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Study of electrophysiology recordings in organotypic cultures

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Study of electrophysiology recordings in organotypic cultures

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Abstract

Epilepsy and its onset, epileptogenesis, have complicated underlying mechanisms that can often be studied in greater detail when in vitro. In vitro hippocampal cultures develop epileptic symptoms in a period of approximately ten to fourteen days in vitro. Working in vitro allows for an easier manipulation of elements such as growth factors that can affect epileptogenesis as well as multiple methods of analyzing data to ensure significant results. The capability of electrophysiology recordings to directly quantify changes in epileptogenesis in vitro is the main focus of this work. Using this method, recordings of different regions of the brain capable of developing epilepsy were performed.

The main concern of working in vitro is the artificial environment created for culture in which they are kept is not parallel to the natural environment they encounter in the cerebrospinal fluid (CSF). Therefore there is reason to be concerned that the media itself could contribute to epileptogenesis. Two distinct culture media, Neurobasal-A (NeurA) and CSF-based medium (CBM) were used to determine if this was the case. We were able to conclude epileptogenesis occurred regardless of the media type, although specific adjustments help to reduce seizures and the associated cell death.

Chapter 1: Introduction

1.1 A history of epilepsy and its onset

Epilepsy is a disorder in which chronic seizures occur in a manner that is often unpredictable due to abnormal activity in the brain [1]. These seizures can be of different type and severity for different individuals that can range from tremors to complete unconsciousness. Furthermore, even a single individual with epilepsy can have multiple types of seizures and other varying symptoms. An epileptic seizure is a temporary occurrence of abnormal, excessive or synchronous neuronal activity in the brain [1]. There is a general lack of understanding of the fundamental mechanisms of epileptogenesis, the pathophysiological process underlying the development of epilepsy [2]. The seizures associated with epilepsy increase in severity and frequency as the disease develops over time [3]. The onset of epilepsy is a vicious cycle where seizures can lead to a progression of the disease and initial seizures are also thought to be one of the causes of epilepsy. Inhibiting the initial seizures is one target of study that could significantly reduce its prevalence.

The main known cause of epilepsy is traumatic brain injury. However, the likelihood of epileptogenesis is variable among levels of injury and individuals. A severe brain injury is characterized by loss of consciousness or amnesia for more than 24 hours, subdural hematoma, or brain contusion [4]. It is known that a clear correlation does exist, as the overall standardized incidence ratio of seizures for severe injury was 17 when compared to population rates [4]. Posttraumatic epilepsy (PTE) is the most common cause of new-onset epilepsy in young adults

with up to 30,000 new cases each year in the United States [5]. The frequency of post-combat PTE is unsurprisingly increased in comparison with the civilian population [5]. Of the subjects of PTE, most receive anticonvulsants to combat the seizures and related symptoms, the role of which is often neuroprotective by trying to minimize the brain damage by preventing early seizures [6]. After a period of roughly 7 days, these anticonvulsants no longer work functionally as a neuroprotective tool, and also are varied in their anti-seizure ability.

It is well known that current treatments after this initial onset vary widely in type and efficacy. The aforementioned anticonvulsants often control or reduce the frequency of seizures in some patients while others show no improvement [1]. This is hypothesized to be due to the level of progression of the disease. Many antiepileptic drugs focus on the reduction of sodium channel activation [1]. This increase in the inactive state of the channel causes a decrease in firing of axons, thereby reducing seizures. Other sets of similar drugs typically all focus on the same goal, reducing firing of cells in the brain. This has side effects commonly including cognitive impairment, fatigue and other similar effects.

Alternatives to, or additions to, medication include dietary restrictions, nutritional supplements, and hormones have also helped certain patients reduce symptoms [1]. The ketogenic diet is one example where decreased carbohydrates and increased fat and protein cause the brain chemistry to be more resistant to seizures, yet only works for a minority of patients [1]. Other options include implantable devices, which show promise but have yet to be fine-tuned. Another option is surgery, typically removing the portion of the brain where the seizures

are thought to originate from. All of these treatments focus on symptomatic approaches rather than focusing on modification of the disease, that is, altering the natural progression of epilepsy onset [2]

1.2 *The role of in vivo and in vitro studies*

Epilepsy causes a series of changes at the molecular, cellular and network levels in the brain [7,8]. These changes need further study to establish underlying mechanisms and pathways that contribute to epileptogenesis and ways to combat or interfere with each. Many experiments currently rely on in vivo testing, relying on mostly rodent models to evaluate response to alterations in brain chemistry due to alterations of natural function or due to influx of drugs. These experiments commonly use in vivo extracellular or intracellular recordings, using electrodes placed directly into the animal's brain [9]. This allows for a measurement of field potentials, as well as other types of signaling such as inhibitory postsynaptic potentials (IPSP's) depending on the goal of the experiment [9]. These types of models most closely mimic clinical features of human epilepsy in terms of having behavioral and electroencephalographic seizures. Although since these models vary widely, each set of experiments requires a new set of parameters and careful evaluation before conclusions can be drawn [10].

The complexity of epilepsy lends itself to requiring a more simplified model that can have smaller adjustments made incrementally to more distinctly understand the mechanisms of epileptogenesis. In vitro models allow for investigations of cellular and molecular mechanisms while still preserving the critical network phenotypic features of epilepsy, the development of spontaneous

seizures, in particular [10]. The advantage of slice preparation, in vitro, is the ability to control external medium, to apply known concentrations of drugs, and to record from known layers or from visually identified neurons [10]. Testing for changes in number of cells dying, or for the general heath of each individual neuron gives in vitro experiments a benefit that doesn't exist in vivo. Previous studies of organotypic slice cultures focus on ictal activity that is the seizure activity itself and electrographic seizures that have occurred over a shorter period of time, typically on the order of days [10]. However, slices can be kept in culture in vitro in some cases up to 12 weeks, during which they develop epileptiform activity in the absence of pharmacological manipulation or orthodromic stimulation [11]. This occurrence has led to the generally accepted hypothesis that the trauma of slice dissection can be the trigger of epileptogenesis in culture slices [12].

