SAN DIEGO STATE UNIVERSITY

Study and Modeling of Calcium Diffusion in Astrocytes

by

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Contents

1. Introduction:

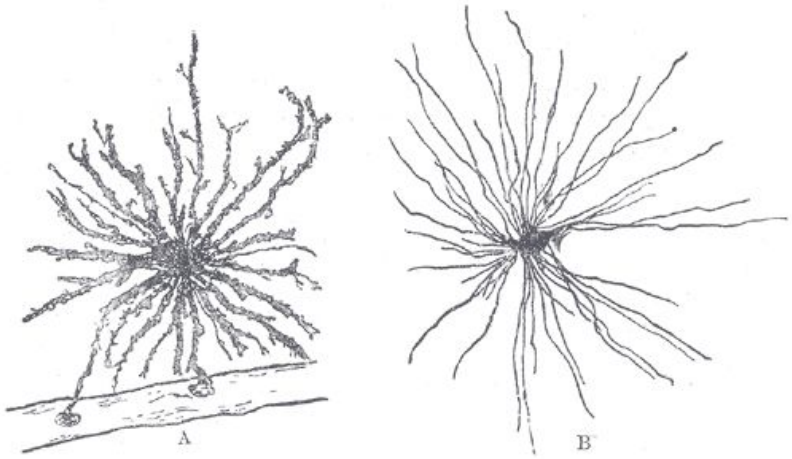
Historically, glia cells have been regarded as support cells in nervous system. They were considered to be gap fillers, whose sole purpose was just to hold neurons together. But work over the last decade suggest that they may play significant role in nervous system.

Now, the function of glia has been reconsidered, and they are now thought to play a number of active roles in the brain, including the secretion or absorption of neurotransmitters and maintenance of the blood–brain barrier, neural modulation and neural function.

Lastly, they make more than 50% of our brain volume; we believe anything which accounts for more than 50% can not be neglected. With this motivation, we have studied glia cells, especially astrocytes, a type of glia cell among many others. We have focused on signal transmission in astrocytes and various mechanisms that might affect these signals. On the basis of our understanding and intuition, we built a 1D model to describe diffusion of calcium in an single cell of astrocyte, which also includes two mechanism; namely, calcium pumps (sink, negative feedback) and input ion channels (source, positive feedback). Finally, we solved our model using Mimetic Discretization Methods and have got satisfactory results with realistic values.

2. Glia cells

Glia cells or simply glia was discovered in 1856 by the pathologist Rudolf Virchow in his search for a “connective tissue” in the brain. Glial cells, also called as Neuroglia are non-neuronal cells that plays a very crucial role in central and peripheral nervous system. The term “Glia” comes from Greek name implies “glue” of the nervous system. They initially got this name, because they seems to fill spaces between neurons to hold them together.



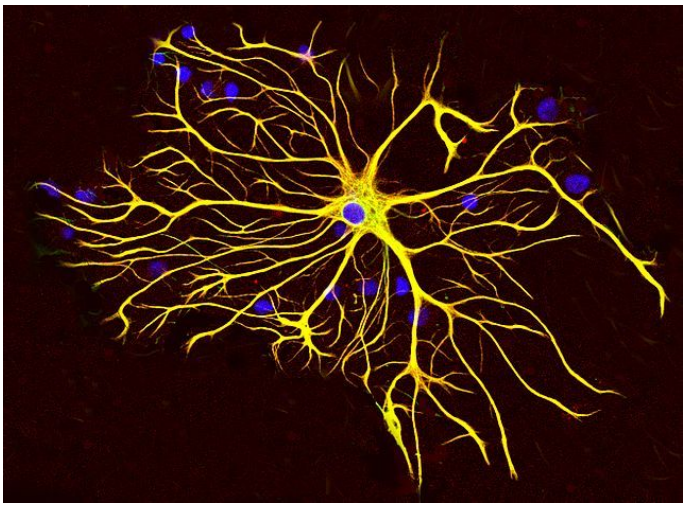
Glia cell of the brain shown by Golgi's method.

