Investigating the role of VPA on epileptic events in the hippocampus

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Abstract

Valproic acid (VPA), one type of antiepileptic drugs (AEDs), is widely used as a treatment for epilepsy. The exact mechanism of this drug remains elusive. However, studies have found that the drug could cause both positive and negative effects on the hippocampus. Temporal lobe epilepsy is a type of epilepsy that originates in the hippocampus, and it is often marked by the occurrence of abnormal voltage patterns such as high frequency oscillations (HFOs) in the hippocampus. HFOs are local field potential (LFP) patterns measured in the hippocampus and they primarily occur in the CA1 region of the hippocampus, which receives excitatory input from the CA3 region. Mathematical models of the hippocampal regions and simulations of the drug effect on the regions could help us validate *in-vivo* and *in-vitro* experimental recordings of ripple phenomena in the hippocampus, and allow for a deeper level of understanding of the drug effect of VPA on the hippocampal area, respectively. In this project, three representative hippocampal models were investigated including a modified model of the CA1 region and two models of the CA3 region to study the relationship of the drug effect of VPA and the resulting neural activities.

1 Introduction

Five million people in the US have been diagnosed with epilepsy, or seizure disorder, at some point in their life. Epilepsy can be caused by stroke, brain tumor, traumatic brain injury, or central nervous system infection, but often the cause is unknown [1]. The most common form of epilepsy, temporal lobe epilepsy, originates in the hippocampus [2]. The hippocampus is a spiral shaped region in the temporal lobe of the brain, which itself contains distinct compartments, including the dentate gyrus, CA1, and CA3. The trisynaptic circuit is one of the main pathways by which information travels through the hippocampus. Information from the entorhinal cortex enters the dentate gyrus of the hippocampus. The dentate gyrus then synapses onto CA3, which synapses onto CA1 [3]. Neural recordings show that epilepsy is often marked by irregular spiking of pyramidal cells in the CA3 and by an increase in the occurrence of high frequency oscillations (HFOs) in the local field potential (LFP) of CA1, so we worked with models of these two regions of the hippocampus to simulate epileptic activity [4, 5].

The CA3 and CA1 are physiologically very similar, and are mainly comprised of three different types of neurons: pyramidal cells, basket cells, and OLM interneurons. The pyramidal cells are excitatory, while the other two cell types are inhibitory (GABAergic). The main difference between

these two hippocampal regions is that the CA3 pyramidal cells have recurrent synapses, but the CA1 pyramidal cells don't. Figure 1 shows the connectivity of the two networks, with inhibitory synapses marked by red X's.

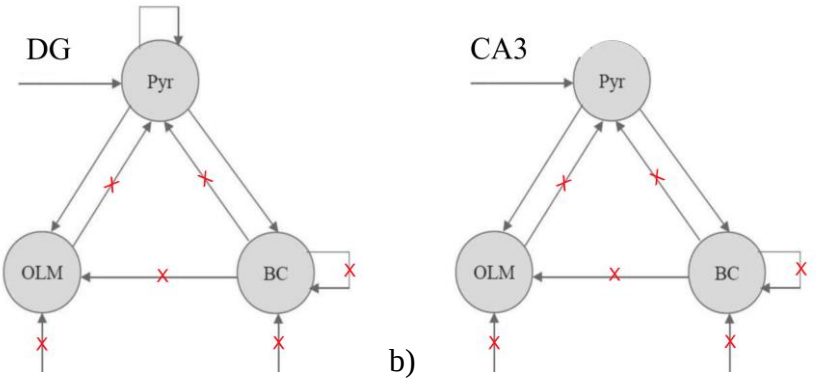


Figure 1: a) CA3 network showing input from dentate gyrus (DG) and recurrent synapses in the pyramidal cells, b) CA1 network showing input from CA3 and no recurrent synapses in the pyramidal cells. Red X's represent inhibitory synapses.

