Modeling BK Channel Function in the Frog Synapse at the Neuromuscular Junction

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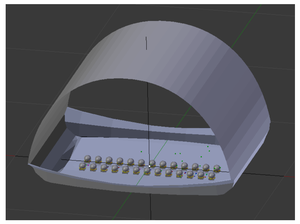
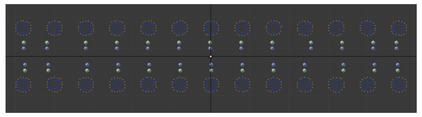
Introduction

- The frog neuromuscular junction provides a large synapse at which to study the timing of channel opening and vesicle release in the presynaptic terminal.
- BK channels, or calcium gated potassium channels, open as a result of voltage change and of the binding of calcium molecules.
- Because of the small size of the synapse and the speed of action potentials, it is hard to study not only at the physiology of the channels but also the timing of the channels opening.
- Construction of a model that is constrained by experimental and data allows us to more accurately determine what happens during synaptic signal transmission.

Methods

We used Blender and CellBlender to construct the main geometry of the model using experimentally gathered data on synapse structure at the frog neuromuscular junction. Our model was simplified and had only one active zone.

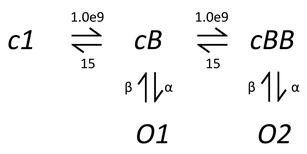




Left: Model of one active zone of the frog neuromuscular junction with vesicles, calcium and BK channels, and calcium molecules. Above: Configuration of vesicles and channels within the active zone. Green channels are calcium channels and purple channels are BK channels.

MCell was used to create the remaining files needed for the simulation. We used python scripts to write out files to describe the molecules in the system and the reactions between them. We used approximate literature values and ratios to determine the on and off rates for binding reactions.

The model of the BK channel is a simple five state gating scheme with three closed states and two open states. Each simulation was run for 1300000 iterations with a .1 nanosecond time step for between 800 and 1500 seeds. Data was then averaged across the seeds to find the average response of each simulation across time.

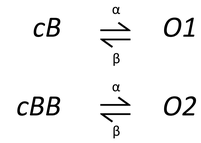


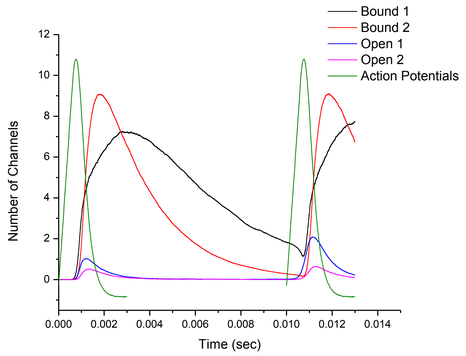
Gating scheme used for simulation. cB and cBB are closed states with one and two calcium molecules bound, respectively. O1 and O2 are voltage-dependent open states.

After running the simulation we gathered visualization data which was uploaded to a modified version of our model. The modified model included 24 active zones. Using this visualization data we were able to create a movie of our simulation taking place.

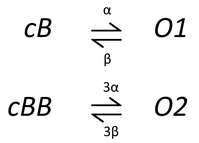
Results

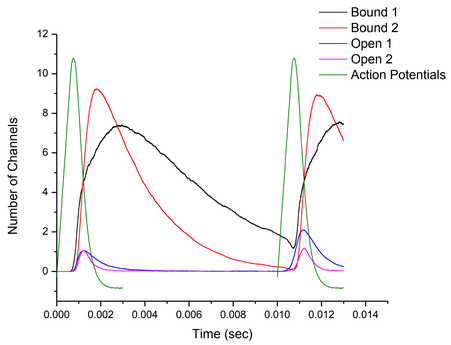
Open and close rates are the same for one or two calcium bound



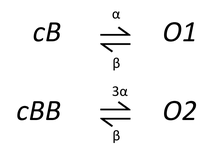


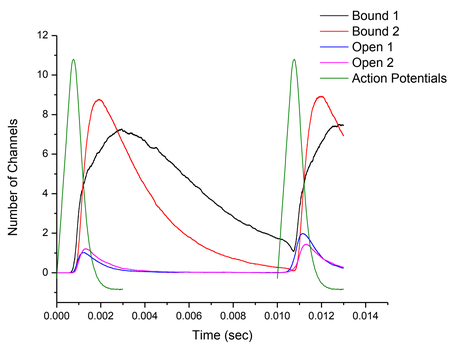
Open and close rates are both three times greater for two calcium bound



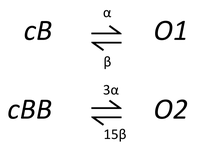


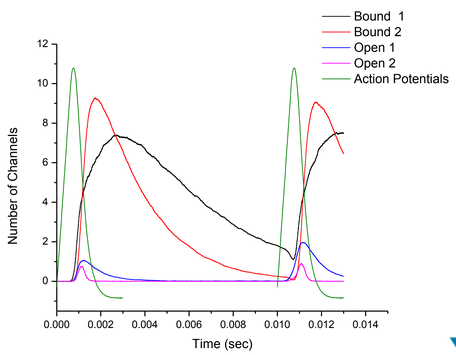
Open rate for two calcium bound is three times greater but close rates are the same





Open rate for two calcium bound is three times greater and close rate is 15 times greater





Discussion

The timings of the channels opening in all four simulations show that the repolarization of the cell is responsible for the opening of the BK channels. This aligns well with pharmacological data.

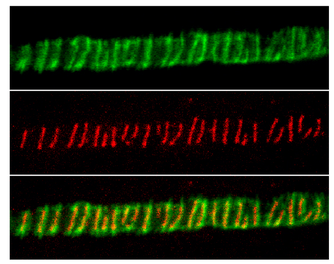
Interestingly, the second pulse always leads to significantly more open 1 states but the same approximate number of open 2 states when compared to the first pulse . This is because the calcium molecules can fall off the channels when the channels are still in their closed states. By the time the second pulse is given, there are almost no channels with two calcium molecules bound, while there are a number of channels left with one calcium molecule bound, which translates to an increased number of open 1 states in the second pulse.

In the second simulation, increasing the open and close rates for the second open state caused an equal increase in the number of channels opened for the first pulse and the second. Despite the ratio between the open and close rates being the same as the first simulation, it is clear that more open 2 states occurred after the increase in rates.

The third and fourth simulations show how changing the off rate changes the amount of time the channels are open. In simulation three, having a very low close rate leads to the open 2 state channels being open for some time after the cell has repolarized. The fourth simulation shows that all open 2 states close before the pulse has been completed.

Future Work

- Incorporate the effect of the flux of potassium into the cell on the shape of the action potential.
- See how changing the number of channels open, and the amount of potassium ions that enter, affects transmitter release.
- Preform super resolution imaging by tagging the BK channels with fluorescent dyes to confirm the density and distribution of BK channels in our model.



Fluorescent staining of a frog neuromuscular junction. Green dye, Alexa 488, is attached to α-Bungarotoxin to indicate the post-synaptic acetylcholine receptors. Red dye, Dylight 650, is combined with Iberiotoxin to display the BK channels. No higher resolution is currently possible, so exact locations of BK channels are unknown.

Acknowledgements

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