Modeling Astrocyte Communication

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Abstract

Calcium wave oscillations are utilized by astrocytes as a method of cellular communication. These waves are dependent on both an intracellular, gap-junction pathway involving $[Ca^{++}]_i$ and $[IP3]_i$ and an extracellular pathway involving $[ATP]_o$ and $[Glu]_o$. Our goal was to incorporate the extracellular pathway into a preexisting mathematical model of the intracellular pathway. The model showed that during an $[IP3]_i$ stimulated calcium wave, $[ATP]_o$ controls the amplitude while $[Glu]_o$ controls the duration of the wave. Also, our model implicated purinergic, PLCB and PLCG activating receptors as potential drug targets to modify glutamate release by astrocytes which may effect neural plasticity.

Keywords: astrocyte; calcium oscillation; glutamate; ATP

1. Introduction

Astrocyte-astrocyte communication is dependent on calcium waves which are temporary elevations in intracellular calcium that travel between adjacent astrocytes [1]. Intracellular calcium can be increased in two ways. First, extracellular calcium can enter the cytosol though ion pores [4]. Second, the endoplasmic reticulum, which serves as an intracellular calcium store, can release calcium into the cytosol [9]. An intracellular calcium wave is capable of creating calcium influxes in adjacent cells [4].

Current research has demonstrated that calcium waves propagate by both the diffusion of calcium and inositol triphosphate (IP_3) through gap junctions as well as an extracellular pathway [4]. Two extracellular signals which have been identified are adenosine triphosphate (ATP) [5,11] and glutamate (Glu) [1]. The presence of these signals contributes to the generation of a calcium wave by creating calcium influxes into the cytosol. ATP stimulates purinergic plasma membrane ion pores which allow calcium to enter the cell from the extracellular fluid. Glutamate stimulates two different receptor types. One type promotes an extracellular calcium influx while the other promotes the endoplasmic reticulum to release calcium into the cytosol. Also, both ATP and glutamate are released during a calcium wave. ATP release is stimulated by the presence of IP_3 [12] while glutamate release is dependent on intracellular calcium [1]. Another important role of extracellular glutamate is the modification of neural synapses. The release of glutamate provides a possible link between glial cells and neural plasticity [10].

IP^3 activity is stimulated by phospholipase β (PLCβ) and phospholipase γ (PLCγ). PLCβ activity is stimulated by a membrane bound g-protein. PLCγ activity is stimulated by intracellular calcium. IP^3 activates a receptor on the endoplasmic reticulum which allows calcium to leak into the cytosol [6].

Mathematical models which mimic calcium waves have been created [2,6,11]. The most comprehensive of these models is presented by Hofer [6]. However, this model neglects calcium fluxes created by extracellular glutamate and ATP. We intend to add glutamate and ATP calcium fluxes to the existing calcium wave model as well as model extracellular glutamate and ATP to provide insights into the role of astrocyte calcium wave oscillations in neural plasticity.

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2. The Model

The base of our model will be the Hofer model [6]. A schematic of our model is shown in figure 1. We used a 3x3 array of cells due to computing restrictions. Since the model could produce calcium waves, the smaller array did not compromise the results. Also, second order partial differential terms were approximated to be: $d^2C/dX^2 = C(j-1, y,t) + C(j+1,y,t) - 2*C(j,y,t)$, where j represents a spatial position on the 3x3 grid. A similar approximation was used for the d^2C/dY^2 terms as well as all IP_3 diffusion terms. These terms were derived by creating a parabolic approximation of concentration as a function of space for a fixed point in time. All boundary conditions were assumed to be 0.

To model $[Glu]_o$ and $[ATP]_o$ the extracellular space within the 3x3 grid of cells was split into four regions (Fig. 2). Each region interacted with adjacent cells. For example, region 1 interacted with cells 1,2,3 and 4. Conversely, cell 2 interacted with regions 1 and 2. Also, it was assumed that each region was independent of other regions, so diffusion between regions was not taken into account. In general, the equations used to model $[Glu]_o$ and $[ATP]_o$ are:

$$d[GluN]/dt = Kglut * ([Ca^{++}1]_i + [Ca^{++}2]_i + [Ca^{++}3]_i + [Ca^{++}4]_i) /4 - GluN \qquad \text{Eq. 1}$$

where N = 1,2,3,4, Kglut is a rate constant for the flow of glutamate into the extracellular environment due to $[Ca^{++}]_i$ and $[Ca^{++}1\text{-}4]_i$ indicates the calcium concentrations within each of the four cells which the region interacts with.

$$d[ATPN]/dt = Katp * ([IP_3]1i + [IP_3]2i + [IP_3]3i + [IP_3]4i) / 4 - ATPN, \qquad \text{Eq. 2}$$

where N = 1,2,3,4, Katp is a rate constant for the flow of ATP into the extracellular environment due to $[IP_3]_i$ and $[IP_3]1\text{-}4$ indicates the IP_3 concentrations in each of the four cells which the region interacts with.