There are different types of glial cells in the central nervous system, glial cells include oligodendrocytes, astrocytes, ependymal cells and microglia, and in the peripheral nervous system glial cells include schwann cells and satellite cells.

For over a century, it was believed that the glia did not play any role in neurotransmission. However, with improved techniques in the 21st century, researchers and scientist has found out that glia cells do have important role to play in assisting/ supporting the neurons to form synaptic connections between each other or possibly between neurons and glia cells themselves.

3. Astrocytes:

Out of different types of glial cells, the most abundant type of cells in central nervous system are Astrocytes. They constitute upto 40% of all glia cells. Astrocytes are star shaped glia cells and are found in proximity to neurons.



An Astrocyte cell from Rat brain. Source: EnCor Biotechnology Inc.

Astrocytes are non-electrically excitable, unlike neuron's, they display a form of excitation that is based on variation of ca^{2+} concentration in cytosol. They express most of their properties with change in ca^{2+} concentration. And, this localized change (increase or decrease) in concentration of ca^{2+} followed by succession of similar events, leads to a wave like pattern, which is called calcium wave.

In last decade, studies and research has shown that astrocytes propagate intercellular calcium waves over long distance in response to stimulation, just like neurons do. Also, on similar lines, astrocytes release a chemical called "gliotransmitter" which can stimulate many processes in nervous system.

4. Ca Signaling/ Wave:

Ca wave is defined as a localized increase in cytosolic ca^{2+} that is followed by a succession of similar events in a wave like fashion. These ca waves can be restricted to one cell (intracellular) or transmitted to neighboring cells (intercellular).

Calcium [Ca^{2+}] is an important second messenger, found in almost all cell types. The dynamics of Ca^{2+} is very important in cellular physiology because Ca^{2+} regulates their activity and interactions.

As we know, astrocytes are excitable cells with ca^{2+} fluctuations being the waves by which they respond, integrate and convey signals. These waves are also called as Ca signals.

Triggering and Transmission of Ca waves:

These calcium waves are triggered whenever there is some sort of stimulation. The recent results have shown that the neurotransmitter can trigger actively propagating Ca^{2+} waves in the cytoplasm of astrocytes.

The law governing Ca^{2+} signal transmission is provided by diffusion equation. Therefore, the extent to which these intercellular Ca^{2+} waves can travel are governed by diffusion parameters of Ca^{2+} ions.

The transmission of Ca waves takes place through two pathways. First is direct communication between cytosol of two adjoining cells through “gap junction” channels. Gap junctions are a specialized intercellular connection between two cells. They directly connect the cytoplasm of two cells, which allows various molecules, ions and electrical impulses to directly pass through a regulated gate between cells.

