The Effect of Anti-Epileptic Drugs on 4-AP Induced Seizures in Rat Brain Slices

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Introduction

Epilepsy is a neurological disorder that affects an estimated 50 million people, nearly 1/3 of whom do not have sufficient control over its symptoms. Although several anti-epileptic drugs (AEDs) have been developed to reduce the onset of seizures, much improvement can still be made in the treatment of symptoms and elimination of side effects. AEDs mainly modulate voltage-gated cation (Na^+, K^+, and Ca^{2+}) channels in neuronal membranes. This electrical behavior creates the neuron's action potential and affects the firing patterns of countless connected neurons in a neural network, producing a certain level of synchrony. Hypersynchrony is strongly linked to the onset of epileptic seizures.

In this project, we aim to discover how the AED Ethosuximide changes the electrical activity between neurons to prevent seizures. We assessed this using extracellular recordings in rat hippocampal brain slices. Seizures were induced using the chemical 4-aminopyridine (4-AP), which blocks potassium channels in neuronal membranes. Ethosuximide reduces calcium channel activity to decrease neuronal synchrony. We plan to introduce this drug to the brain slice during the seizures and observe the effects, then wash away the drug to see if the seizures return. This model will reveal whether epileptic patients suffering from seizures similar to those caused by 4-AP will benefit from taking ethosuximide.

Methods

Experiments were performed in 15-day-old rat hippocampal brain slices of 500µm thickness. Extracellular recordings were conducted in the slice. In this technique, the tip of a metal microelectrode is placed in the area of interest in the brain slice. In our experiments, electrical activity was recorded from the entorhinal cortex surrounding the hippocampus. The electrode conducts the electrical activity from the neuronal connections in the extracellular space to an amplifier, which provides electrical measurements through computer software (MultiClamp Commander 700B and RTXI – Realtime Experimental Interface).

The brain slices rested in low-magnesium Artificial Cerebrospinal Fluid (ACSF) with 100µM 4-AP. The reduction in magnesium aids in the generation of seizures by 4-AP. The fluid was oxygenated and continuously flowing to keep the slice alive and active. Once seizures were observed, a 350µM solution of Ethosuximide in 4-AP containing ACSF replaced the original ACSF. We were then able to observe Ethosuximide's effect in the presence of 4-AP on a brain slice that was already seizing.

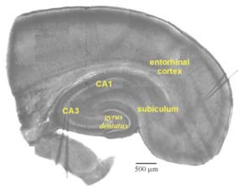

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Figure 1. A rat hippocampal slice. Neurons studied were located in the medial entorhinal cortex surrounding the hippocampus, mainly in layers 2-3 and 5-6.

Experimental Results

Results were very hard to come by in this project. We did 80 experiments over 29 days in the past six months. Of those 80 slices, we only saw seizures in three of them. Below is the data set from our successful experiment on March 21st 2011. The entire data set and its spectrogram is shown below, as well as a piece of the total data set showing a seizure before drug was added and another that shows activity after the drug was added.

Figure 2. Experimental setup. The slice is placed in the center underneath the microscope, with the electrode mounted in the manipulator on the left. The image is projected onto a television screen (not shown) for better view.

