Simulating calcium buffering and handling at nerve terminals with CellBlender/MCell

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1 Introduction

This paper aims to investigate the calcium buffering aspect of synaptic transmission through a computational perspective. Utilising CellBlender with the MCell backend, we will create the model *in silico* and simulate the activation and release of synaptic vesicles. As an example of the utility of these technologies, we present a bio-realistic model of a neuron synapse and demonstrate these results in animated form.

Blender is a 3D graphics modelling software which is free and traditionally used for applications such as digital media, games, visual art. MCell is a cell simulation command line utility which is capable of providing Monte Carlo simulations of a cell at the molecular level. CellBlender is a Blender addon which allows the user to access features within MCell through a Blender interface. Together with the general purpose language Python, this paper utilises these technologies to produce an accessible guide for simulating the calcium buffering and handling at nerve terminals.

The advantages of such an approach are many-fold. Firstly, the user does not need to be familiar with programming through a command line interface to do cellular level modelling, enabling a wider audience to access the power of a simulation environment. Secondly, the simulation results are presented in an animation, which is an information-dense medium and allows the user to quickly interpret the model results. Finally, once the simulation has been set up, it is an easy task for the user to adapt the model parameters and geometric configurations to answer their scientific questions.

The steps used to create the model are available at SITE, should the reader attempt to replicate the results.

2 Background Information

2.i Synaptic Transmission - An Overview

- An action potential travels along the membrane of the presynaptic cell until it reaches the synapse.
- This depolarisation causes voltage gated calcium channels to open.

- Calcium ions flow from the extracellular space into the presynaptic terminal due to the concentration gradient.
- The high calcium concentration activates proteins on neurotransmitter vesicles, causing them to fuse with the synaptic cell membrane and emptying their contents (about 4000 molecules) into the synaptic cleft.
- The neurotransmitter molecules (AMPA, NMDA) bind to their respective receptors on the postsynaptic terminal. At least two neurotransmitter molecules must bind to the receptor in order for it to activate. Most neurotransmitters are lost to the extracellular environment where they are retrieved by glial cells.
- Activation of a receptor will cause something to occur in the cell. For example, ligand-gated ion channels such as AMPA and NMDA receptors open ion channels which permit sodium, potassium and calcium ions to enter the postsynaptic terminal.
- The influx of ions will cause a change in voltage at the postsynaptic terminal, which produces a postsynaptic potential.

In our model we describe only the chemical aspects. The electrical aspects (i.e. the action potentials) are not described.

2.ii Synaptic Structure

The synapse consists of the presynaptic terminal (the bouton) and the postsynaptic terminal (the spin).

Axons of neighbouring neurons are interspersed with small swellings along their length, and at the terminal end of the axon. These swellings are termed boutons or axonal varicosities, and at these locations synaptic connections may form. The bouton contains the synaptic vesicles and associated apparatus for vesicle release.

Dendrites of neurons are covered in spines, which are small protrusions consisting of a bulbous head and a thin body, as per Arellano et al. (2007). Their primary role is to form excitatory synaptic connections, and as such the spine head region is covered in neurotransmitter receptors.

2.iii Vesicles

At each synapse, there are vesicles filled with neurotransmitters. The majority of vesicles are undocked (not at the presynaptic membrane) and docking is a process which takes several minutes.

Each vesicle is filled with about 4700 neutrotransmitter molecules, as per Bruns and Jahn (1995).

Docked vesicles can fuse with the snyaptic membrane. SNARE (soluble N-ethylaleimide-sensitive factor attachment receptor) proteins hold the vesicle in place. The SNARE complex consists of the subunits synaptobrevin, syntaxin and SNAP-25. The SNARE complex acts to 'unzip' the vesicle when an appropriate protein is activated. For our synapse, this protein is synaptotagmin-1, which is receptive to calcium ions.

Each synaptotagmin-1 unit has four smaller subunits. Each subunit may be activated by a single calcium ion. The consensus on the number of calcium ions required to activate the synaptotagmin-1 unit is 2, according to Dittrich et al. (2013).

There is no widely accepted consensus on how many synaptotagmin-1 proteins are required to activate the SNARE complex and cause vesicle fusion. For example, it is cited in van den Bogaart et al. (2010) that a single SNARE complex is sufficient for vesicle release. However, Dittrich et al. (2013) state that at least three SNARE complexes are required for vesicle release.

Based on the existing literature, we suggest the following compromise. At least two calcium ions must bind to the synaptotagmin protein in order for it to engage with and activate the SNARE complex. A vesicle will require three such activated SNARE complexes in order for vesicle release (or fusion) to occur.

2.iv Receptors

We are interested in investigating the glutamate neurotransmitter, and the corresponding receptors are the AMPA and NDMA receptors.

The AMPA receptor is a heterotetramer, and is composed of four types subunits - GluR1, GluR2, GluR3 and GluR4. It is a fast-opening receptor, with a response time of CITATION.