One of the treatments for epilepsy includes anti-epileptic drugs (AEDs). It works to prevent seizures from happening in the brain. In general, AEDs decrease the likelihood of occurrence of seizure by reducing and modifying the excitability of neural networks that are associated with causing seizures. The exact mechanisms of AEDs, however, are not yet fully understood [8]. One type of AEDs is known as valproic acid or VPA, which has been found to increase gamma-aminobutyric acid (GABA) levels and also inhibit the conductance of sodium channels [9]. GABA is a type of inhibitory neurotransmitter that can inhibit the communications between neurons by influencing the membrane potential of the neurons. Sodium channels, on the other hand, allow flows of positively charged sodium ions into the neurons causing depolarization or action potential. Increasing the amount of GABA or blocking the sodium channels or having both could result in a lower excitability of the neurons [8]. However, studies have found that long-term exposure to the drug could lead to complexities such as interference of memory and learning processes [9]. Indeed, in an animal study conducted by Sgobio et al. using mice suggests that VPA treatment could have both positive and negative effects on hippocampus [9]. In our project, we aim to study the drug effect of VPA on the neural activities of CA1 and CA3 regions by performing simulations in NEURON.

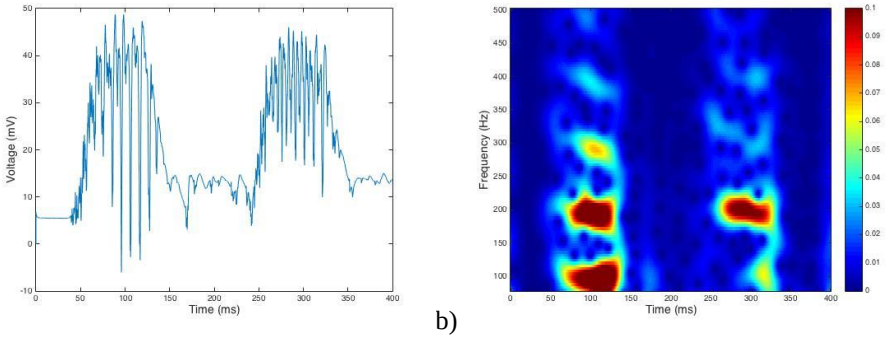
2 Methods

Three representative hippocampal models, one on CA1 and the other two on CA3, were explored and employed for our simulations in NEURON. The overall goal was to first simulate epilepsy-like activities in all models by investigating and altering the parameters such as number of pyramidal neurons versus basket neurons, ion channel conductance, and excitatory/inhibitory synaptic strengths etc. After having the epilepsy-like activities successfully simulated in all models, we proceeded to simulation of the drug effect of VPA for the models. As aforementioned, VPA works by increasing the GABA level available to the cells, and by blocking the sodium channels to decrease the excitabilities of neurons. Hence, we simulated the drug effect by decreasing the sodium channel conductance and/or increasing the level of GABA for the modified models.

2.1 CA1 modified Fink model

The CA1 model we worked with, by Fink et al., was designed to demonstrate HFOs [5]. The model contains 80 pyramidal cells and 20 basket cells. Although the model does not contain OLM cells, the connectivity is sufficient to demonstrate HFOs. HFOs are LFP traces that come in two varieties, ripples and fast ripples. Ripples are 80-250 Hz, and fast ripples are greater than 250 Hz. Both types of HFOs, but especially fast ripples, occur more frequently in epilepsy [5]. Figure 2 shows a sample voltage trace and spectrogram of an LFP measurement from CA1. As shown in the spectrogram, both bursts of activity demonstrate ripples, but only the first burst demonstrates fast ripples. The first burst represents activity more likely to be seen in a model of epilepsy. In order to mimic the

action of VPA, we varied the weight of the GABAergic basket cell synapses by 30%. This weight change was chosen to approximate the effect of VPA because Phiel et al. demonstrated a 15-45% increase in GABA when VPA was acutely administered to rodents [10].



a) b)

Figure 2: a) Sample voltage trace of LFP in CA1 model, b) Sample spectrogram of the same data. Looking at the spectrogram, we see ripples (~100 Hz and ~200 Hz) in both bursts of activity, but fast ripples (~300 Hz) only in the first burst. We expect to see data resembling the first burst more frequently in a model of epilepsy.

2.2 CA3 Migliore bursting model

One of the CA3 models that we explored was called the Migliore bursting model. The CA3 pyramidal neurons have been known to exhibit distinct firing characteristics and bursting phenomenon in some CA3 cells is one of them [11]. This bursting phenomenon in the CA3 has been found to be closely linked to sharp wave ripples seen during ictal state [11]. A burst can be triggered either spontaneously or by a short pulse of injected current [11]. In our case, we assumed that it was only triggered by a pulse of injected current, which was fixed at 1 nA for a duration of 20 ms for all cases. All variables were adapted from the original model, and specifically the sodium channel kinetic is given by the Hodgkin-Huxley equations:

$$I_{Na} = g_{Na} m^3 h\,(V - V_{Na})$$

where V_{Na} was fixed at 50 mV and the values for m and h used were based on experimental results [11]. Since the synaptic conductance used in this model was simplified and not type-specific, we were only able to explore the effect of varying sodium conductance on the output action potential.