1.3 Culture media and its potential role in epileptogenesis

The difference for in vitro models compared to in vivo, is organotypic slices are maintained in an artificially controlled environment. The basic features of this environment are crucial for their survival. Specifics may differ but being kept in culture is to mimic the environment in which the cells in the hippocampal slices would normally be accustomed to. After dissection and slicing, the ability of the slices to survive depends on temperature, gas content, and humidity. The actual medium in which the slices are kept while in culture is one of the most important components for keeping the cells alive. There is concern, however, that it may be possible that these environmental factors drive epileptogenesis in

organotypic cultures. Our hypothesis focused on testing the most artificial aspect of organotypic culture environment: the culture medium [13].

It has long been recognized that mammalian tissues must be bathed in a mixture of metabolic substrates, hormones, and growth factors to maintain them in vitro longer than 24 hours [13]. Early versions of these types of culture media were based on the composition of blood plasma, often supplemented by actual blood derived serum [14,15]. These samples of animal serum can have variable concentrations of hormones and metabolites, which caused the need for chemically, defined media [16, 17]. We have previously used both serum-supplemented and chemically defined media to maintain organotypic hippocampal cultures, and found that epileptogenesis occurs in both types of medium [18,19]. This chemically defined culture medium is based on the composition of blood plasma, not of CSF. This difference relates to one of the proposed causes of epileptogenesis; the opening of the blood-brain barrier (BBB) due to a brain insult [20,21]. Direct exposure of brain tissue to components of blood that do not naturally cross the BBB, or to compounds that are present in blood at different concentration than in CSF, may contribute to epileptogenesis [22, 23]. Therefore, it may be possible that epileptogenesis in organotypic cultures is not triggered by trauma (dissection), but by exposure of hippocampal tissue to a cocktail of compounds that are present at a much lower concentration or not present at all in normal CSF [13].

Composition of culture medium that is typically used for culture of postnatal neurons, and that we have used, as have many other researchers, for

organotypic hippocampal cultures is Neurobasal-A [13]. It can readily be seen that concentrations of glucose, potassium, and magnesium in Neurobasal-A are substantially different than those found in CSF [24-26], and may contribute to in vitro hyper-excitability [22,27]. In addition, many amino acids are contained in Neurobasal-A at significantly higher concentrations than in CSF [28-31]. Altered concentrations of amino acids such as glycine, serine, leucine, isoleucine, valine, phenylalanine and others are found in metabolic epilepsies [32,33]. This may play a role in the development of spontaneous epileptiform activity in organotypic cultures [13].

In part of this work, we tested the hypothesis that composition of culture medium has an effect on epileptogenesis in organotypic hippocampal culture. We also examined the influence of individual components of media on epileptogenesis in this model [13].

1.4 Electrophysiology introduction

Electrophysiology experiments have been performed to determine characteristics of cells and regions of cells, specifically neurons, since the early to mid 1900's. A micropipette can be inserted into the axon in order to perform voltage clamp experiments to test changes in cellular membrane impedance as well as action potentials [34]. The discovery of this technique led to major breakthroughs in the understanding of neuronal modeling and ion currents by using the giant squid axon due to its size and ability to be studied more easily [35]. The development of electrophysiology has increased exponentially since then and multiple types of recording setups such as multiple electrode array

(MEA's), biphasic stimulation electrodes, patch clamp recordings and others allow for a variety of studies to be performed to expand our knowledge of neural pathways. These tools can also be used to study the effects of behavioral changes, drug application, and signaling pathways in individual cells or cell regions. Methods similar to the ones used in this experiment using a single microelectrode rather than two contributed to the understanding of signaling pathways in epileptogenesis [3].

The experiments presented focus on large regions of activity in organotypic hippocampal slices. These recordings allow visual representation of the activity occurring within the slice. This emphasis was on determining if interictal activity (between seizures) or ictal activity (during seizures) was present [13, 36]. Although described further in experimental methods, organotypic hippocampal slices are used for these experiments. The regions we are testing for this activity using electrophysiology recordings are the CA3 and CA1 regions of the hippocampus (Figure 1). These regions are crucial for study due to the connection of axons from CA3 to CA1 allowing for the testing of population spikes that tend to propagate throughout the entire slice.

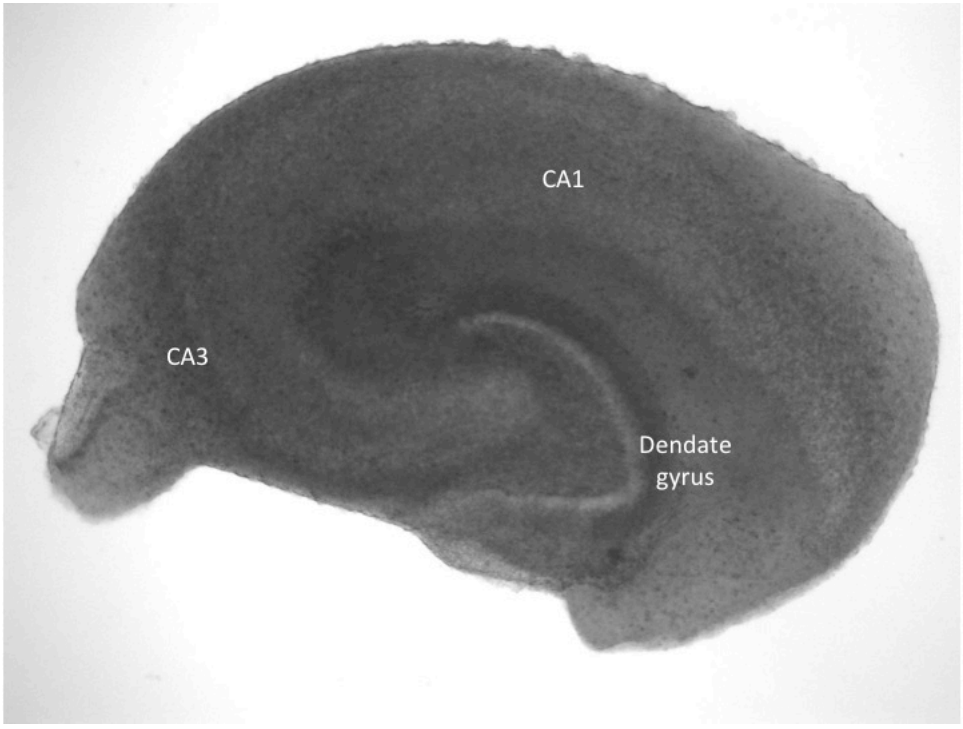


Figure 1: The CA3, CA1, and dentate gyrus regions of the hippocampus.