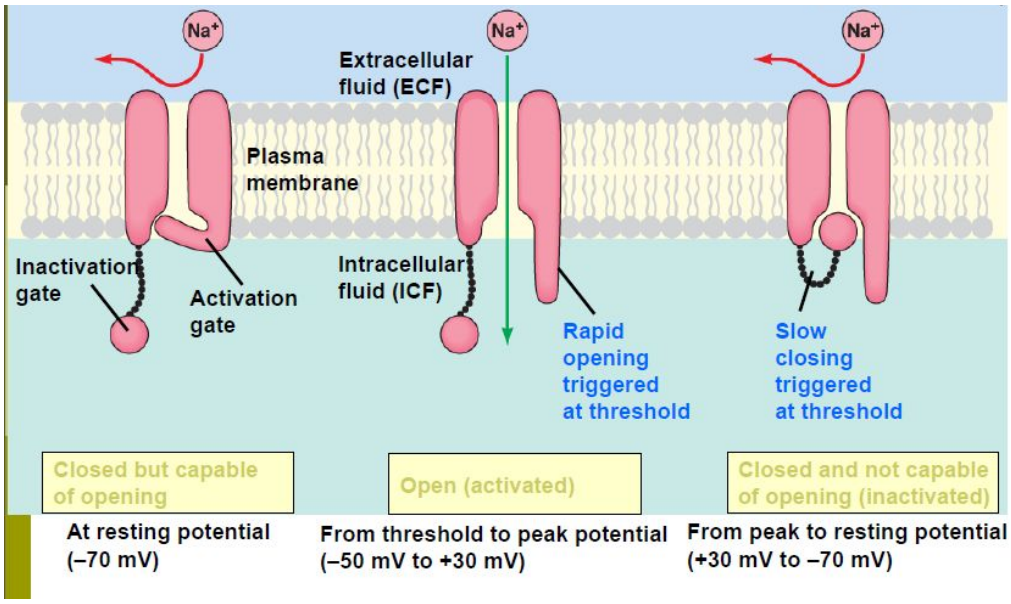
Other type of pathway is indirect communication, where there is no physical connection between two cells. This type of communication depends upon the release of gliotransmitters that activates membrane receptors on neighbor cells. Once membrane receptors are activated, these cells respond with increase in intercellular ca^{2+} elevations. Gliotransmitter are chemicals released from glia cells that facilitates neuronal communication between neurons and other glia cells, and this communication is facilitated through calcium waves/ signals.

These waves propagating between different cells are called Intracellular calcium waves (ICWs). ICWs are spatially and temporally complex events involving the recruitment of elementary Ca^{2+} release sites, which then propagate through cell by amplification mechanism. This amplification mechanism involves four different types of components, two of which depend on positive feedback and other two depends on negative feedback.

5. Voltage Gated Ions channels:

In neurons (and other cells, like glial cells), possesses a cell membrane that is mostly lipid, and, ions like Na and K cannot cross the lipid membrane. Transportation and transmission of ions are carried out by channels, which are tiny openings in the membrane formed by protein pores.

These channels are often gated i.e. open and closed depending on the potential difference between inside and outside of cell. Voltage gated channels are those in which membrane potential of cell determines whether they are opened or closed.



These voltage gated channels exhibit three states:

a) Resting State:
b) Active State:
c) Inactive State:

6. Mathematical Formulation:

Diffusion of calcium in astrocytes follow fick's law of diffusion, so we have our model based on diffusion equation, which is given below, where u is concentration of calcium ions and α is diffusion coefficient.

$$u_t = \alpha\, u_{xx}$$

$$\frac{\partial u}{\partial t} = \alpha \frac{\partial u}{\partial x}\frac{\partial u}{\partial x}$$

$$\frac{\partial u}{\partial t} - \alpha \frac{\partial u}{\partial x}\frac{\partial u}{\partial x} = 0$$

In our model, we have two components which affect the local calcium concentration inside the cell. One of which is called ion channel, which behave as a positive feedback for the cell and other is called calcium pump, which behaves as a negative feedback. These are also called source and sink. So, after incorporating these two components, our model can be written as:

$$u_t = \alpha\, u_{xx} + S1 + S2$$

where, S1 represents source term (ion channels) and S2 represents sink term (calcium pumps).

1. Ca Pumps – They pump out calcium from cytoplasm at a rate given by D_p. Calcium pumps are modeled as:

$$J_p = -D_p * [Ca^{2+}]$$

where, D_p is diffusion coefficient that quantifies the rate at which calcium is pumped out of the cytoplasm and J_p is flux from calcium pump.

2. Input Ion channels – Ion channels are modeled as:

$$J_i = IN * D_i * ([Ca^{2+}]_{ext} - [Ca^{2+}])$$

where, D_i is diffusion coefficient scaling the ion channels state, $[Ca^{2+}]_{ext}$ is a (constant) external calcium concentration, and $([Ca^{2+}]_{ext} - [Ca^{2+}])$ is the driving concentration difference for ion transport through the channels.

