

# The Effects of Intracellular Astrocytic Calcium Concentrations on Neurons

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March 17, 2010

## **Abstract**

The aim of this project is to assess the effects of intracellular astrocytic calcium transients on neurons. It is known that when amyloid beta (AB), a neurotoxic protein, is applied to a co-cortical cell culture of astrocytes and neurons, astrocytes exhibit a marginal increase in cell death but there is a significantly higher death of neurons. I modelled this phenomena by injecting an astrocytic current that is dependent on intracellular calcium concentration into a neuronal network. I discovered that this current globally inhibits neuron spike rates within the network. This reduction and loss of spikes forms the basis for neuronal death when AB is applied to cortical cell cultures of astrocytes and neurons.

# Contents

<b>1</b>	<b>Introduction</b>	<b>3</b>
<b>2</b>	<b>Background</b>	<b>4</b>
<b>3</b>	<b>Methods</b>	<b>5</b>
3.1	Calcium in Steady State Input . . . . .	6
3.2	Oscillating Calcium Transient Input . . . . .	6
3.3	DiGarbo Model . . . . .	6
3.4	Neuron Network: Hodgkin Huxley . . . . .	7
<b>4</b>	<b>Results &amp; Observations</b>	<b>9</b>
4.0.1	Simple Excitatory Synapse with a Current that takes in a fixed value of calcium Concentration . . . . .	9
4.0.2	Feedback Inhibition Model using steady state astrocytic current . . . . .	10
4.0.3	Feedforward Inhibition Model using steady state astrocytic current . . . . .	11
4.1	Sinusoidal and Exponential Calcium Transients . . . . .	12
4.2	Oscillating Input . . . . .	14
4.2.1	Model using DiGarbo et al's calcium dynamics and 3 neuron feedforward inhibition network . . . . .	15
<b>5</b>	<b>Discussion</b>	<b>16</b>
<b>6</b>	<b>Future Work</b>	<b>16</b>
<b>7</b>	<b>References</b>	<b>18</b>

# 1 Introduction

It has been noted that the accumulation of amyloid beta (henceforth known as AB) on neuritic plaques is one of the chief indicators of Alzheimer's disease. Amyloid beta is a neurotoxic protein, specifically the AB peptide 1–42 or the fragment 25–35. In Alzheimer's disease, mutated human AB precursor proteins (APP) and mutant presenilin 1 (PS1) that caused an increase in AB production and plaque deposition were noted (Kuchibotla, 2009). Thus, there exists an overaccumulation of AB on neuritic plaques in mouse models with Alzheimer's (Abramov, 2004). The effects of AB have long been studied, and there is a wide array of literature studying the effects of AB on intracellular calcium levels - which is what I am interested in. However, there are plenty of conflicting results; some papers argue that calcium levels are increased upon amyloid peptide application (Stix and Reiser, 1998), where some argue that calcium levels are decreased (Meske et al, 1998). It is, indeed, difficult to draw a solid conclusion with all the conflicting literature.

What is clear, however, is that astrocytes are indeed the primary target of AB and that AB induces spontaneous calcium waves (Abramov et al, 2003; Kuchibotla et al, 2009; Chow et al, 2010). It was also found experimentally in our lab (Chow et al, 2010) that cultures with AB had a larger percentage of cells that produced intracellular calcium transients ( $92 \pm 3.5\%$  v  $81.5 \pm 5.3\%$ ).

Kuchibotla et al (2009) demonstrated the existence of AB induced calcium waves by blocking all neuronal activity with tetrodotoxin (TTX) thus showing a direct correlation between AB and astrocytic calcium waves. The full length peptide AB 1-42 and 25-35 caused sporadic cytoplasmic calcium signals in astrocytes that persisted for hours by inducing a pathway for calcium influx across the plasma membrane. In Abramov's experiments, it was reported that astrocyte cell death was marginally increased, but about half the neurons had died. It was also observed in Kuchibotla's results that astrocytes exhibited a resistance to cell death upon application of AB whereas neurons died. This experiment was replicated and shown in Figure 1.

In this project, I aim to show what Newman et al (2003) discovered experimentally: that the amplitude of neuronal inhibitory responses is closely correlated to the magnitude of astrocytic  $\text{Ca}^{2+}$  increase.