Chapter 2: Experimental Methods

2.1 Culture Media preparation

Customized culture media were prepared with different compositions and concentrations of electrolytes, amino acids, and glucose (SigmaAldrich) as described in the text. Osmolarity of all media was matched to Neurobasal-A (240 – 260 mOsm/kg) by adjusting NaCl concentration. All culture media were supplemented with bovine serum albumin (250 mg/L, physiological range of albumin in healthy CSF is 70 – 266 mg/L [34-37]), insulin (3.5 mg/L), selenium (14 μg/L), from Sigma, and glutaMAX (0.5 mM) and gentamicin (30 mg/L), from Life Technologies [13].

2.2 Organotypic hippocampal slice preparation

Hippocampi were dissected from postnatal day 7-8 Sprague-Dawley rat pups (Charles River Laboratories), cut into 350 μm slices on a McIlwain tissue chopper (Mickle Laboratory Eng. Co., Surrey, United Kingdom) and placed onto poly-D-lysine (Sigma-Aldrich) coated glass cover slips in 6-well tissue culture plates. Slice cultures were maintained in various culture media at 37 °C in 5% CO_2 on a rocking platform. Medium was changed twice a week. All animal use protocols were approved by the Institutional Animal Care and were conducted in accordance with the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals [13].

2.3 Electrophysiology in a laboratory setting

The system used to manage these recordings had a myriad of components that needed to be optimized to be able to mimic the brain environment properly and allow for accurate recordings (Figure 2). The chamber where the recordings took place is shown placed in a faraday cage. Micromanipulators controlled the tungsten microelectrodes (0.1 MOhm) for placement into the aforementioned CA1 and CA3 regions of the culture slices. The culture slices were originally placed on cover slips when dissections occurred and were transferred to a 35 mm petri dish for recording.

The electrodes are connected to the PZ2 preamplifier, which relays the output recording to the RZ3 amplifier. Additionally, in order to maintain the conditions necessary inside the chamber, there is an inflow of gas from the tank and water from the water heater. The main focus of the system was to mimic the temperature, humidity, and gas content of the environment that hippocampal cells would be subjected to while performing electrophysiology recordings.

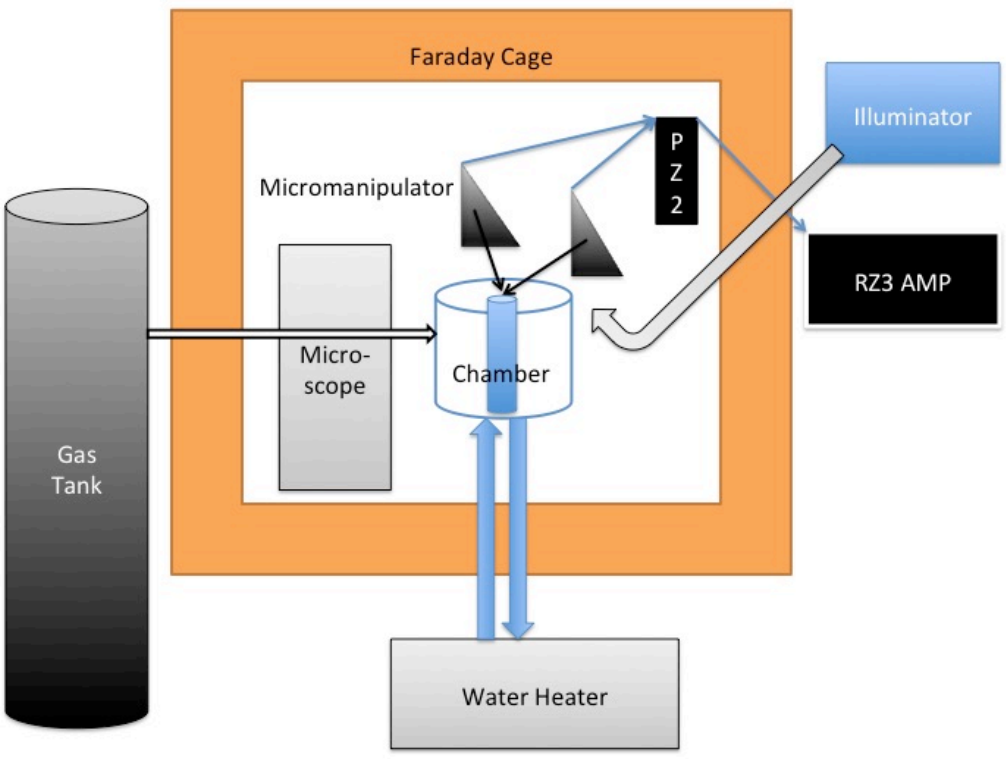
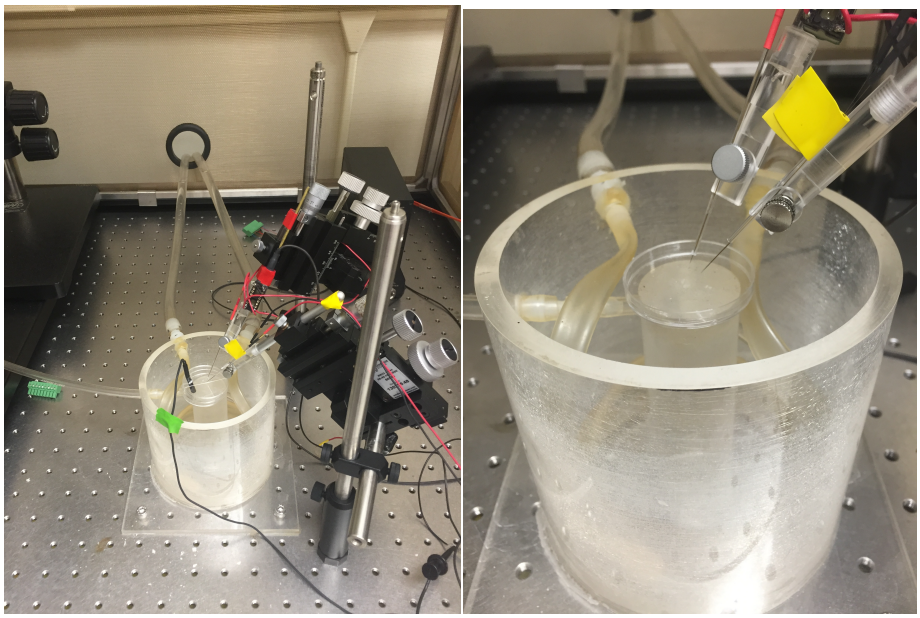