At each time step, an increment (or possibly decrement) to local calcium concentration will be computed according to the sum of the fluxes associated with 3 channel types:

$$\nabla[Ca^{2+}] = \nabla t\,(J_p + J_i)$$

This $\nabla[Ca^{2+}]$ is integrated with lateral transport governed by Fick's equation, and the evolution of the state computed over time.

Parameters: We have used following parameter values,
Diffusion coefficient (α) = 5.3 x 10^{-6} cm^2/s
Length of cell (L) = 80 μm
Ext Ca^{2+} concentration = 1.2 mmol
Initial Ca^{2+} concentration (IC) = 0.6 mmol
Diffusion constant for Ca pumps (D_p) = 5.3 x 10^{-6} cm^2/s
Diffusion constant for ion channels (D_i) = 5.3 x 10^{-6} cm^2/s

7. Numerical Solution:

We have solved our model numerically using Mimetic Discretization Methods. After discretizing our domain, the model can be written in matrix-vector format given by following equation,

$$U = L * U + S1 + S2$$

where, L is our laplacian matrix and S1 and S2 are vectors that represent flux from source and sink components in our model.

We construct laplacian (L) matrix using MOLE (Mimetic Operators Library Enhanced) library. It is done by using 1D Mimetic laplacian operator. This operator can be called using a command called "**lap(k, m, dx)**". This returns a (m+2) by (m+1) one dimensional mimetic laplacian operator.

L = lap (k, m, dx);

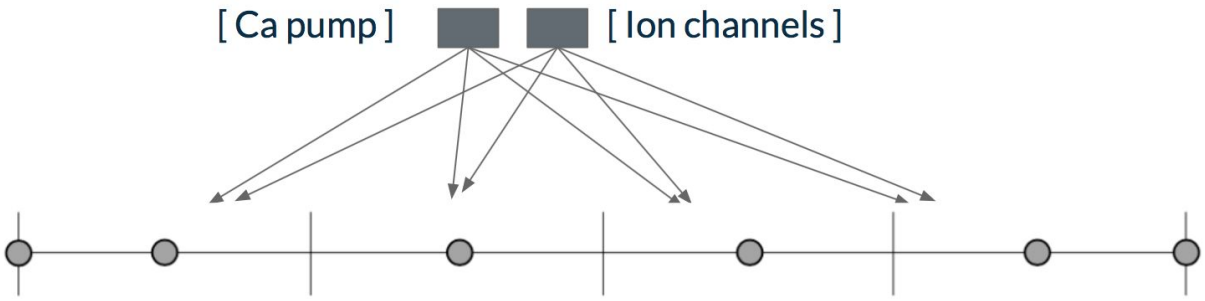
where,

k is operator's order of accuracy
m is the number of cells required to attain the desired accuracy
dx is the step size along x-axis

Generating a array of 1D staggered grid points and storing them in "grid" is done in following manner, where west and east are two ends of our grid and dx is the step size on x-axis.

```
grid = [ west  west+dx/2 : dx : east-dx/2  east ];
```

We have Ca pumps and ion channels acting on every grid point and it can visualize as shown below. In future, we would like to come up with a model in which these channels will be activated or inactivated at some random grid points with some realistic logic attached to it.



1D staggered grid with Ca pumps and ion channels acting on every grid point

Flux from Ion channels and Ca pumps are represented by S1 and S2 respectively. S1 and S2 are vectors which are calculated using equation mentioned in our model description in previous section.