The mechanisms of calcium influx due to $[Glu]_o$ and $[ATP]_o$ are not completely understood [4]. For this reason, these calcium fluxes were modeled using simple rate constants which do not take into account the dynamics of the individual receptors. The following equations were used to model the calcium influxes due to $[Glu]_o$ and $[ATP]_o$.

$$VglutN = (K10 + K11) * GN \qquad \text{Eq. 3}$$
$$VatpN = K12 * AN \qquad \text{Eq. 4}$$

where N=1,2.3,4, K10 is the rate constant for the flow of calcium into the cytosol from the extracellular environment due to glutamate, K11 is the rate constant for the flow of calcium into the cytosol from the endoplasmic reticulum due to glutamate and K12 is the rate constant for the flow of calcium into the cytosol from the extracellular environment due to ATP.

These two calcium fluxes where added to the net calcium flux equation (Eq. 1 from [6]) for each cell.

The unknown rate constants were determined by fitting solutions from the model to experimentally observed values. The extracellular glutamate concentration caused by calcium was known to be between 1 – 100um [7] and the extracellular ATP concentration caused by IP_3 was known to be between 1 - 90 μm [8]. Kglut and Katp were both chosen to give peak values of $[Glu]_o$ and $[ATP]_o$ to approximate the experimentally determined levels. The calcium flux caused

by extracellular glutamate and ATP are both thought to be in the range of 0.1-1um [1,5]. K10, K11 and K12 were chosen to give calcium fluxes in that range. A summary of novel model parameters is presented in Table 1. All remaining parameter values can be found in the Hofer model paper [6].

Table 1. Novel Model Parameters

Parameter	Symbol	Value
Rate constant of glutamate release due to $[Ca^{++}]_i$	Kglut	2.0 sec^{-1}
Rate constant of ATP release due to $[IP3]_i$	Katp	1.0 sec^{-1}
Rate constant of calcium influx from ECF due to $[Glu]_o$	K10	0.5 sec^{-1}
Rate constant of calcium influx from endoplasmic reticulum due to $[Glu]_o$	K11	0.5 sec^{-1}
Rate constant of calcium influx from ECF due to $[ATP]_o$	K12	1.0 sec^{-1}

Model solutions were obtained using xppaut in a similar manner previously described [6] with the exception that an alternating direction implicit scheme was not used for the diffusion terms.

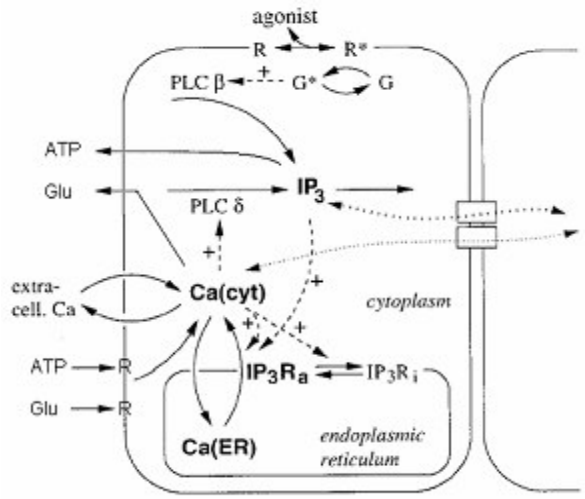


Fig 1.

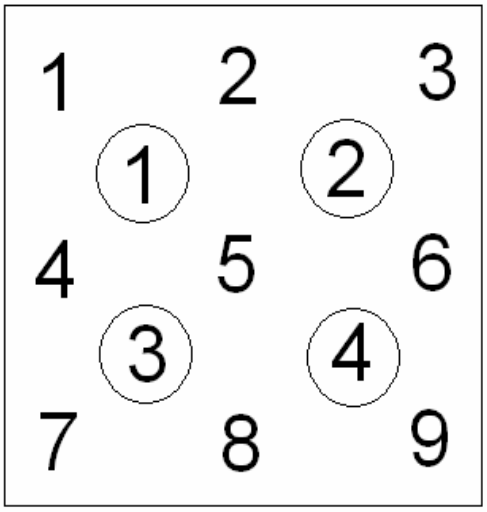


Fig 2.