Figure 2: Schematic of recording system: indicating recording chamber, components leading into it such as the gas and heated water, and the recording setup including electrodes from micromanipulators, the preamplifier, and amplifier.

In order to ensure the culture medium would remain at the required 37 °C, a Thermo Scientific HAAKE S7 heated circulating bath was used to create a constant temperature of the water within the chamber. The chamber was filled with water to a level of just below the location of petri dish placement on the column. Given that the chamber is well insulated, it was not difficult to ensure the water temperature inside the chamber stay constant with the set temperature of the heated bath (Figure 3). However, given the space of air above the water in the chamber would hold a different temperature, a series of tests needed to be performed to ensure the culture itself would be at 37 °C. This required the heated

bath to be set at 45 °C and for the chamber cover to remain on as often as possible, especially during recordings which is not indicated in figure 3. Additionally to raise the chamber to the correct temperature required 35 minutes of pre-heating of the water heater and chamber.



(a) (b)

Figure 3: (a) Photograph of recording chamber and micromanipulators. (b) Zoomed in image showing microelectrodes and example placement.

Secondly, in order to guarantee the correct percentage of gases in the chamber, we had to pump in 5% CO_2, and 20% O_2, and a balance of nitrogen. The carbon dioxide component is the main focus due to its increased content in hippocampal regions compared to the outside air. The difficulty of infusing this gas into the chamber is twofold: it has to be inputted below the surface of the water as to bubble up and create the necessary 100% humidity. This is once again to mimic the natural condition and to avoid the slices drying out and dying, but the gas content must also be present in the correct concentration so that the cells

survive. This balance is crucial although if the bubbling increases too much then the petri dish can be disturbed causing the slice and electrodes to move. Movement of the petri dish can cause movement of the electrodes in the slice and often mean the entire recording will be void due to cell damage within the slice. In some cases this movement can even cause damage of the electrodes, therefore this must be avoided. The slice could also detach from the cover slip if this movement occurred. In order to combat this problem, the correct amount of gas input should be set and humidity should be calculated with a hygrometer. Then once this is done, the slice with the petri dish present can be introduced to the chamber and secured via malleable wax.

The next challenge to these electrode recordings is the placement of electrodes within the thin hippocampal slices. The region of placement is the CA3 and CA1 area of the slice, for each electrode, respectively. Locating these regions often requires reference of a more fine-tuned microscope than the one present in the chamber itself, therefore it is advisable to view the slices quickly under a microscope with OptixCam OCView or similar software that allows you to visualize the slice on a computer screen and pinpoint the regions of electrode placement before using a standard microscope within the Technical Manufacturing Corporation faraday cage. The microscope within the cage has a reduced magnification and no connection to a computer or similar system because it would create impeding noise in the system. The use of a fiber optical illuminator can also be helpful in order to visualize the slice better in dim lighting and its snakelike attachments can be moved in and out of the cage easily since it

can cause electrical noise if left within. Once this is established the microelectrodes can be placed into the slice with the use of micromanipulators. The electrodes are quite thin and vibrate when moved which makes the insertion process even more difficult as they are still moving when placement is being attempted. To combat this, a thin glass pipette was used to surround the electrode so its movement was variable (Figure 4). Even so this process is often difficult at first to ensure the electrodes don't penetrate the slice fully and cause damage to the slice and the electrodes. In this regard, patience is an essential tool to have effective placement.

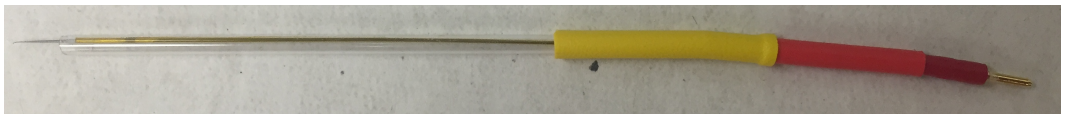


Figure 4: Micro-electrode contained within a glass pipette

Lastly, in order for these recordings to be useful, noise needs to be minimized as much as possible due to its magnitude relative to activity. One of the main issues originally was the noise created from the vibration of the electrodes themselves as they are incredibly thin and shake upon any disturbance, which as aforementioned was improved by the use of a pipette surrounding. Anything over the range of 10-20mV will interfere with the viewing of interictal activity although interictal activity usually has a magnitude on the order of 100mV and will still be visible. The faraday cage itself is the best way to eliminate noise but it needs to be shut completely and remain that way during the entirety of the recording. A proper ground between the ground in the solution containing the slices, the ground for each of the electrode pre-amps and a

complete ground in the system is the best way to minimize noise. Ground within the system to a stable point rather than to an exterior ground created the most effective minimization of noise in our system.

2.4 Experimental setup organization

In order to ensure the fair comparison of data and no bias between different recordings, we put in place a set of requirements for each data set. Each data set included an equal number of slices treated with NeurA and CBM, six of each. Recordings were taken throughout the period of 0-14 DIV in each of the sets of slices in the greatest variety possible to ensure unbiased results (Table 1). There were 12 data sets used which included 12 slices per data set as indicated. Some slices were excluded from analysis due to factors such as slice detachment or inadequate humidity and slice death due to drying out during recordings.