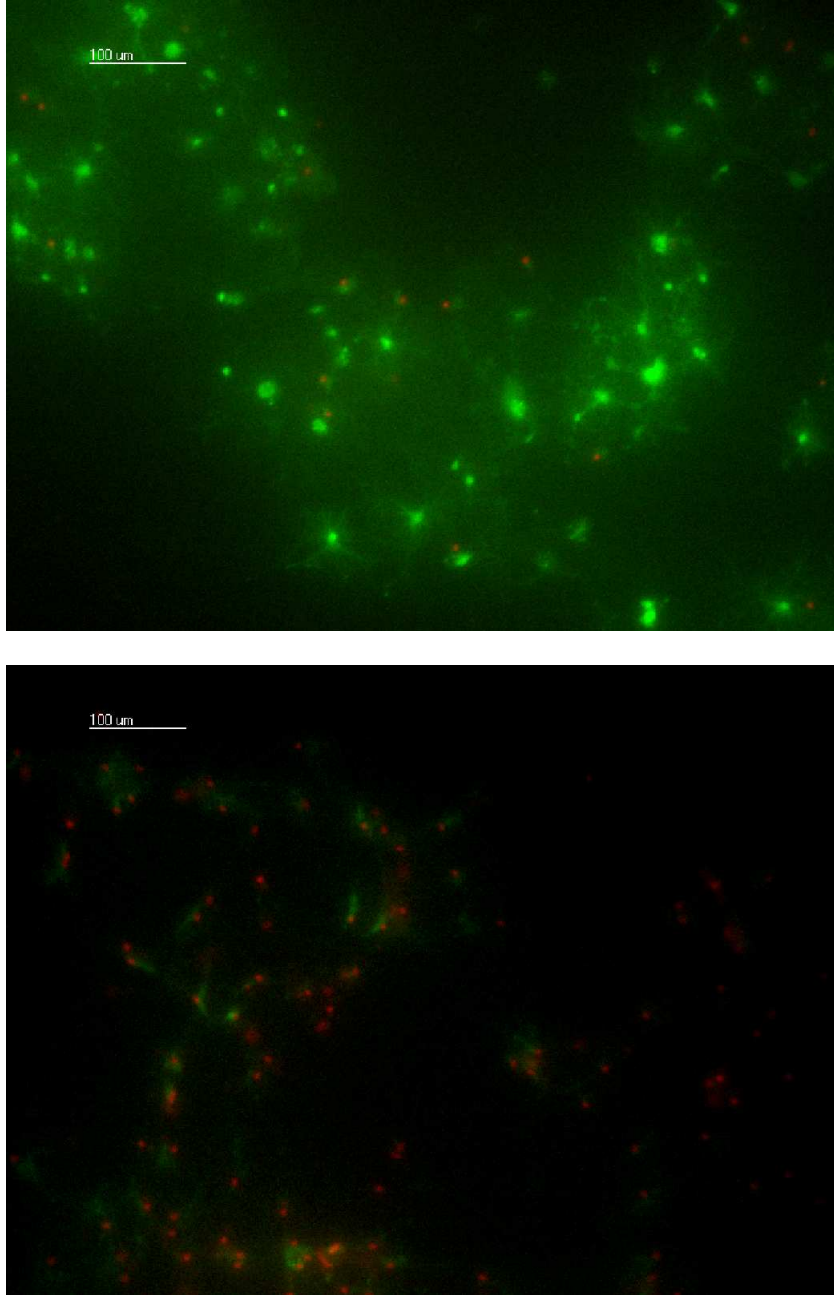


Figure 1: Experimental data. 5 $\mu$ M of AB was applied and after 24 hours a significant percentage of neurons had died (numbers unavailable).

## 2 Background

Chemical, electrical and mechanical stimulation of astrocytes cause intracellular  $\text{Ca}^{2+}$  levels to increase (Newman, 1997), thus astrocytes exhibit  $\text{Ca}^{2+}$  excitability. The main type of calcium wave we are interested in is intracellular calcium transients. Propagated increases in intracellular  $\text{Ca}^{2+}$  in astrocytes arise from the release of  $\text{Ca}^{2+}$  from internal stores, mediated by IP3 (Newman, 1997). Note that IP3 is a glycoprotein that

releases Ca, and IP3R is a membrane receptor. Intracellular calcium transients associated with A $\beta$  exposure in astrocytes results in calcium dependent glutathione depletion in neurons and the formation of reactive oxygen species in astrocytes that directly produce neuronal death (Abramov et al., 2003, 2004).

AB spontaneously induces calcium transients in astrocytes. This is the result of calcium being released from internal stores which is activated by increases in IP3. IP3 elevation is generated because of phospholipase C (PLC) which is activated by G protein coupled receptors (Haydon, 2001). ATP release is also dependent on PLC, and is released from internal stores and released into extracellular space (Newman, 2003). ATP released at synapses is then converted to adenosine. Note that adenosine is a potent inhibitory neuromodulator which increases postsynaptic potassium conductance and decreases presynaptic calcium conductance. Thus, we see that the inhibitory current induced in the neuron is caused by the intracellular calcium transients in the astrocytes, which is what I have modelled for this project.

### 3 Methods

The way in which I went about modelling the process of seeing how neurons can be synaptically suppressed is by injecting an inhibitory astrocytic current into the network of neurons. I successfully achieved this in 2 ways thus far. The third method is still a work in progress, the results of which may be seen in this report as well.

- 1) Steady state astrocytic calcium values
- 2) Non-steady state i.e. oscillating astrocytic calcium
- 3) More complex models such as Li-Rinzal and a model used by DiGarbo et al, 2009.

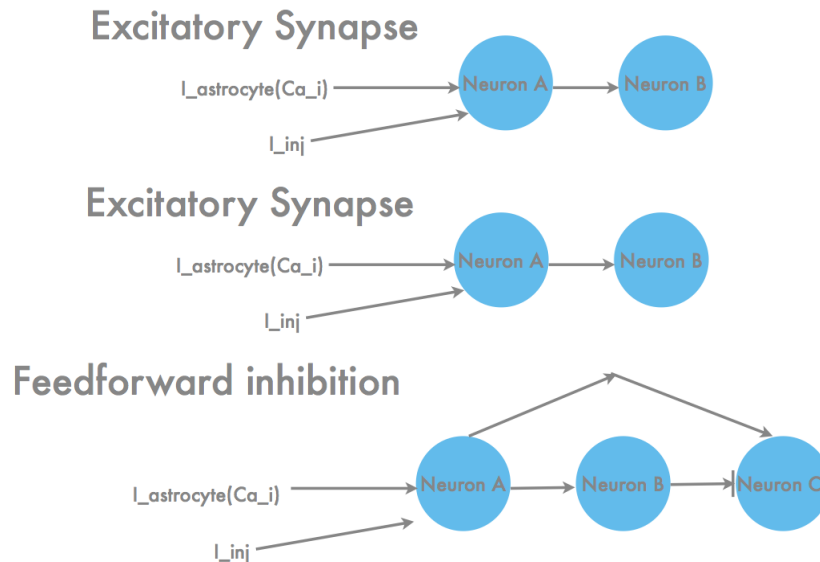


Figure 2:

### 3.1 Calcium in Steady State Input

I coupled the neurons to the astrocytes by simply injecting an inhibitory astrocytic current governed by a varying value of  $Ca_i$ , adapted from equation 1. I varied the values of  $Ca_i$  from 0 – 30 $\mu M$

$$I_{astrocyte} = A_{astrocyte}(Ca_i)$$

### 3.2 Oscillating Calcium Transient Input

$$\begin{aligned} \frac{dCa}{dt} &= \frac{Ca_i^2}{\beta i^2 + Ca_i^2} - Ca_i^2 \\ \frac{di}{dt} &= k(Ca_i - i) \end{aligned}$$

I used these set of equations given for a simple oscillator. The reason that I used this instead of a more dynamically complex model such as the Li-Rinzel/DiGarbo models, was purely to test whether intracellular calcium transient spikes do actually inhibit neuron spiking before moving on to more complex models.

### 3.3 DiGarbo Model

In the DiGarbo paper, the astrocytic current is given by Equations 1 and 2.

$$\begin{aligned} I_{astrocyte} &= A_{astrocyte} H[\ln(y)] \ln(Ca_i) \\ y &= [Ca^{2+}]_i - 196.69 \end{aligned} \tag{1}$$

where H is the Heaviside function; the value of  $A_{astrocyte}$  was given by experimental results in DiGarbo et al's paper. In their model  $A_{astrocyte} = 2.11 \mu A cm^{-2} \mu M - 1$  for a cell of 25 $\mu cm$  radius.

$$\frac{dCa}{dt} = V_{leak} + V_{CCE} + V_{ATP(P2X)} - V_{OUT} + V_{ER(rel)} - V_{SERCA} \tag{2}$$

$$\frac{dCa_{ER}}{dt} = \beta(V_{SERCA} - V_{ER(rel)}) \tag{3}$$

$$\frac{dR}{dt} = V_{IP3R(rec)} - V_{IP3R(inact)} \tag{4}$$

$$\frac{dI}{dt} = V_{PLC\beta} + V_{PLC\delta} - V_{IP3R(deg)} \tag{5}$$

The terms in Equation 1 dictate the time evolution of calcium concentrations in the cell:

$$\left\{ \begin{array}{l} V_{leak} = k_0 \\ V_{CCE} = \frac{K_{CCE}H_{CCE}^2}{H_{CCE}^2 + Ca_{ER}^2} \\ V_{ATP(P2X)} = K_{ATP(P2X)}G(ATP_{ex}) \\ G(ATP_{ex}) = \left( \frac{ATP_{ex}}{H_{ATP(P2X)} + ATP_{ex}} \right) \\ V_{out} = k_5(Ca_i) \\ V_{ER(rel)} = \frac{K_1 + K_2RCa_i^2I^2}{(K_a^2 + Ca_i^2)(K_{IP3}^2 + I^2)}(Ca_{ER} - Ca_i) \\ V_{SERCA} = k_3Ca_i \\ V_{IPR3(rec)} - V_{IP3(deg)} = k_6 \frac{K_I^2}{(K_I^2 + Ca_i^2) - R} \\ V_{PLC\beta} = K_{ATP(P2Y)}X_F \end{array} \right. \quad (6)$$

where  $V_{leak}$  is simply the leakage flux,  $V_{CCE}$ , this is the calcium entry pathway called the Capacitative Calcium Entry,  $V_{ATP(P2X)}$  where  $G(ATP_{ex}) = \left( \frac{ATP_{ex}}{H_{ATP(P2X)} + ATP_{ex}} \right)$ . This models the influx of calcium mediated by ionotropic ATP receptors P2X, where,  $V_{out}$ , the efflux of calcium,  $V_{ER(rel)}$  flux from the endoplasmic reticulum,  $V_{SERCA}$ , efflux from the sarcoplasmic endoplasmic reticulum is given by  $V_{IPR3(rec)} - V_{IP3(deg)}$  and  $V_{PLC\beta}$ .

We assumed that the reaction was in equilibrium. The concentration of metabotropic receptors is given by  $X_F = \frac{[ATP]_{ex} - R_{mb}}{R_o}$  so that the time evolution of the reaction of ATP is given by:  $\frac{dX_F}{dt} = K_F[ATP]_{ex} - X_F(K_R + K_F[ATP]_{ex})$ . The solution of this equation is given by  $X_F(0) = 0$  which yields:

$$X_F(t) = \frac{[ATP]_{ex}}{K_D + ATP_{ex}} \left( 1 - e^{-t/\tau} \right) \quad (7)$$

However assuming that  $1 - e^{-t/\tau} \approx 1$ , the expression simply goes to

$$X_F(t) = \frac{[ATP]_{ex}}{K_D + ATP_{ex}} \quad (8)$$

$$V_{PLC\delta} = \frac{V_7Ca_i^2}{K_{Ca^2} + Ca_i^2} \quad (9)$$

The parameters for this model are given in the paper.