3 The Modelling Approach

The specific details on how to use CellBlender to create a model achieving the goals outlined above are contained in a separate document "A Guide on Modelling Synapses with CellBlender and MCell", which the reader should refer to for all technical details.

Here, we will discuss the scientific details of the modelling approach.

3.i Summary of the Modelling Approach

The modelling occurs in two phases - presynaptic, and postsynaptic.

In the presynaptic phase, we simulate calcium ions moving through the presynaptic bouton and then binding to the SNARE complexes (in the model, we treat the SNARE-synaptotagmin interaction as a single unit). After each SNARE complex is activated, the model produces a tag molecule indicating the event has occurred. The tag molecule has no model properties otherwise.

A script (inspired by the approach taken in Ma (2014)) was written to scan over the model files and detect the times at which the tag molecules are produced. After a certain number of tag molecules accumulate on a vesicle, it is then declared to be activated, and this time is reported in the script. The user can then manually enter these times into the second phase of the simulation.

In the postsynaptic phase of the simulation, we simulate the vesicle fusion using the vesicle fusion times produced by the script analysis. The neurotransmitter molecules in the vesicle are created in the model at the times that the vesicles are released, and they can diffuse through the synaptic cleft to the receptors on the spine head.

Finally, these two phases are combined to provide a single contiguous simulation of calcium handling at the synaptic terminal.

The instructions are supplied as a PDF document, as well as an IPython Notebook.

3.ii Scale, Size and Time

In order for the simulation to be realistic, the dimensions and units within Blender must match those of the MCell simulation. It is possible to create a shape of any size in Blender, but this size may not be appropriate for the simulation scale.

In MCell, the units of space are µm, the unit of time is s, diffusion constants are $\mathrm{cm}^2\,\mathrm{s}^{-1}$. Unimolecular reactions have rate units s^{-1}, volume-volume or volume-surface bimolecular reactions have units $\mathrm{mol}^{-1}\,\mathrm{l}\,\mathrm{s}^{-1}$ and surface-surface bimolecular reactions have units $\mu\mathrm{m}^2\,\mathrm{mol}^{-1}\,\mathrm{l}\,\mathrm{s}$. One Blender unit is equivalent to 1 µm in MCell.

It is also important to note that CellBlender does discrete simulation - the state of the modelling environment is calculated at a certain time. A short time interval is allowed to pass, and CellBlender again recalculates the state of the modelling environment. The end product is a series of frames which can be played sequentially as an animation.

3.iii Translating the Biology into Blender

All models are simplifications of reality, and this model is no different. In this section we outline the simplifications made for the purposes of modelling.

We simulate only a single synaptic connection. This consists of a single presynaptic bouton, and a single postsynaptic receptor. These are created *in silico* using Blender geometry objects, and are static objects within the simulation. The surfaces of these objects may be defined to prevent molecules passing through.

Vesicles are simulated as spherical Blender objects situated within the presynaptic bouton. These are also static objects within the simulation. Vesicle fusion is defined in a stylised manner as follows. On the vesicle surface we define several SNARE molecules. Each SNARE molecule needs to accept 2 calcium ions to become 'activated'. In order for vesicle fusion to occur, at least 3 molecules must be activated. Note that the actual process of vesicle fusion is not simulated - this functionality is not directly implemented in CellBlender, and an external script was written to simulate this.

Molecules in CellBlender are point objects with a defined interaction radius (left at defaults). No steric interactions were simulated. Molecules react in user-defined reactions, and do not have any intrinsic chemical, biological or steric properties.

4 Making the Model Realistic

In this section we have sought to specify the quantities and parameters used in our model to the extent that these data exist. It is important that our model mimic the reality to a reasonable amount to ensure the integrity and usefulness of the model.

4.i Model Components

The model as implemented in the accompanying guide consists of the following components:

1. Presynaptic bouton

2. Spinehead
3. Voltage-gated calcium channels (2)
4. Vesicles (2)
5. Glial cells

It consists of the following molecules/proteins:

1. VGCC - the calcium channels responsible for permitting flow of calcium into bouton. Has open and closed states.
2. Ca - calcium ion
3. CaBS - a SNARE complex which has a single calcium ion bound
4. TAG - a SNARE complex which has two calcium ions bound
5. NT - a neurotransmitter molecule (represents glutamate)
6. LGIC - a neurotransmitter receptor residing on spine head (receptive to NT). Has open and closed states.

4.ii Model Equations

The model as implemented in the accompanying guide consists of the following reactions:

Equation	Description
VGCC_C $\rightarrow$ VGCC_O	Calcium channel opening
VGCC_O $\rightarrow$ VGCC_C	Calcium channel closing
VGCC_O $\rightarrow$ VGCC_O + Ca	Calcium influx into bouton
Ca + CaBS $\rightarrow$ CaBS_Ca	First calcium binding to synaptotagmin/SNARE complex
CaBS_Ca + Ca $\rightarrow$ TAG	Second calcium binding to synaptotagmin/SNARE complex
NT + LGIC_C $\rightarrow$ LGIC_O	Neurotransmitter binding to receptor

4.iii Reaction Specifications

We can summarise the model mechanism with chemical reactions. Some of these reactions have been highly simplified for simulation ease.