DIV	1	2	4	5	13	14
Number of NeurA Recordings	1	1	1	1	1	1
Number of CBM Recordings	1	1	1	1	1	1

Table 1: Idealized recording setup based on equal distribution of recordings on different DIV's for a single batch of 12 slices from the same rat pup.

2.5 Data input and analysis

The electrophysiology recordings themselves are inputted from the electrodes that are connected to an amplifier (RZ2, Tucker Davis Technologies) fitted with high-impedance multiple-channel pre-amplifier stage (PZ2-64, Tucker Davis Technologies) (band-pass 1 Hz-3 kHz, gain ×1000). Sampling rate was 6

kHz per channel. The amplifier is connected to our laboratory computer. Software is then used to input the signal, filter it further with an adjustable preset NeuroFilter, convert it to a single channel and then store the data (Figure 5).

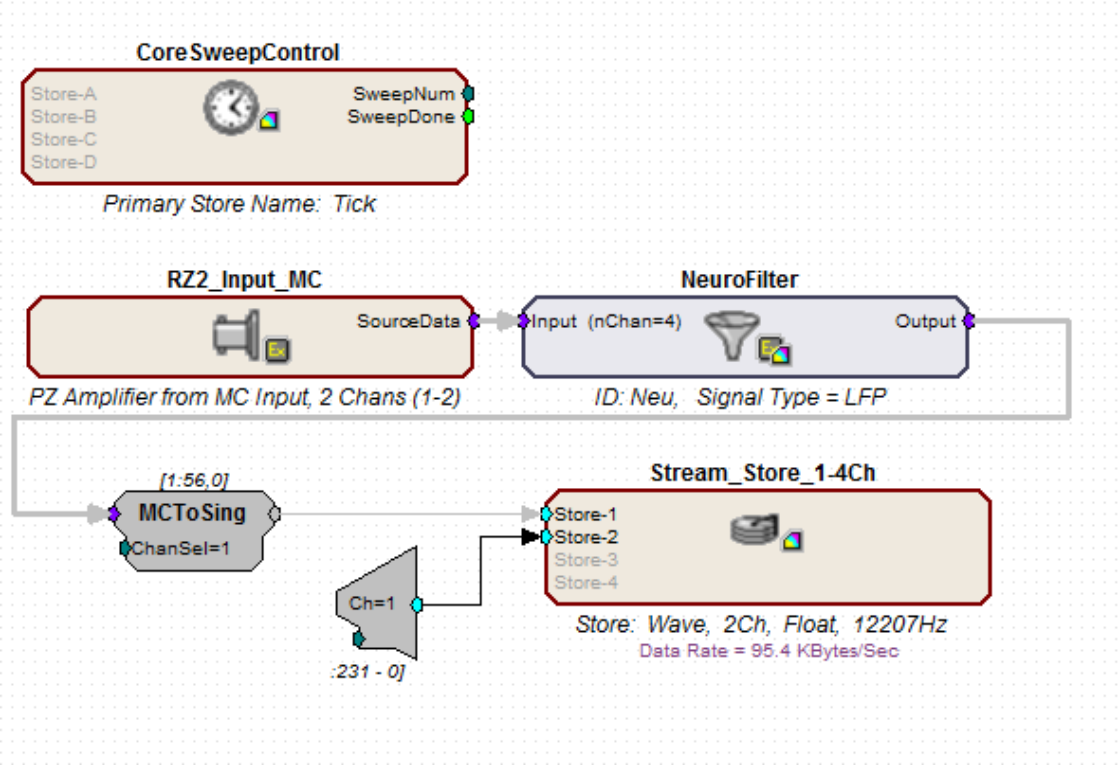


Figure 5: Image of circuit used for data input from amplifier and initial filtering

Once the data is stored in a data tank, Matlab programming was used to evaluate the presence of ictal activity. These electrographic seizures, as the analog to epileptic seizures in vivo, can be defined in two ways. They can be paroxysmal events of much larger amplitude than the background multiple unit activity and lasting longer than 10 s, or shorter paroxysmal events that occurred with event frequency of at least 2Hz for at least 10 s [13].

2.6 Other supporting information setup

Electrophysiology recording information was supplemented by the analysis of lactate dehydrogenase (LDH) and lactate levels in culture medium, as well as by brightfield microscopy. LDH levels indicate the amount of dead or plasma membrane-damaged cells in the culture while lactate levels indicate the amount of ictal activity present. Together, this information can help draw conclusions about the effects of different media and media components tested. These factors were tested by collection twice a week and using LDH-Cytotoxicity Detection kit (Roche) and L-lactate Assay kit (Eton Bioscience) respectively, according to the manufacturers' protocols. Lactate concentrations were calculated relative to known lactate standards, while LDH concentrations were calculated a and normalized to the 0 - 3 DIV average of LDH concentration in control culture supernatant [13].

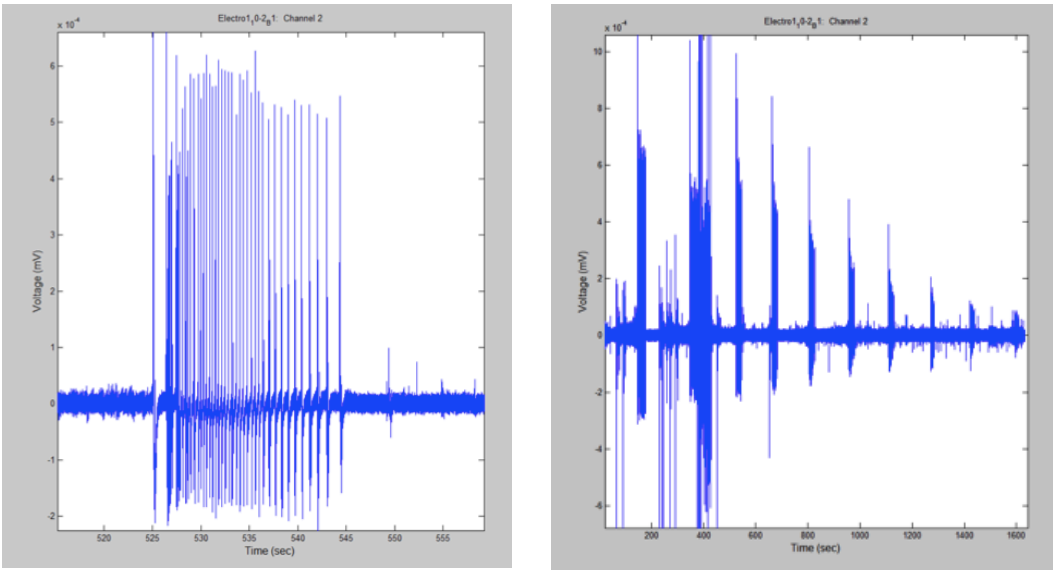
Brightfield microscopy was also used to supplement data collected through electrophysiology recordings. Culture health was evaluated based on three morphological characters: blurriness of the culture edge, brightness of the slices, and integrity and distinctness of the neural layers. Blurry edges indicate that the slice attached well on the substrate, while distinct edges indicate that the slice didn't integrate well with the substrate. Some unhealthy slices would lose the attachment and even float. Unhealthy slices will look darker than healthy slices due to dead cell accumulation. Healthy slices have well-preserved cytoarchitecture with CA1, CA3 neural layers and dentate gyrus (DG) [13].