2.3 CA3 Safiulina single pyramidal neuron model

CA3 neurons have been observed to generate bursting spikes and regular firing trains. This model simulates the functional role of glutamatergic and GABAergic signaling inputs on subsequent mossy fibers (MF)-CA3 pyramidal cell spiking [12]. Output signals from a single CA3 pyramidal neuron were recorded following the input of 10 random poissonian generated activations. These activations could be set to either GABAergic or glutamatergic inputs at frequencies between 20-40 Hz. Up to 30 synaptic inputs from the Dentate Gyrus (DG) were modeled, with 52 basal dendrites and apical dendrites. This model was implemented in NEURON version 7.3 with the following parameter consideration and active dendritic properties: voltage-gated sodium (NaV) conductance, specific potassium conductance including delayed rectifier (KDR), M-current (KM), fast-inactivating A-type (KA), and slowly-inactivating D-type (KD), three voltage-gated Ca2+ conductance (CaV N-, L- and T-type), two Ca2+-dependent potassium conductance (KC and KAHP), and a hyperpolarization-activated conductance (Ih) [13].

Voltage dependent inactivation of slow-inactivating potassium conductance modeled in CA3:

$$n = \frac{1}{1+\alpha n} \quad where \quad \alpha n = e^{0.51(v+27)} \qquad \tau n = 100\frac{\beta n}{1+\alpha n} \quad where \quad \beta n = e^{0.36(v+27)}$$

3 Results

3.1 CA1 modified Fink model

Beginning with the initial parameters in the CA1 model, we increased the weight of GABAergic basket cell synapses by 30% to mimic the action of VPA and then decreased the weight by the same amount to simulate a more epileptic case. When GABA was increased, the ripple activity around 100 Hz disappeared from the second burst, which is consistent with our assumption that increasing GABA (a mechanism of VPA) would reduce potential epileptic activity. Conversely, when GABA was decreased, the 100 Hz ripple activity in that burst was greatly strengthened, which could be indicative of epilepsy. Interestingly, there was no significant change in fast ripple activity when varying GABA. Spectrograms demonstrating these results are shown in Figure 3.

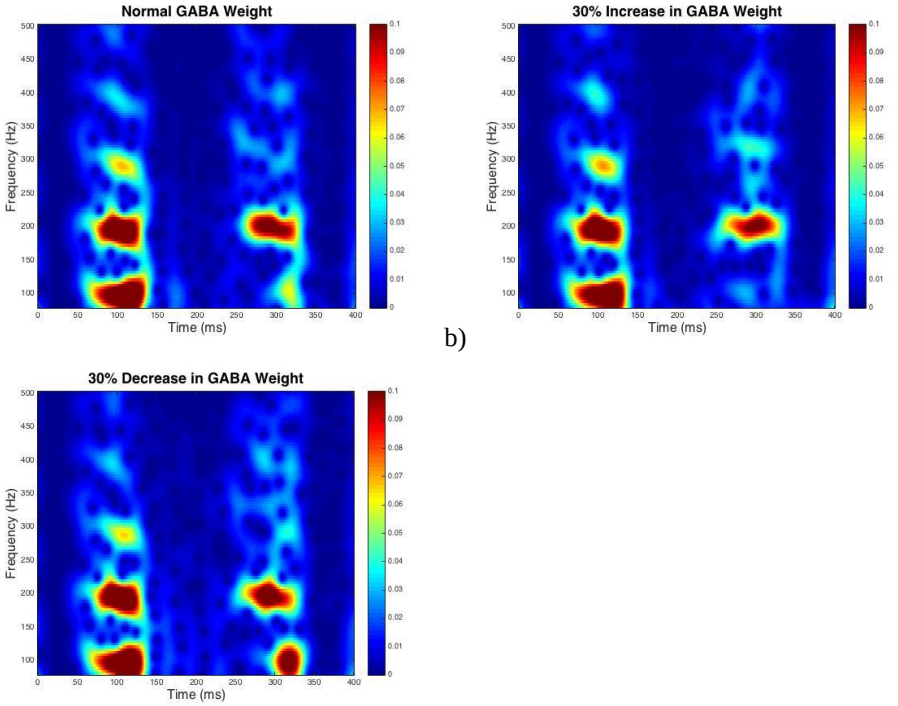
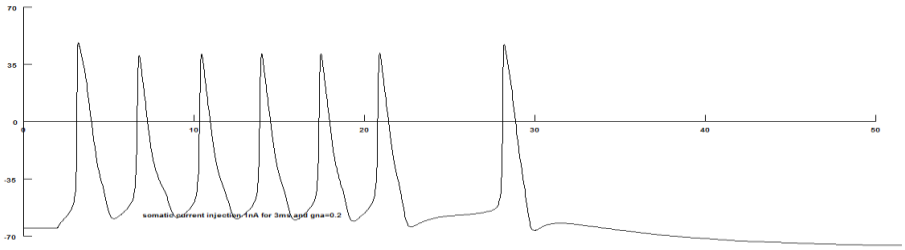