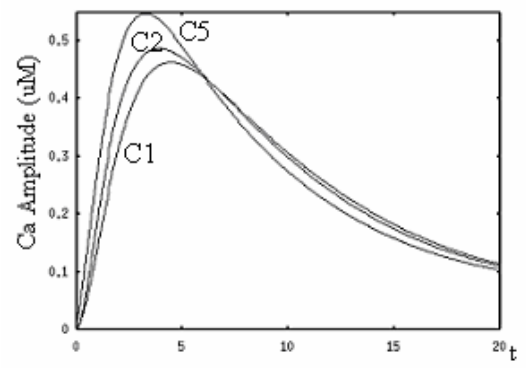
3. Simulation Results

Simulations were performed with two main questions in mind. First, how are the calcium wave dynamics effected by $[Glu]_o$ and $[ATP]_o$ calcium fluxes? Second, what are potential drug targets which can alter the amount of glutamate released into the extracellular environment hence altering neural synapse activity?

Unless otherwise noted, all simulations were performed with an initial stimulus of $[IP3]_i$ for cell five equaling 10 μm to mimic the response of agonist receptor stimulation (Refer to Fig 1). Our simulations show that calcium waves can be generated by a diffusion model which takes into account $[Glu]_o$ and $[ATP]_o$ and that these waves can propagate with a velocity in marginal

agreement with experimentally observed values (40 μm/sec vs. 20 μm/sec) with the distance between cell centers estimated to be 40 μm [6]. (Fig. 3)

Simulations show that the peak $[Glu]_o$ and $[ATP]_o$ are in the appropriate ranges [7] (Fig. 4). The calcium fluxes caused by $[Glu]_o$ and $[ATP]_o$ are also in the appropriate ranges [1,5] (Fig. 5). The flux caused by $[Glu]_o$ is delayed compared to that of $[ATP]_o$. This is due to the nature of the model stimulus. The $[ATP]_o$ flux is dependent on $[IP_3]_i$. Since the initial stimulation is a large increase of IP_3 in cell 5, ATP is immediately exported into the extracellular environment whereas glutamate release must wait until $[Ca^{++}]_i$ increases. This effect also results in the calcium flux associated with glutamate to show similar lag when compared to that of ATP (Fig 5). The effect of this lag is that the initial rise in calcium elevation results from $[ATP]_o$ while later portion of the elevation is sustained by $[Glu]_o$.



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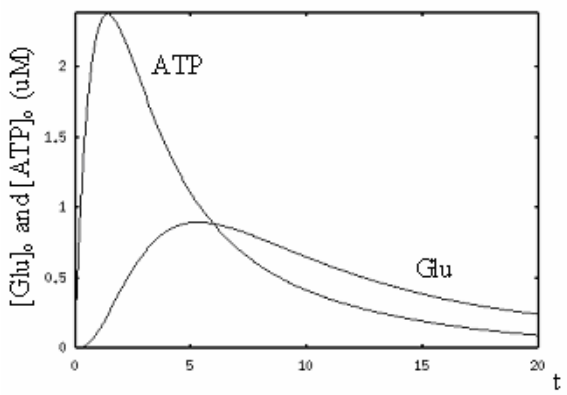

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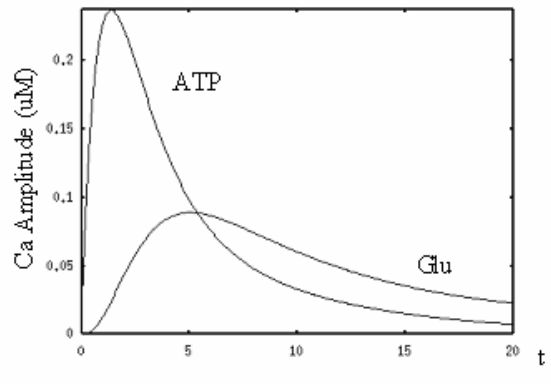

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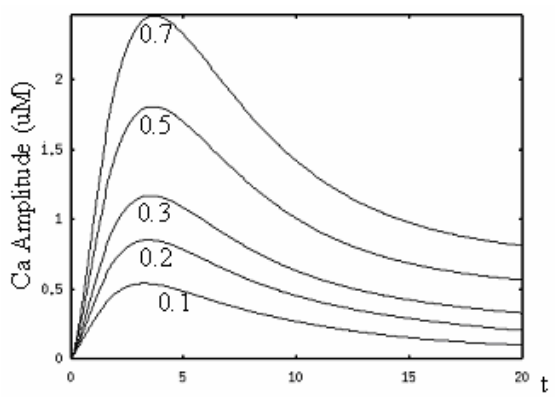


Fig 6.