### 3.4 Neuron Network: Hodgkin Huxley

I used the often used Hodgkin Huxley model, and kept  $I_{inj} = 30\mu Acm^{-2}$ . To create excitatory and inhibitory synapses, I used the following equations:

$$\begin{aligned}
I_{syn} &= g_{syn}(t)(V_{post} - E_{Cl}) \\
g_{syn}(t) &= g_{GABA}r(t) \\
\frac{dr}{dt} &= \alpha_r[T](1 - r) - \beta_r r \\
[T] &= \frac{T_{max}}{1 + \exp\left(\frac{-(V_{pre} - V_P)}{K_p}\right)}
\end{aligned}$$

With parameters of

$$\begin{aligned}
E_{Cl} &= 32mV \\
\alpha_r &= 2.4mM^{-1}ms^{-1} \\
\beta_r &= 0.56ms^{-1} \\
[T]_{max} &= 1mM \\
K_p &= 5mV \\
V_P &= 72mV \\
g_{Glu} &= 0.3mS/cm^2 \\
g_{GABA} &= 0.01mS/cm^2
\end{aligned}$$



## 4 Results & Observations

### 4.0.1 Simple Excitatory Synapse with a Current that takes in a fixed value of calcium Concentration

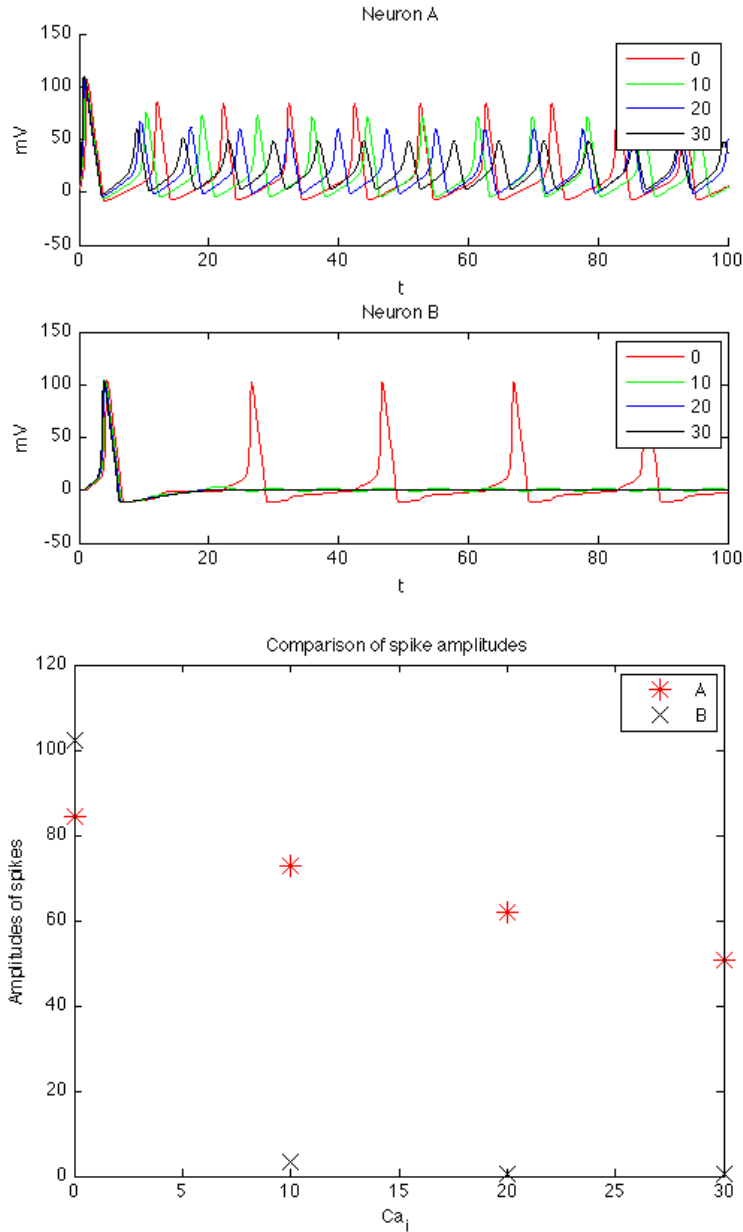


Figure 3: Excitatory synapse with steady state input

FIGURE 2: Values of  $Ca_i = 0$  make the control state i.e. a regularly spiking HH neuron. However, with increasing concentrations of calcium, we see that the spiking amplitudes are increasingly dampened as given by the negative slope in the graph of  $Ca_i$  vs amplitude of spikes. This is expected since calcium inhibits spiking in neurons.

What is interesting is that for Neuron B the amplitudes are completely inhibited for  $Ca_i > 10$  meaning that there is threshold of calcium concentration at which the neuron spiking is suppressed. This is a point to note and can be looked further into for Future Work.