Chapter 3: Results and Discussion

3.1 NeuroA vs CBM

3.1.1 Electrophysiology recording results of NeuroA vs CBM

As mentioned in the methods section, the requirements for electrographic seizures are frequency and magnitude dependent, requiring a frequency of 2Hz and a magnitude normalized at roughly 100mV. These metrics are evaluated using Matlab coding custom-designed for the purpose of seizure detection (Figure 6a). Often the recordings, especially in later DIV's, have seizures that initially are within both the frequency and magnitude requirements but upon further inspection fail either one or both of these criteria (Figure 6b). Therefore it is important to use a method to analyze data that is unbiased as in the custom code we developed that measures magnitude of ictal activity, frequency of ictal activity, and duration of single seizure. If all of these parameters are met, then the program indicates that it is an electrographic seizure and outputs its duration.



(a) (b)

Figure 6: (a) Single seizure (b) Entire recording

The initial analysis had each data set organized with specific information from each slice and recording (Table 2). This information included the type of medium the slice was in, number of days in vitro, overall recording time, number of seizures, and length of time seizing. Overall results could then be interpolated from this basic information to create a more quantified set, more clearly shown later.

Data set number:	"1"				
Slice Region:	"CA1"				
Name of slice	Type (NeurA or CBM)	DIV (0-14)	Recording time (s)	Number of seizures	Length of time seizing (s)
"Slice A1"					
"Slice B1"					

Table 2: Information to be documented from each data set

There was a large amount of variability in each data set for these parameters (figure 7). This data set was chosen to be representative of the variability and general trend of seizure duration. It is apparent by the data points for two slices at 1 DIV and 12 DIV, with 10 seizures and 34 seizures respectively,

that the amount of seizures increased over time. Yet as visible by the general changes in number of seizures over time of DIV when observing each data point in figure 7, the changes are less significant and conclusive when only looking at one data set.

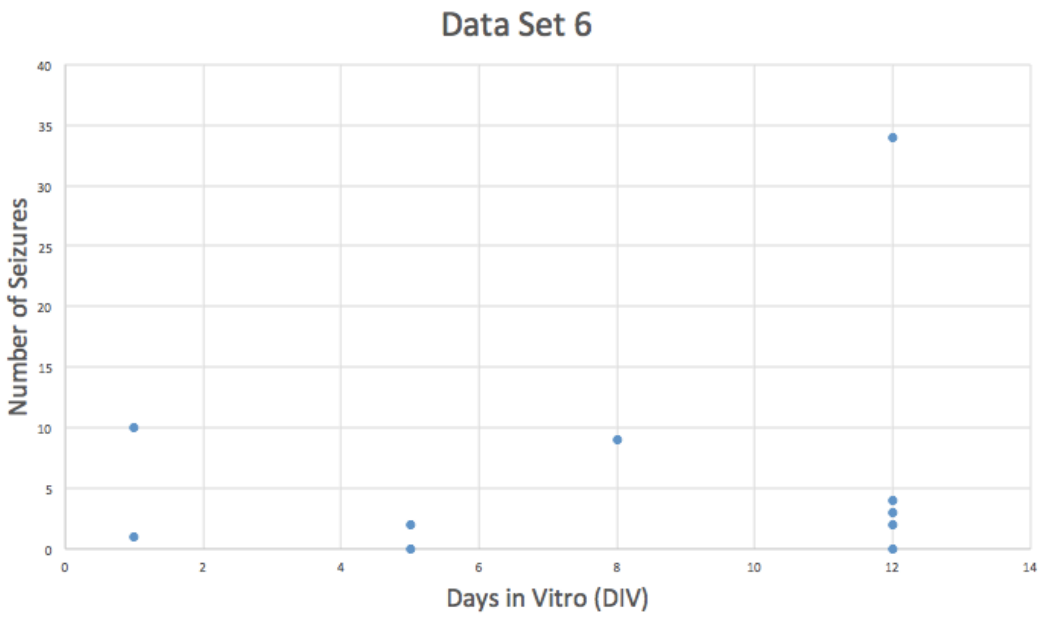


Figure 7: Data points showing the number of seizures for each slice present in the data set.

We then quantified electrographic seizures (ictal events) in NeurA and CBM cultures from 0 to 14 DIV from the 12 data sets. Data were grouped into four time periods, 0-3 DIV, 4-7 DIV, 8-10 DIV, and 11-14 DIV (for each condition at each time point, n = 17, 9, 8, 10, respectively). Significant differences were found in number of seizures only in the 0-3 DIV time period where no seizures occur in the CBM medium in CA1 or CA3, although this significance can be misleading due to the low number of seizures in both types of media (Figure 8). Consequently, significant differences were found in average seizure duration in the 0-3 DIV time period in CA1. However, significant

differences were found in average seizure duration in both the 0-3 DIV time period, and the 4-7 DIV time period in CA3.