Observations have been made which indicate that purinergic receptor sensitivities are not uniform throughout a population of astrocytes [3,5]. To simulate the effect of different purinergic receptor sensitivities we evaluated the effects of ranging K12 from 0.1–0.7 sec^{-1} (Fig. 6). Simulations show that increasing K12 can raise the amplitude of $[Ca^{++}]_i$. The increased purinergic receptor sensitivity also translated into an increased glutamate release into the extracellular fluid (results not shown).

Increasing the stimulus intensity can also raise the amplitude of $[Ca^{++}]_i$. Consequently, it is expected that a larger stimulus increases the amount of glutamate released. A simulation was

performed using three stimulus intensities 5, 7 and 10 μm which confirmed this expectation (Fig. 7). Increasing the stimulus intensity/response may be a strategy utilized by astrocytes to regulate neural synapses [10].

As mentioned previously, the wave propagation velocity was slightly faster in our model (40 μm/sec) compared to experimentally observed values (20 μm) as well as values from the base model (10um/sec) [6]. While we can not explain the difference between experimentally observed values, the difference between the base model exists mostly as a result of including ATP calcium fluxes. As shown in Fig. 8, removing the ATP calcium flux slows wave propagation down to approximately 25 μm/sec. It should also be noted that removing the glutamate calcium flux as well causes $[Ca^{++}]_i$ to decrease more rapidly (Fig. 9) which supports our observation that the glutamate calcium flux increases the duration of the calcium wave. Removing the calcium flux caused by $[ATP]_o$ (Fig. 10) or $[ATP]_o$ and $[Glu]_o$ both decrease the amount of glutamate released during a calcium wave (result not shown).

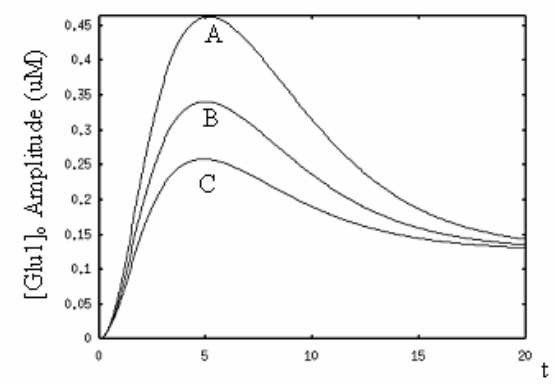
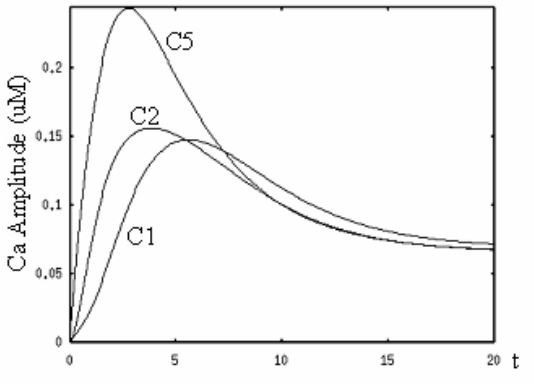


Fig 7.



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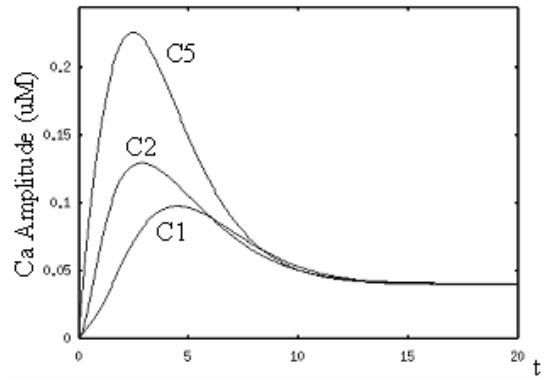