#### 4.0.2 Feedback Inhibition Model using steady state astrocytic current

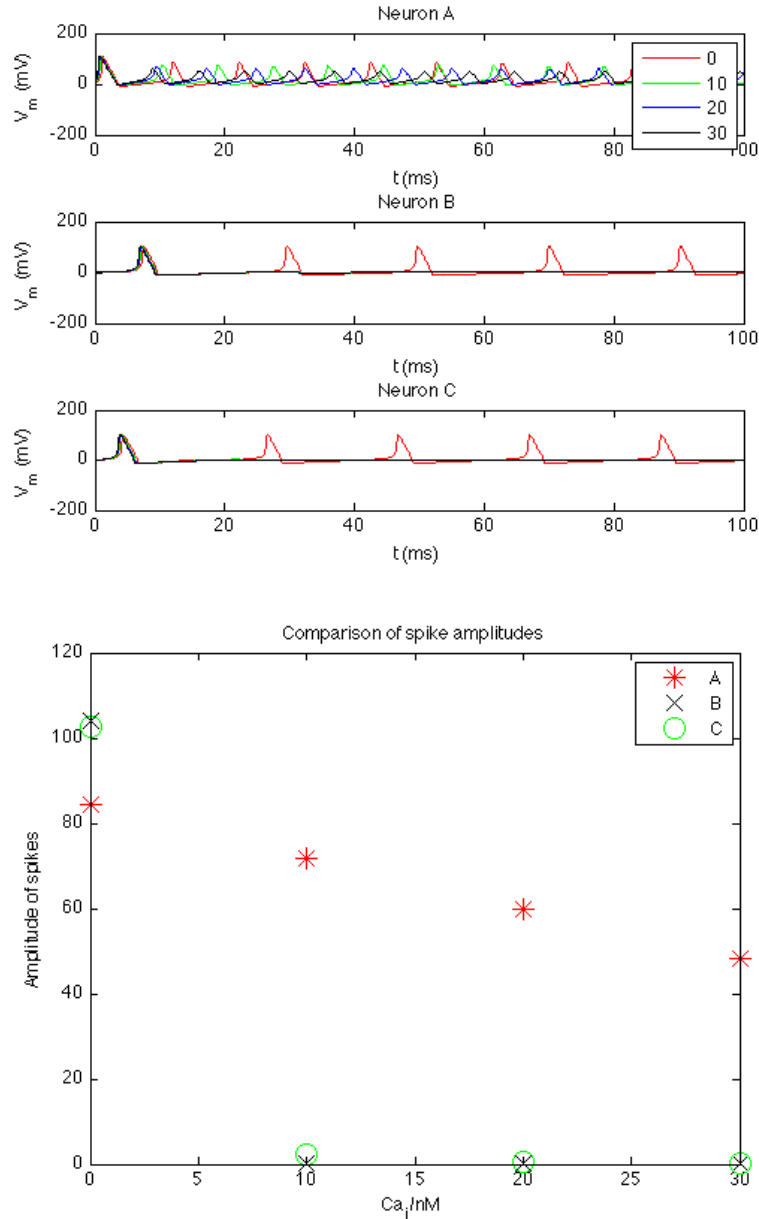


Figure 4: Feedback inhibition network with steady state input

FIGURE 3: Feedback inhibition yields similar results to that of Figure 2. The fact that Neuron C's spike train has a phase lag compared to Neuron B's spike train at  $Ca_i = 0$  is simply due to the property of the network and not the calcium input.

### 4.0.3 Feedforward Inhibition Model using steady state astrocytic current

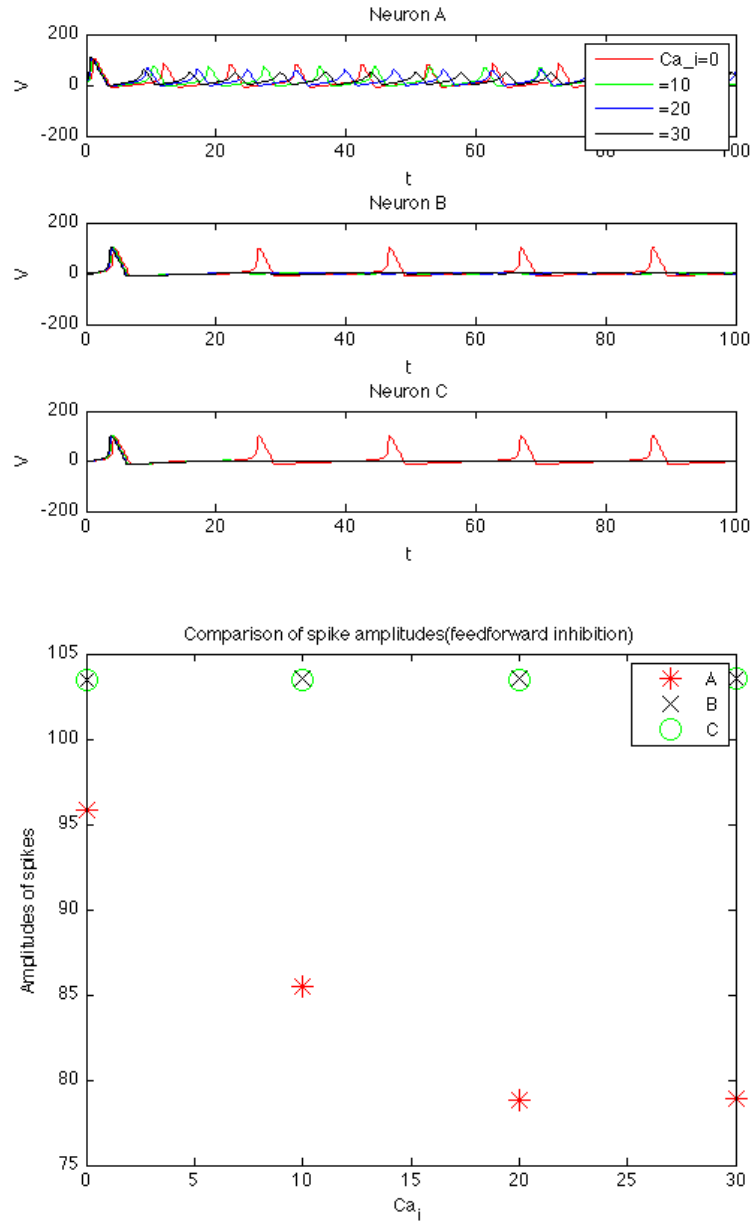


Figure 5:

FIGURE 3: Feedforward inhibition yields similar results to that of Figure 2 input. Interestingly, however, the relationship between amplitude and calcium concentration for Neuron A does not have a linearly decreasing slope as in Figures 1 and 2. The reason behind this is unclear but the fact remains that a general increase in calcium concentrations yield a decrease in spike amplitudes and inhibition in subsequent neurons

## 4.1 Sinusoidal and Exponential Calcium Transients

I modelled calcium transients as sinusoids and exponential calcium transients to test the viability of having a non-steady state input into the neural network.

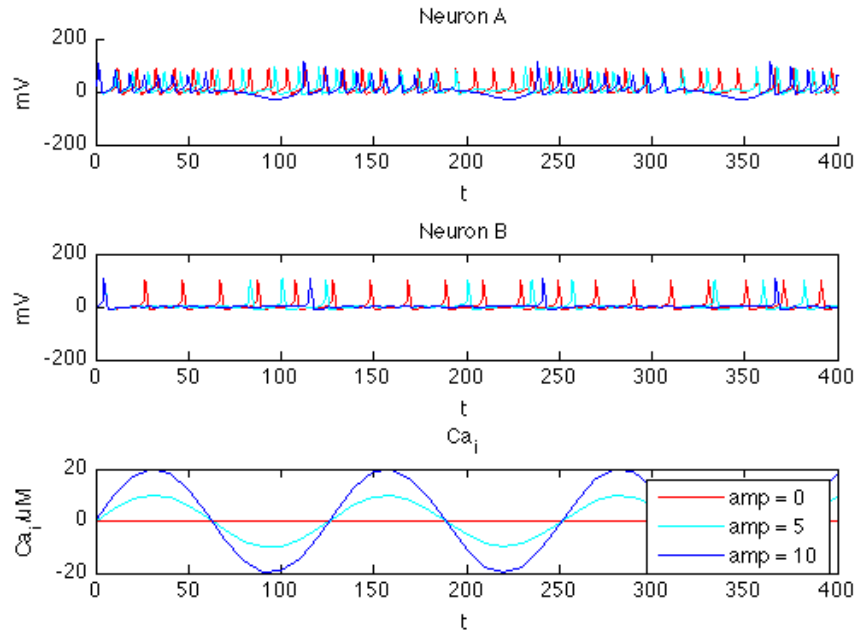


Figure 6: Sinusoidal calcium input into excitatory synapse

FIGURE 6: We note that for an increase in calcium concentrations from 0ms to 60ms, spike rates are inhibited. When the calcium concentration decreases from approximately 60ms to 125ms, the neuron begins to fire once more. This is especially obvious for the sinusoid with an amplitude of 5, shown in light blue. For an amplitude of 10, modelling the calcium transient as a sinusoid does not appropriately reflect the expected result - the neuron spikes are out of phase with the drop in  $Ca_i$

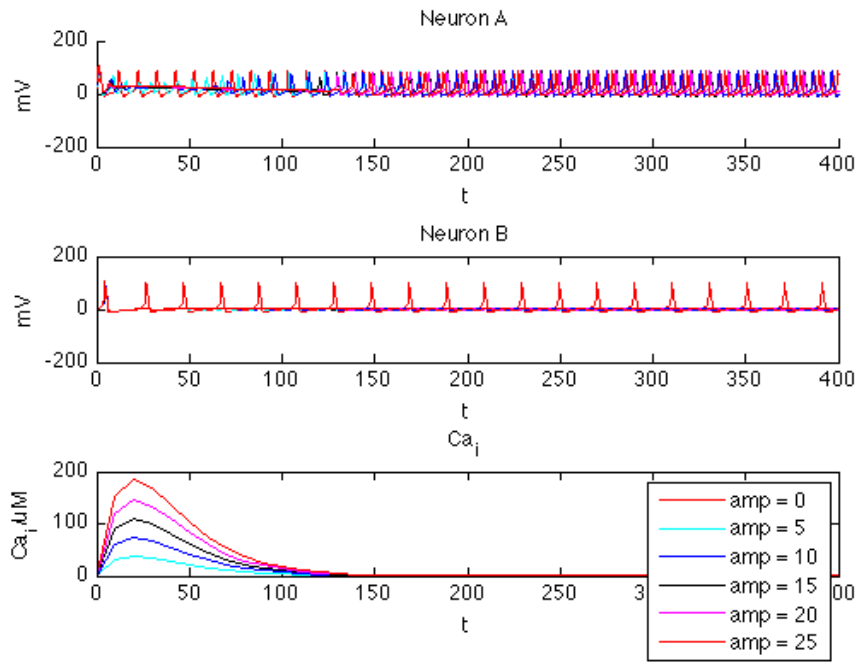


Figure 7:

FIGURE 7: This calcium signal was given by the more physiologically feasible signal  $Ca_i = amp(te^{-t/20})$ . For increasing amplitudes of the calcium signal, the spike amplitude of A decreases but then spikes again when the calcium concentration goes down to 0. This is further verified by a more accurate calcium oscillator given in the next section.