Boundary Conditions:

In our model, we have Dirichlet boundary conditions. And, these can be imposed very easily using MOLE library. Both the sides (west and east) have zero imposed on them using following lines of code:

```
U (1) = 0;
U (end) = 0;
```

Initial Conditions:

We have assumed initial condition to be at 0.6 mmol and that is imposed in following manner,

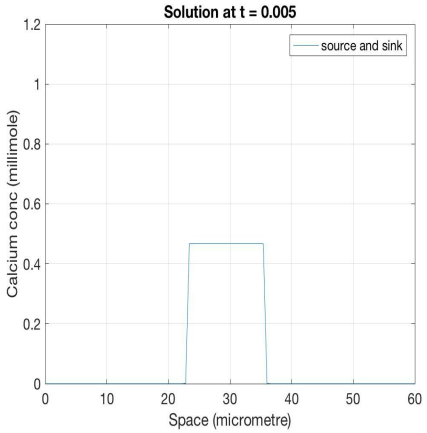
```
U = zeros (m+2, 1);
U ( k, 1 ) = 0.6;
```

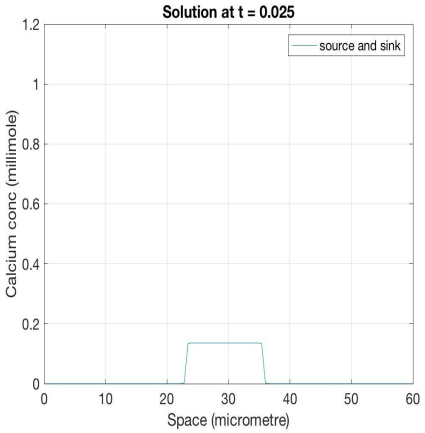
where, k goes from 1 to m+2. We can also impose IC to limited grid points.

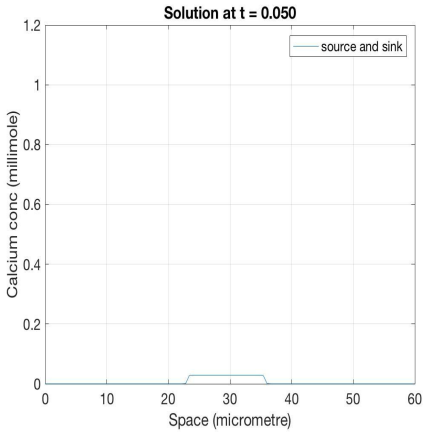
8. Results and Discussion

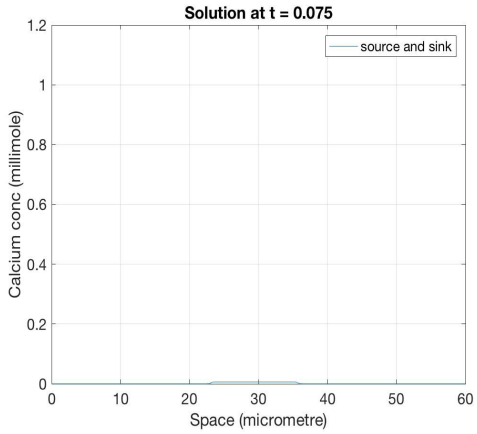
Plot shown below describes the diffusion behavior of our model. The x-axis represents the space in micrometers. It goes from 0 to 60 micrometer, which is the typical length of astrocyte cell. And, y-axis represents calcium concentration in millimoles.

It can be noted that diffusion of Calcium is taking place at a scale of few millisecond, that's exactly what happens in astrocytes. This shows that our model closely resembles the diffusion in astrocytes.