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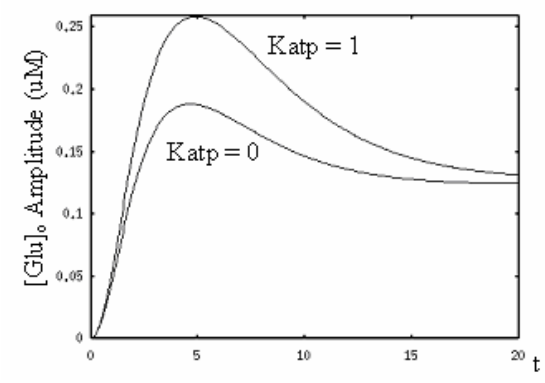

Fig 10.

As an aside, it should be noted that two experimental observations were successfully mimicked by our model which adds further validation to the results produced thus far. Experiments investigating the effects of ATP on calcium waves have demonstrated that in the absence of diffusion through gap junctions, an extracellular ATP stimulus of 10 μm is capable of generating a calcium wave [5]. This result was also produced by our model in figure 11. In our simulation, the initial stimulus was an extracellular ATP concentration in region one and two of 10uM. Figure 11 shows the progression of the calcium wave from cell one to cell seven. The

implication of this result is that gap-junction diffusion is not necessary for calcium waves to exist so long as a sufficient ATP stimulus is present.

Experiments investigating the effects of glutamate on calcium waves have also demonstrated that an extracellular glutamate stimulus of 100 μm is sufficient to create a calcium wave [1]. This result can be produced by our model and is shown in figure 12. In our simulation, the initial stimulus was an extracellular glutamate concentration in region one and two of 100 μm. Figure 12 shows the progression of the calcium wave from cell one to cell seven. This result shows that at high concentrations, glutamate has the ability to stimulate calcium waves.

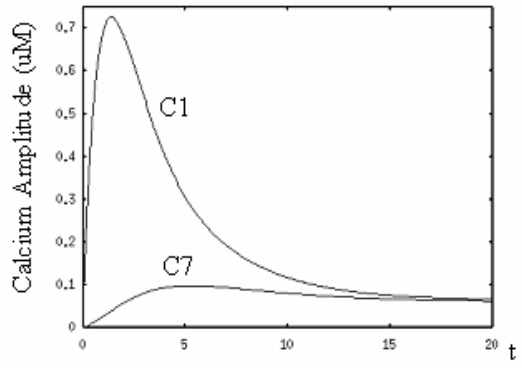

Fig 11.

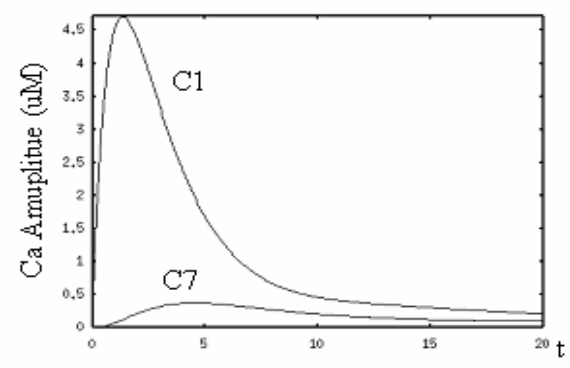

Fig 12.

4. Conclusions

Our simulations have demonstrated that the pre-existing model for calcium wave oscillations can be modified to include calcium currents caused by $[Glu]_o$ and $[ATP]_o$.

During an experiment where calcium wave oscillations are being stimulated by increasing $[IP3]_i$, altering purinergic receptor affinity/sensitivity can exert the greatest effect on the initial rise in amplitude while altering glutamate receptor affinity/sensitivity can exert the greatest effect on the duration of the resulting calcium wave. These insights can be evaluated in vivo using calcium imaging techniques in conjunction with different combinations of glutamate / purinergic receptor antagonists.

Also, our simulations have identified several drug targets which have the potential to modify how astrocytes modulate neural synapses through the release of glutamate. In general, any stimulus which alters the amplitude of the calcium wave can alter the amount of glutamate released into the extracellular fluid. Possible drug targets include purinergic receptors, PLCβ activating receptors, PLCγ activating receptors and to a lesser extent, glutamate receptors. Inhibition or excitation of any of these receptors will alter the release of glutamate during a calcium wave which will modify the dynamics of adjacent neural synapses.