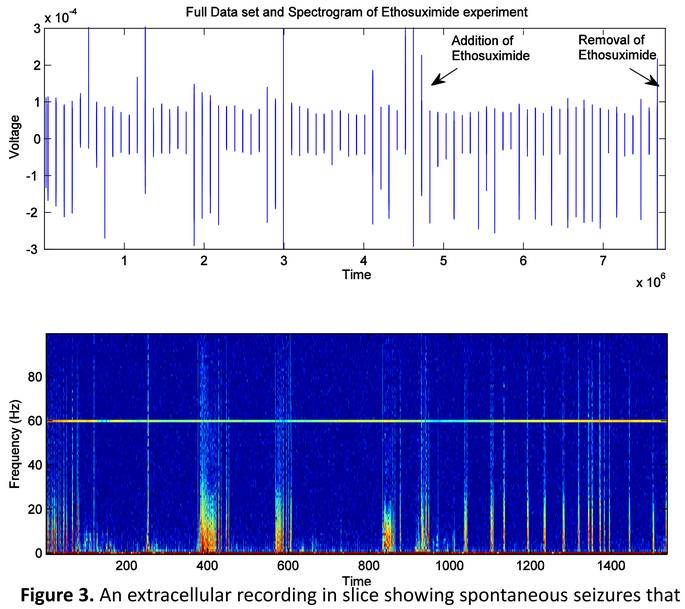

Figure 3. An extracellular recording in slice showing spontaneous seizures that occurred because of the 4AP and low Mg2+. The drug Ethosuximide was added where marked. The spectrogram for the data is shown below.

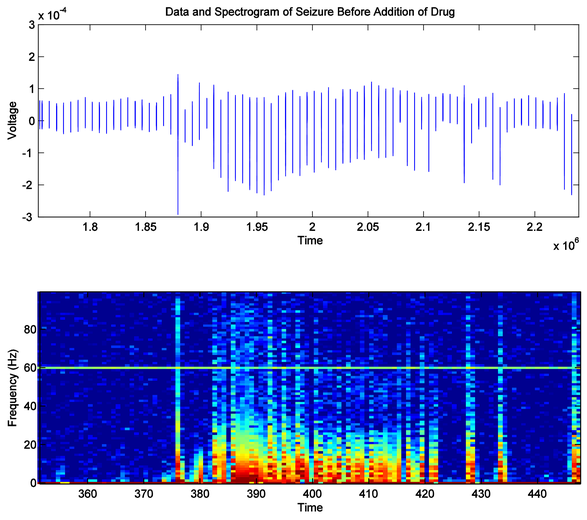

Figure 4. A close up voltage trace of a seizure and its corresponding spectrogram. This seizure was taken from the part of the data set before the drug was added.

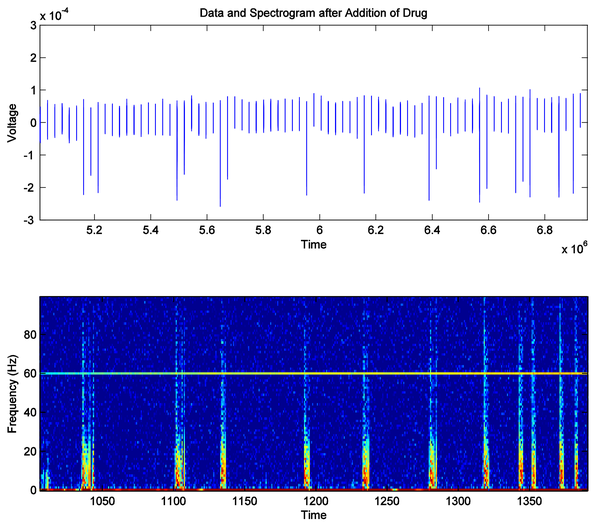

Figure 5. A close up voltage trace and spectrogram of the data after the drug Ethosuximide was added.

Discussion

At the moment, our data is inconclusive. We didn't get enough slices to seize in our experiments. A few problem solving strategies we tried were increasing the slice thickness, increasing the concentration of 4AP, and remaking solutions to be sure they had the correct components and concentrations of components. From the data set shown it looks as though Ethosuximide is used as an anti-epileptic drug because it doesn't allow a seizure to propagate, but we can not conclude this because our sample size is much too small.

Getting seizures in slice can be very difficult, as we found with this project. To get more accurate seizures in the future, it may be useful to try a pico-spritzer approach using double puffs of NMDA in the presence of 4AP and low Mg2+. The NMDA acts like glutamate on the NMDA receptors and causes action potentials. This gives a foci for the seizure at the spot where the NMDA is puffed and the seizure that arises looks just like a spontaneous seizure.

We plan to execute more experiments using these techniques during the rest of the semester in hopes of obtaining more seizure data to attain more conclusive results.

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