Figure 3: Spectrograms of CA1 LFP a) in the original model by Fink et al., b) in response to a 30% increase from the original model in the weight of GABAergic synapses, c) in response to a 30% decrease from the original model in the weight of GABAergic synapses. In the second burst of activity, ~100 Hz ripple activity disappears when the weight is increased (mimicking VPA mechanism), and is much stronger when the weight is decreased (indicative of epilepsy).

3.2 CA3 Migliore bursting model

In the simulation for the Migliore bursting model, a relatively high magnitude of sodium conductance was first used, i.e. 0.2 mS/cm^2 in this case, to stimulate a "high frequency" spiking pattern as shown in **Figure 4**. The magnitude of sodium conductance was then gradually decreased to simulate the drug effect. At g_{Na} = 0.1 mS/cm^2, there was a slight but not significant change in terms of spiking frequency and peak. However, at a magnitude of 0.05 mS/cm^2, the "high frequency" spiking pattern began to diminish. Eventually, at a value of 0.015 mS/cm^2 which also corresponds to the sodium conductance value in the literature [6], only a short pulse of burst was observed indicating the effectiveness of the drug. One explanation for why there is sporadic spiking after initial burst could be due to the long injected current step under which recurring single action potentials may be produced [6].

a)



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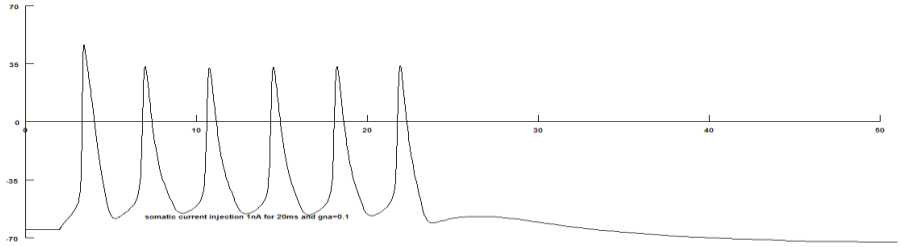

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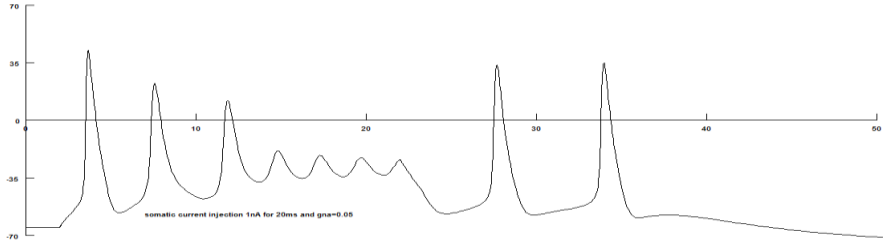


d)

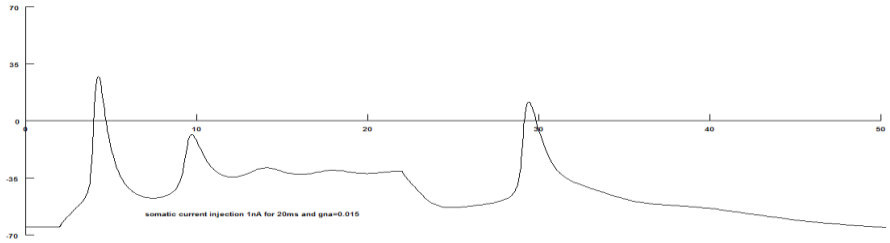


Figure 4. g_{Na} = a) 0.2, b) 0.1, c) 0.05, d) 0.015 mS/cm^2. The y-axis indicates V_{soma} and it ranges from -70 to 70 mV. The x-axis indicates simulation time and it ranges from 0 to 50 ms.