Recall that our vesicle fusion mechanism has been simplified to a reaction involving SNARE complexes and calcium ions. We can take the rate of calcium binding to synaptotagmin as a proxy for this value. The authors in Ma (2014) provide a rate value of $1 \times 10^8\,\mathrm{mol}^{-1}\,\mathrm{l}\,\mathrm{s}^{-1}$ for what they call the 'synaptotagmin-like calcium binding site'.

The rate of calcium influx into the bouton was difficult to determine, due to the simplified mechanism of the calcium channel in the model. We revert to the reference guide by Czech et al. (2009) and use their rate value of $1 \times 10^3\,\mathrm{mol}^{-1}\,\mathrm{l}\,\mathrm{s}^{-1}$.

The vesicle unzip time has been estimated at 200 µs, from Llinas (1999). This value is a rough approximation only, since the homeostasis conditions of a squid differ greatly from that of a human. We assume that the unzipping occurs linearly over this time interval.

The rate of diffusion of the neurotransmitter glutamate was estimated at $4 \times 10^{-6}\,\mathrm{cm^2\,s^{-1}}$. The rate of glutamate receptor binding was estimated at $4.6 \times 10^{5}\,\mathrm{mol^{-1}\,l\,s^{-1}}$. These values were taken from Rusakov (2001) by averaging the upper and lower bounds on the diffusion and rate constants.

The rate of diffusion of calcium was estimated at $5.3 \times 10^{-6}\,\mathrm{cm^2\,s^{-1}}$ and was drawn from Dittrich et al. (2013).

Parameter	Value	Reference
Rate of calcium influx	$1 \times 10^{3}\,\mathrm{mol^{-1}\,l\,s^{-1}}$	Czech et al. (2009)
SNARE complex binding rate	$1 \times 10^{8}\,\mathrm{mol^{-1}\,l\,s^{-1}}$	Ma (2014)
Glutamate binding rate	$4.6 \times 10^{6}\,\mathrm{mol^{-1}\,l\,s^{-1}}$	Rusakov (2001)
Rate of glutamate diffusion	$4 \times 10^{-6}\,\mathrm{cm^2\,s^{-1}}$	Rusakov (2001)
Rate of calcium diffusion	$5.3 \times 10^{-6}\,\mathrm{cm^2\,s^{-1}}$	Dittrich et al. (2013)
Vesicle unzip time	200 µs	Llinas (1999)

4.iv Object Specifications

Morphology data on the size of the bouton and the sizes and quantities of the synaptic vesicles was kindly provided by CITATION.

The mean estimate for the bouton volume was given as $0.36\,\mu\mathrm{m}^3$. We can approximate the radius of the bouton by treating it as a hemisphere to obtain a value of 0.7 µm.

The mean estimate for the synaptic vesicle radius was given as 0.017 µm.

The size of the postsynaptic spine head was estimated to have the same dimensions as the presynaptic bouton.

Finally, we estimated the cleft width at a constant 0.023 µm - this is an average of lateral and central cleft width measurements.

Parameter	Value	Reference
Estimate for bouton volume	$0.36\,\mu\mathrm{m}^3$	CITATION
Derived estimate for bouton radius	0.7 µm	Derived using hemisphere approximation
Estimate for synaptic vesicle radius	0.017 µm	CITATION
Synaptic cleft width	0.023 µm	CITATION

4.iv.1 Molecule Quantity Specifications

From Bruns and Jahn (1995) we have drawn an estimate of 4700 neurotransmitter molecules per vesicle (in this case the data is drawn from a vesicle containing the glutamate molecule).

From Stricker et al. (1996) we have taken the estimate of 100 neurotransmitter receptors. The data was originally taken from a mouse hippocampus.

From Wilhelm et al. (2014) we have drawn an estimate of 26000 SNAP-25 proteins in a synapse. There are two SNAP-25 proteins per SNARE complex, and about 750 vesicles in total. From this we can estimate about 15 SNARE complexes per vesicle.

Dittrich et al. (2013) state that the number of calcium ions required to activate a synaptotagmin protein (and hence activate the SNARE complex) is a minimum of 2. They also state that 3 such activated SNARE complexes are required to cause vesicle release.

CITATION estimated a mean of 750 vesicles at the bouton; however we have only modelled two due to computing constraints.

Parameter	Value	Reference
Number of Neurotransmitter molecules per vesicle	4700	Bruns and Jahn (1995)
Number of Neurotransimtter receptors per spine head	100	Stricker et al. (1996)
Number of SNARE complexes per vesicle	15	Wilhelm et al. (2014)
Number of calcium ions to activate synaptotagmin/SNARE	2	Dittrich et al. (2013)
Number of SNAREs to induce vesicle fusion	3	Dittrich et al. (2013)
Number of vesicles	750	CITATION

5 Results

6 Conclusion

7 Acknowledgements

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