References

[1] A.H. Cornell-Bell, S.M. Finkbeiner, M.S. Cooper, S.J. Smith, Glutamate induces calcium waves in cultured astrocytes: Long-range glial signaling. Science. 247 (1990) 470-3.

[2] G.W. De Young, J. Keizer, A single-pool inositol 1,4,5-trisphosphate-receptor-based model

for agonist-stimulated oscillations in Ca2+ concentration. Proc Natl Acad Sci U S A. 89(20) (1992) 9895-9.

[3] S. Fedoroff, Prenatal ontogenesis of astrocytes, in: S, Fedoroff, A Vernadakis (Eds.), Astrocytes: Development, morphology and Regional Specialization of Astrocytes, Vol. 1, Academic, Orlando (1986) 229-237.

[4] R.D. Fields, B. Stevens-Graham, New insights into neuron-glia communication. Science. 298(5593) (2002) 556-62. Review.

[5] PB Guthrie, J Knappenberger, M Segal, M.V. Bennett, A.C. Charles, S.B. Kater, ATP released from astrocytes mediates glial calcium waves. J Neurosci. 19(2) (1999) 520-8.

[6] T Hofer, L. Venance , C. Giaume, Control and plasticity of intercellular calcium waves in astrocytes: a modeling approach. J Neurosci. 22(12) (2002) 4850-9.

[7] B. Innocenti, V. Parpura, P.G. Haydon, Imagining extracellular waves of glutamate during calcium signaling in cultured astrocytes. J Neurosci. 50(5) (2000) 1800-8.

[8] E.A. Newman, Propagation of intercellular calcium waves in retinal astrocytes and Muller cells. J Neurosci. 21(7) (2001) 2215-23.

[9] H.R. Parri, V. Crunelli, The Role of Ca++ in the generation of spontaneous astrocytic Ca++ oscillations. Neurosci. 120 (2003) 979-92.

[10] V. Sourdet, M. Russier, G. Daoudal, N.Ankri, D. Debanne, Long-term enhancement of neuronal excitability and temporal fidelity mediated by metabotropic glutamate receptor subtype 5. J Neurosci. 23(32) (2003) 10238-48.

[11] Y. Timofeeva, S. Coombes, Wave bifurcation and propagation failure in a model of Ca(2+) release. J Math Biol. 47(3) (2003) 249-69.

[12] Z. Wang, P.G. Haydon, E.S. Yeung, Direct observation of calcium-independent intercellular ATP signaling in astrocytes. Anal Chem. 72(9) (2000) 2001-7.

Figure Captions

Fig 1. Schematic of the complete simulated model [6]

Fig 2. Spatial arrangement of cells (Numbers) and Extracellular regions (Circled Numbers)

Fig 3. $[Ca^{++}]_i$ as a function of time for cells 1, 2, and 5.

Fig 4. $[Glu]_o$ and $[ATP]_o$ as a function of time for extracellular region one.

Fig 5. Calcium flux due to $[Glu]_o$ and $[ATP]_o$ as a function of time in cell 5.

Fig 6. $[Ca^{++}]_i$ for K12 =0.1, 0.2, 0.3, 0.5 and 0.7 sec^{-1} as a function of time in cell 5.

Fig 7. $[Glu]_o$ for initial conditions A: IP3 = 10uM, B: IP3 = 7 μm and C: IP3 = 5uM

Fig 8. $[Ca^{++}]_i$ as a function of time for cells 1, 2, and 5 when Katp = 0 sec^{-1}

Fig 9. $[Ca^{++}]_i$ as a function of time for cells 1, 2, and 5 when Katp and Kglu = 0 sec^{-1}.

Fig 10. $[Glu]_o$ for Katp = 1 sec^{-1} and Katp = 0 sec^{-1}

Fig 11. $[Ca^{++}]_i$ as a function of time for cells 1 and 7. Pip3 and Pca = 0, [ATP1,2]o = 10uM

Fig 12. $[Ca^{++}]_i$ as a function of time for cells 1 and 7. Pip3 and Pca = 0, [Glu1,2]o = 100uM

Bio Sketch

I am a graduate student at Arizona State University in the bioengineering department. I received a bachelor of science in biomedical sciences from the University of Guelph in Ontario, Canada. I am currently involved in a study that will test the viability of heat shock protein 20 as a means of reducing gliosis caused electrode arrays implanted into rat motor cortex.