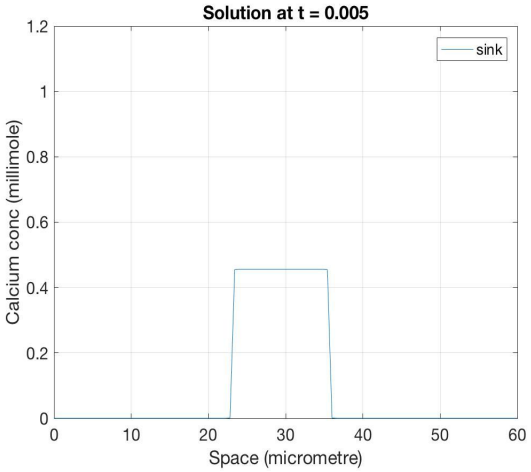


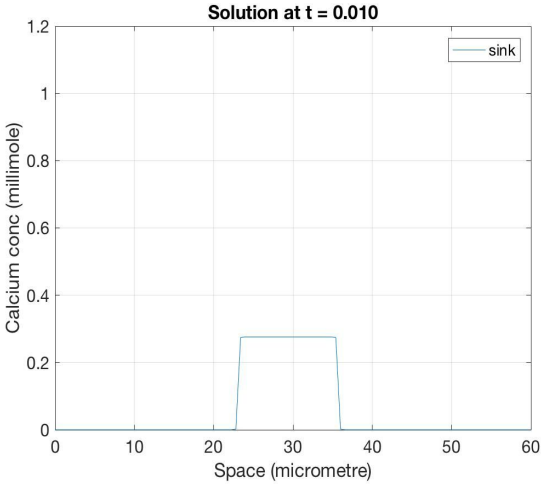


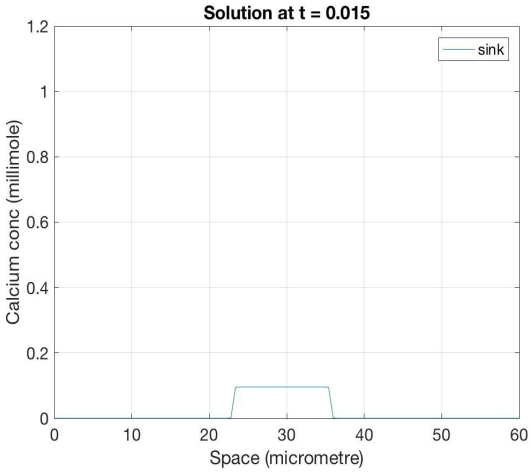


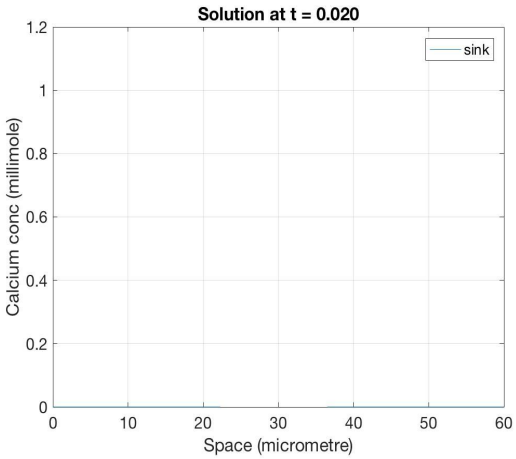
Above shown plots are of model with source and sink component in it. Addition of source and sink term, kind of stabilize our model in way that diffusion happen in few milliseconds.

Below shown plots are of model without source (ion channels) component, and it is clear that diffusion is taking place at much faster rate than previous experiment. This is because we have calcium pumps in our model, which is pumping out calcium and thus cell is left with less calcium for diffusion.









9. Future Work

With our model and Mimetic Discretization Methods, we have achieved satisfactory results with realistic parameters values. Taking our project forward, we would like to improve/ refine our model, in particular make it more subtle and accurate so that it can explain the dynamics of calcium signaling/ waves in astrocytes.

Astrocytes and other related cells (neurons) exhibit many complex molecular and cellular mechanisms which enable them to interact with each other and process information. We also plan to understand, model and then finally incorporate these mechanisms in our model.

In intercellular communication pathways, when calcium waves propagate from one cell to neighbouring cells, it is very likely that wave will die after some time as local concentration goes down. We need to have a regenerative mechanism in place which can keep this waves going for longer distance by maintaining local calcium concentration. Regenerative mechanisms will be an very important part of our model.

Astrocytes function in large networks (even much bigger than neurons network), working on similar lines, we plan to simulate network of such cells on a scale of 1000s cells.

All above mentioned goals, will make our model computationally heavy and time consuming. In order to tackle time constraints and computational problem we also plan parallelize our model using MPI, OpenMP or even GPUs.