3.3 Safiulina CA3 model

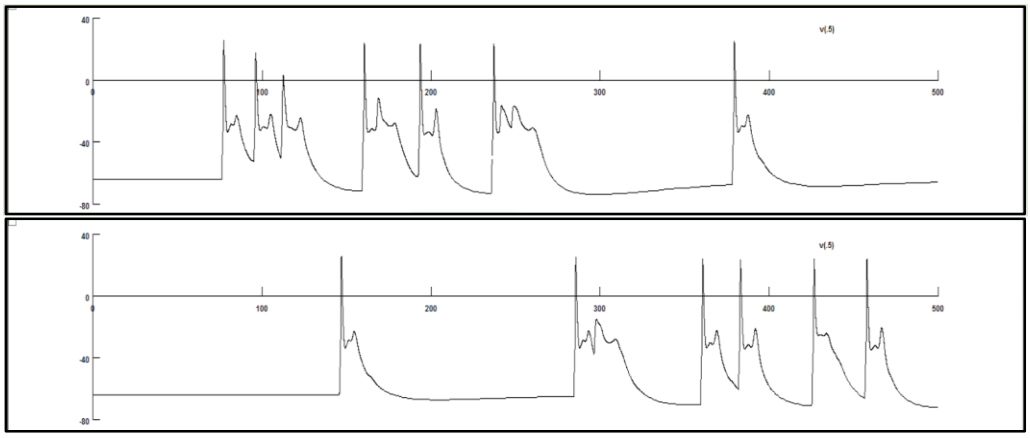


Figure 5: Normal spiking from pyramidal neuron modeled in CA3.

The Safiulina CA3 model generates normal spiking patterns associated with a healthy region of the hippocampal network. Healthy CA3 spikes are comprised of approximately 30mV action potentials that exhibit low and stochastic frequency shown in **Figure 5**.

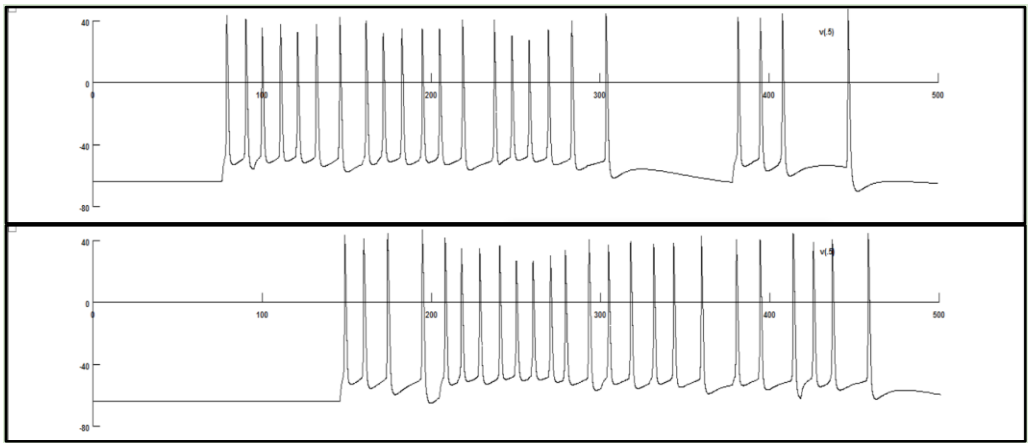
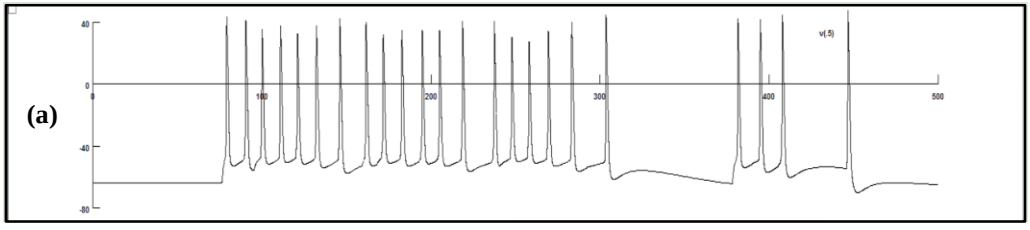


Figure 6: Epileptic spiking from disease-state CA3 pyramidal neuron.