## 4.2 Oscillating Input

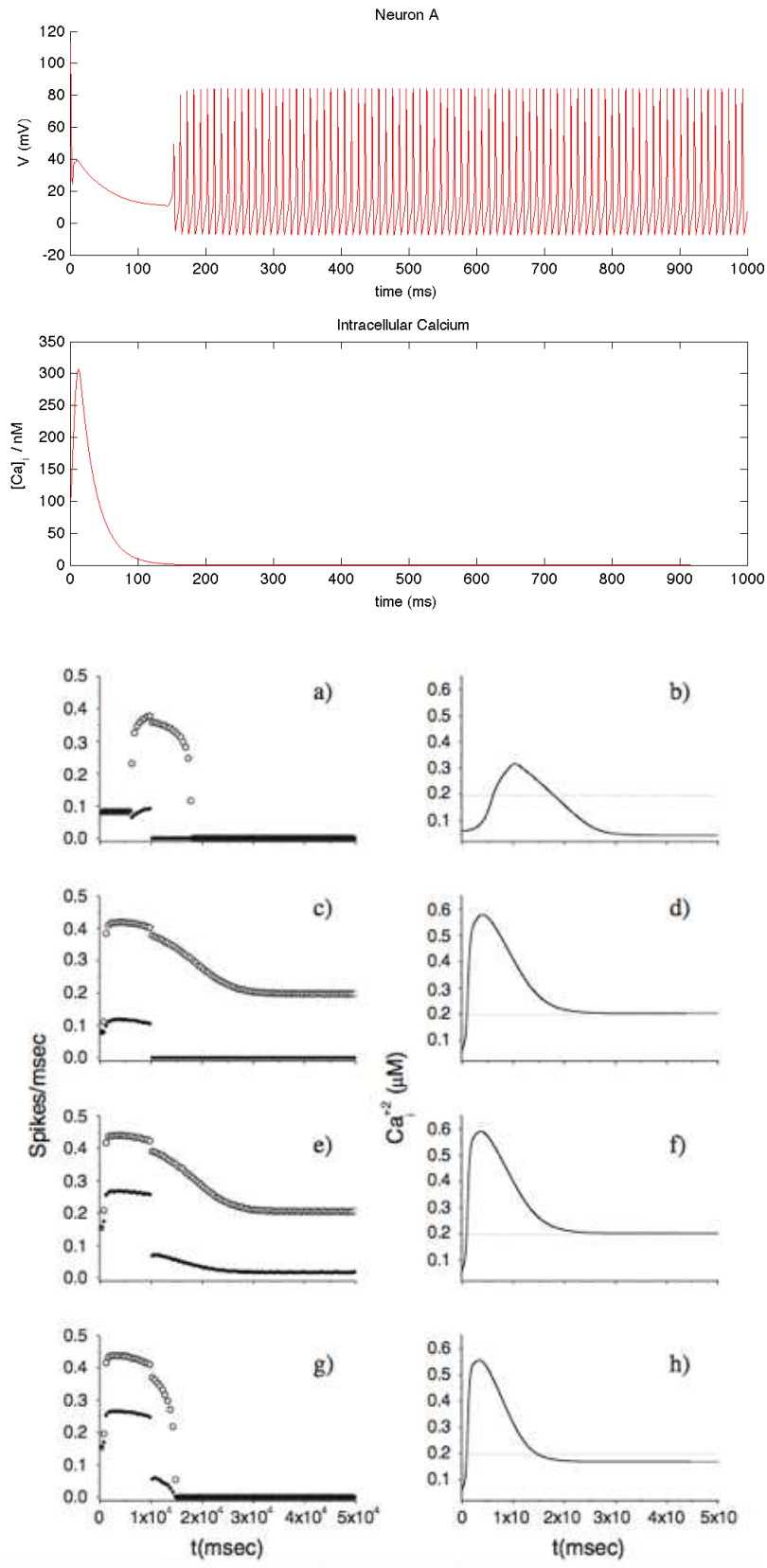


Figure 8:  $K=0.04$  results in a transient spike in calcium concentration

FIGURE 8: I validated my generated calcium signal by comparing it to the given calcium curves in the DiGarbo paper, given above. This result proves that astrocytic currents do actually inhibit neuronal spikes in that at 150ms when the calcium transient dies completely, the neuron immediately begins to fire again.

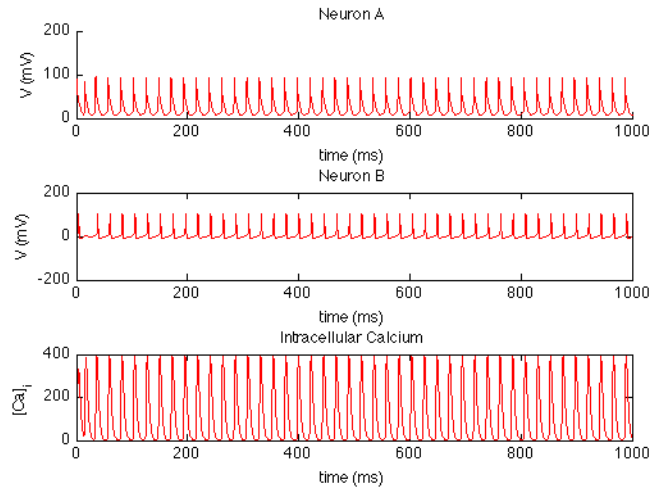


Figure 9: For value of  $K=0.4$ , an oscillating calcium transient is induced and input into an excitatory synapse

FIGURE 9: In this figure, an oscillating calcium transient is induced. Every peak of calcium corresponds to a trough in Neuron A's spike train, and vice versa. Neuron B follows Neuron A's spike patterns because this is an excitatory synapse. This faithfully replicates Newman's experimental findings.