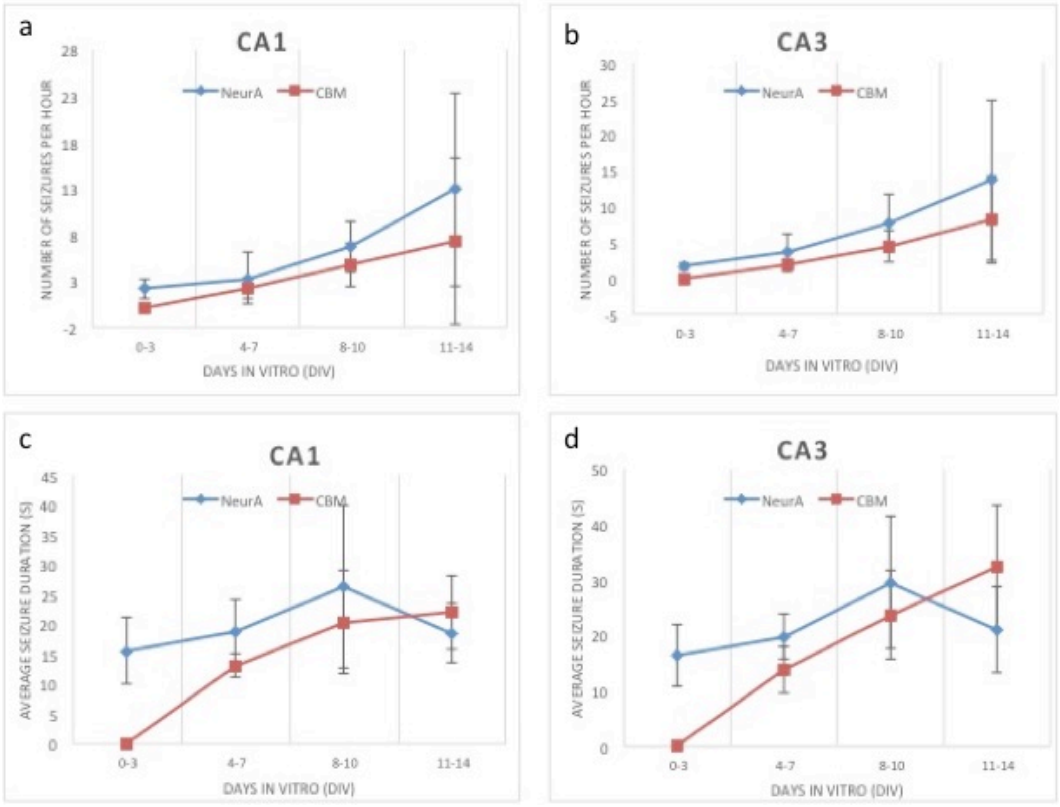
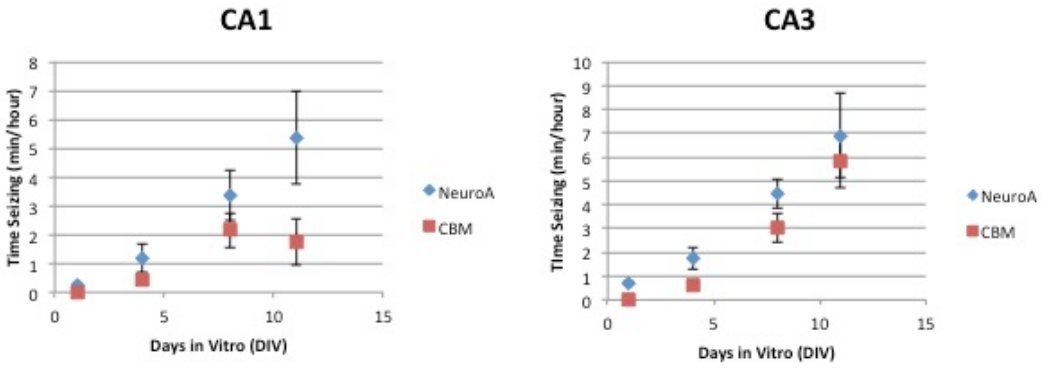


Figure 8: (a) Number of seizures per hour for NeurA and CBM in CA1 (b) Number of seizures per hour in NeurA and CBM in CA3. (c) Average seizure duration in seconds for NeurA and CBM in CA1 (d) Average seizure duration for NeurA and CBM in CA3.

In order to best quantify the amount of pseudo-epileptic activity, it seemed crucial to make a correlation between number of seizures and average time seizing, indicating the amount of actual seizure activity unbiased by length of individual seizure. There are significant differences in the average time seizing in 11-14 DIV time period in the CA1 region, and the 4-7 DIV time period in the CA3 region.



(a) (b)

Figure 9: (a) Average time seizing in minutes per hour for NeurA and CBM in the CA1 region. (b) Average time seizing in minutes per hour for NeurA and CBM in the CA3 region.

3.1.2 Supporting information

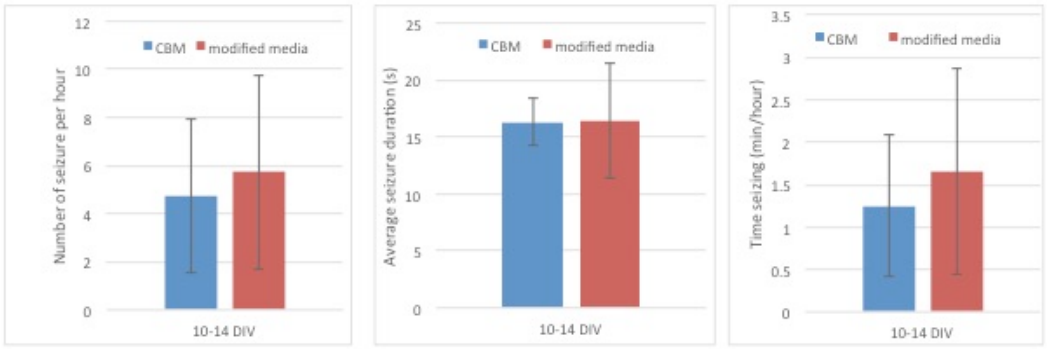
These two main types of media have certain crucial qualities. Simplified NeurA medium was created using necessary amino acids determined by previous LDH and lactate analysis. CBM was created with the proper concentrations of electrolytes to reflect concentrations in rat CSF, while glucose concentration was changed to match typical concentrations in aCSF for acute slice experiments [13].