Our disease-state model of the CA3 network produces spiking patterns characteristic of epilepsy. Pathological spiking patterns gathered experimentally from epileptic patients exhibit higher than normal action potential amplitudes of approximately 35 mV. The frequency of spiking is higher and more synchronous than the observed normal CA3 behavior as shown in **Figure 6**. Additionally, disease-state CA3 networks appear unresponsive to GABA inhibition. This epileptic result was generated by modifying the Safiulina model. Specifically, basket cell inhibition was turned off and sodium conductance marginally increases, both of which are real, physiological events leading up to an epileptic event.



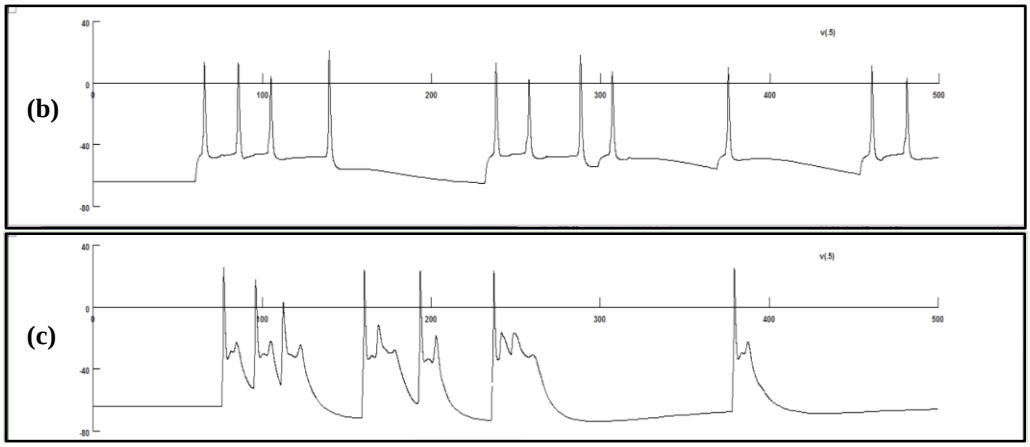


Figure 7: Administration of VPA drug shows partial recovery of CA3 from disease-state. (a) Epileptic (b) Recovered (VPA) (c) Normal

Computational modeling of VPA drug treatment on the disease-state CA3 simulation shows mild recovery of normal hippocampal firing pattern. In the recovered simulation, measured spiking frequency decreases to approximately 20 Hz and stochastic firing returns. However, action potential amplitudes are lower than normal and the previously observed, healthy spiking train is not fully recovered. Only a partial recovery is observed because our simulation of VPA drug treatment has a normalizing effect on sodium conductance but seems to be ineffective at normalizing inhibitory basket cell function. Moreover, a fully recovered pattern may not be produced here because, as discussed for figure 6 above, the modified Safiulina model seems to be unresponsive to GABA once entering the epileptic behavior, suggesting that VPA's expected normalizing of inhibitory neurons, and the subsequent release of GABA, is not being observed in this recovered spiking pattern. See **Figure 7.**

4 Conclusion

The manner in which VPA treats epilepsy is not fully understood, so we took some of the known effects of VPA and applied them to existing hippocampus models to simulate untreated and treated epilepsy. By increasing the weight of GABAergic synapses to mimic VPA in our CA1 model, some of the ripple activity was decreased, suggesting that VPA's upregulation of GABA could treat epilepsy by having a direct effect on the GABAergic synapses in CA1. In addition, the results we obtained from simulating the drug effect of VPA on the neural activity at a disease state in both the Migliore bursting model and the Safiulina model of the CA3 suggest that by decreasing the sodium channel conductance, the bursting activity was able to recover from the disease state to a normal state. Unfortunately, due to time constraints, the role of the GABAergic synapses was not investigated in the Migliore model. In the Safiulina model, however, the network appears unresponsive to changes in the GABAergic synaptic input.

We simulated the drug effect of VPA on all models by making the assumption that the drug only affects the sodium conductance and the GABA level within the CA1 and CA3 areas. To further our understanding of the effect of VPA, we hope to investigate the role of the synaptic connection between the CA3 and CA1 regions and examine how VPA acting at that junction affects epileptic activity as a whole. In addition, a more robust model for CA3 could be developed by adding more complexities such as increasing the number of synaptic inputs and incorporating basket cells and interneurons.

Acknowledgments

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