#### 4.2.1 Model using DiGarbo et al's calcium dynamics and 3 neuron feedforward inhibition network

I modelled calcium dynamics in an astrocyte using the model as in the DiGarbo paper. In the first 3 figures, I coupled an astrocytic current  $I_{astrocyte}$  that used using this aforementioned model to simulate the intracellular calcium dynamics to a feedforward inhibition network of 3 Hodgkin-Huxley neurons A, B and C.

All the simulations for this part of the project have  $I_{inj} = 30\mu A^{-1}cm^{-2}$  and varying parameters of  $g_i$ ,  $g_e$  and  $ATP$  currents. However, there was no apparent difference in spike rate or spike amplitude. Because of this, I then moved on to simply using 2 neurons instead of 3 in an excitatory synapse - without a calcium dynamics model using simple a linear astrocytic current i.e. according to  $I_{astrocyte} = A_{astrocyte}(Ca_i)$  with a fixed value of calcium - as shown above. Figure 10 is an example of how an increase in calcium transients did not induce inhibition in Neuron A and subsequent neurons in the network.

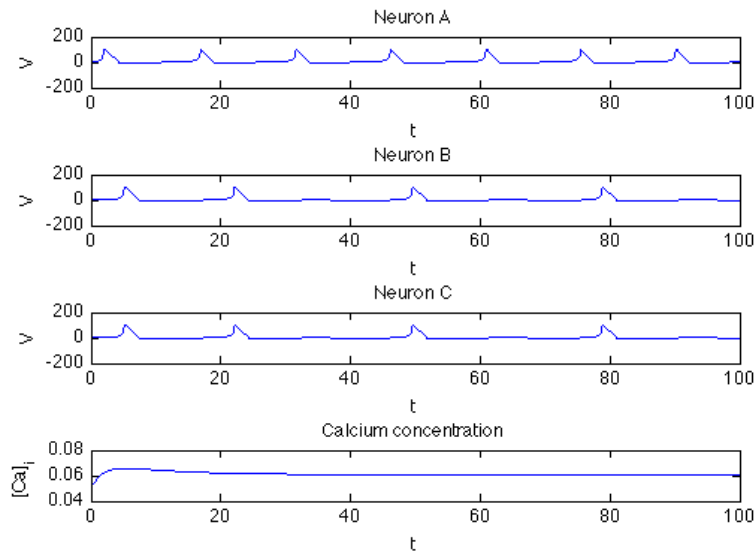


Figure 10: Parameters:  $[ATP]=2.2\mu M$ ;  $gGlu=0.3$ ;  $gGABA=0.01$

## 5 Discussion

In summary, AB induces spontaneous intracellular calcium transients which, through a series of G protein reactions in the astrocyte, then subsequently release ATP into the extracellular space. This ATP is then metabolized into adenosine which then binds onto the A1 adenosine receptor on the neuron, causing an inhibitory current which consequently suppresses the neurons and causes apoptosis.

I have modelled the induced calcium transients in the astrocytes as steady state and non-steady state signals. The main result is that an increase of calcium concentration causes the neuron to stop firing, and when the calcium concentration decreases again, the neuron begins to fire once more. Now that this has been established, we can now couple Li-Rinzel calcium dynamics in the astrocyte to govern the intracellular calcium oscillations and expect to see the same result, only that it will now be more biologically feasible. I also conclude that using the DiGarbo model might not have been the best estimation of calcium dynamics for a network, given how the three neuron network did not respond to it.

An interesting point that was noted during discussion was that the timescale of such a model that takes place over 5-10seconds at most. It was found that calcium waves took minutes to form after AB had been applied (Chow et al, 2010), suggesting that my model will need to run for at least 5 minutes to replicate experimental results.

## 6 Future Work

The main feature of neuronal inhibition that I would like to focus on is incorporating an A1 adenosine receptor model into this network of astrocytes and neurons in addition to calcium kinetics. I would also



want to account for ATP ejection from the astrocyte, its diffusion across the extracellular space and its conversion to adenosine by considering the kinetics of this reaction.

For calcium dynamics, I want to use the mathematical model based on the paper “Astrocytes optimize synaptic transmission” by Nadkarni, Jung and Levine (2008) to couple to the network of HH neurons. The model employed is the Li-Rinzel model, which is a 2-variable version of the 19 state DeYoung-Keizer model.

In approaching how astrocytes increase synaptic transmission, Nadkarni and Jung broke it down into modelling the:

- 1) Presynaptic pyramidal neurons - using Pinsky-Rinzel model
- 2) Postsynaptic neuronal response - using Wang-Buzsaki model for the membrane potential of the interneuron
- 3) Astrocytic calcium dynamics - using the Li-Rinzel model. This model ignores calcium fluxes across the cell membrane, and assumes that various transition rates are independent of one another
- 4) Presynaptic calcium - using the Bertram-Sherman-Stanley model to characterize vesicle release

I will only use method 3) for the astrocytic calcium and not consider whether neurons pre- or post-synaptic for ease of purpose.

For A1 adenosine receptors, I will have to read more papers regarding this topic, paying special attention to a paper by Linden (1991) that details the mechanism of an A1 receptor.

For ATP diffusion, I will refer to previous work regarding ATP diffusion done by other members of the lab.

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