Cultures kept in NeurA and CBM media had no significant differences in LDH release. However, significantly lower lactate release was observed in CBM than in NeurA starting from 7 DIV on. Additionally confocal images showed no significant differences in the number of surviving neurons between NeurA and CBM in either CA1 or CA3.

3.2 CBM vs. modified medium

3.2.1 Electrophysiology recording results of CBM vs. modified medium

Experiments have shown that the reduction of certain amino acids in media has played a significant role in the onset of epilepsy. The reduction of glycine and L-serine concentrations to 0.01 mM and increase of [Mg^{2+}] to 2mM significantly decreased cell death and reduced ictal activity [13]. These two modifications were integrated into a "modified medium", and compared with CBM. Electrographic recordings, as done previously in CBM and the modified media, were recorded from 10 DIV to 14 DIV (Figure 10). Since recording data from both the CA1 and CA3 regions proved to be entirely similar, only the CA3 regions were included. There were no significant differences in number of seizures per hour, average seizure duration, or overall time seizing.



(a) (b) (c)

Figure 10: Comparison of CBM and modified media from left to right includes: (a) number of seizures per hour, (b) average seizing duration, and (c) time seizing.

3.2.2 Supporting information

Additionally microscope images at 14 DIV revealed that cultures in modified media had brighter neural layers than cultures in CBM. Cultures maintained in modified medium released significantly less LDH than slices in CBM at 14 DIV. No significant differences were observed in lactate release between two groups [13].

Chapter 4: Electrophysiology for MEA recordings

4.1 Methods and Results

Electrophysiology recordings were used for the determination of MEA efficacy. The MEA device is used to take recordings constantly in terms of time without adjustments of electrodes or concerns of the cells within the slice dying. Although this yields certain results, it is necessary to get confirmation that these slices on the MEA are developing epilepsy as expected before further conclusions can be drawn. The electrophysiology recording system implemented for single slice recordings was adjusted to take recordings from multiple hippocampal slices in this MEA device (Figure 11). A series of experiments was run using an adjusted version of the recording system to test the epileptic output of four slices already placed on this device by placing electrodes in each of the slices CA1 region.

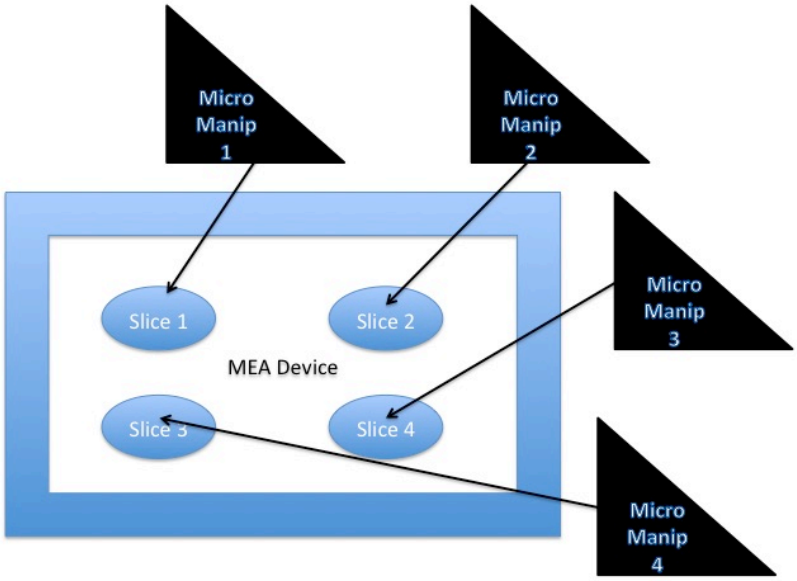


Figure 11: Schematic of MEA device containing four slices and indicated electrodes and micromanipulators for recording.

The initial difficulty regarding the recording of four different slices is the positioning of the microelectrodes themselves in relation to each other. This was exacerbated by the placement of micromanipulators as indicated by the tight spacing of micromanipulators in the schematic. The chamber as used before, needs to continue to have the same conditions that are required for slice survival. This becomes more difficult when you need a larger opening in the cover of the chamber to allow the entry of all four electrodes into the system while still covering enough of the chamber to keep 100% humidity and correct temperature and gas content.

Recordings were taken using these new parameters and while certain slices yielded electrographic seizures, the majority either dried out or were

damaged by the movement of the MEA device itself. Over the course of several months, optimization was attempted yet this system proved difficult to generate consistent recordings. This setup failed to produce sufficient data but yielded important information about the alternate uses for an electrode recording system.

Chapter 5: Conclusions

Electrophysiology experiments in conjunction with lactate analysis, LDH analysis, and bright field microscopy were able to show not only the effects of different media on epileptogenesis but also the affects of certain amino acids and electrolytes within the media itself. The different media within the facets of this experiment, whether it is CBM, NeurA, or the modified medium could not prevent the onset of epilepsy, even when optimized. Therefore medium composition is unlikely to be the cause of epileptogenesis in the organotypic hippocampal culture model of epilepsy.

The scope of electrophysiology experiments is wider than the initial single slice recordings but as shown by the MEA recording experiments, requires fine-tuning to be effective. Electrophysiology recordings have a multitude of uses but without proper adjustment of the overall recording system are best suited for single slice recordings. This would include overhauling the entire system to make one more appropriately fit for the demands of a larger testing setup.

Chapter 6: Future Work

There is concern that if the population spikes are of a high enough magnitude that the highly sensitive microelectrodes could record ictal activity that isn't in the location of electrode placement. In most recordings where electrodes are placed in both the CA1 and CA3 regions of the hippocampal slices, population spikes occur in both regions with a time delay between each region. This is caused by the relatively slow nature of neuron firing. However, if the second electrode is simply recording the population spikes in the first region, then these results would be skewed as this result. It is crucial to further test this hypothesis by utilizing stimulation electrodes in a similar media with and without a hippocampal slice present to test the range that a voltage spike similar to a population spike will travel through the media.

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Vita

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