors while manually extracting data from the local field potential. Similarly, the actual induction start time was determined by watching the real-time videos and watching for a sudden change in the rat's behavior to determine when the induction stimulation's effect began. In the process of determining the actual induction start time, the seizure beginning was being approximated. This was also a potential source of error. In order to decrease the potential for error, the local field potential data was compared with the real-time video data to verify the accuracy of the data that was being collected. Additionally, the real-time video capture was not optimal because there was a glare that is apparent in the recordings and this increased the difficulty of extracting data from the real-time videos and even made some videos inefficient for data extraction. This problem was dealt with by choosing the right videos for data extraction. For instance, some videos captured the rat while having a seizure where the glare of the camera is located. These types of videos were not used for data extraction because it is unclear when the seizure starts and ends.

Initial results have been very promising but more animal subjects will need to be tested to verify the efficacy of this protocol. The next step is to integrate the edited videos into electroencephalogram (EEG) so that the EEG can be analyzed in real-time while watching the videos. The EEG should correspond with the videos because, after being integrated into real-time data, the EEG will reflect the data values that were extracted from the real-time videos. Also, histological methods will continue to be implemented in order to acquire additional verification.

Additional rat subjects will be operated on and electrodes will be installed in their brains. This will help prepare the next group of subjects for data collection and analysis.

Conclusion

Based on the data that was collected from the hippocampi, it can be concluded that there are certain frequencies of stimulation that have a greater effect on the termination or disruption of epileptic seizures than others. For this reason, seizures can be mitigated or terminated if an effective frequency is utilized. In this experiment, 15 Hertz was the frequency that was found most effective for the therapeutic stimulation and this is supported by the seizure duration. The therapeutic induction was sent at 10:59:50 and the rat returned to normal behavior at around 11:00:07. The seizure ended approximately 17 seconds after the therapeutic induction was sent. This is supported by the LFP plot which shows a decrease in electrical activity after the therapeutic induction was sent. It can also be concluded that the control seizures are more severe than the experimental seizures. This is due to the therapeutic stimulation that is used in the experimental seizures. Verification of accurate electrode insertion procedures was also acquired based on the microscope images. Additional results are necessary to verify the efficacy of this protocol and the results that have been acquired so far which indicates the necessity for this procedure to be repeated.

Acknowledgements

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References

1. Current epilepsy treatment and new medications; WebMD, 2012.

2. D'Ambrosio, R. , J.W. Miller; What is an epileptic seizure? unifying definitions in clinical practice and animal research to develop novel treatments; PubMed, 10(3), 61-66, 2010.

3. Incidence and prevalence; Epilepsy Foundation, 2012.

4. Mogul, David J. Faculty research programs; Center for Integrative Neuroscience and Neuroengineering Research, 2013.

5. Paxinos, G. , C. Watson. The rat brain; Academic, 1986.

6. Sobayo, T. , A.S. Fine , D.J. Mogul; A study of multi-site brain dynamics during limbic seizures; PubMed, 7557 – 7559, 2011.