Presynaptic calcium-activated potassium channels and their role in normal synaptic function, neuromuscular disease, and treatment

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Introduction

Large conductance calcium-gated potassium (BK) channels have been previously found to be co-localized with voltage-gated calcium channels (VGCCs) in the active zone (AZ) of neuromuscular junctions (NMJ) and speed the repolarization of action potentials (AP). However, little is known about the BK channel's structure-function relationship with VGCCs and docked synaptic vesicles. Studying BK channel effects on the calcium ion entry and AP waveform is crucial to the overall understanding of the NMJ and neuromuscular diseases such as Lambert-Eaton myasthenic syndrome (LEMS) in which there is a reduction in the number of presynaptic VGCCs in the AZ. Based on recent data, we hypothesize that the structure-function relationships between BK channels and VGCCs within NMJ AZs critically regulates nerve terminal function.

Frog NMJ Model

To study the structure-function relationship of the BK channel with the AZ, we used a recently developed computer model (Figure 2) based on CellBlender and MCell. Our model's AZ geometry was constructed with CellBlender and includes various molecular structures such as VGCCs, BK channels, and calcium ions. MCell is an optimized stochastic reaction-diffusion simulation software used to accurately model molecular behavior and biological systems.

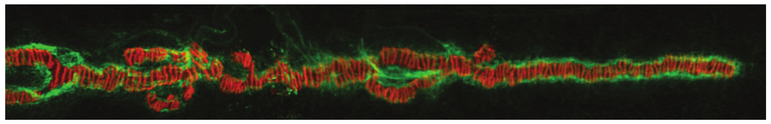

Figure 1. Frog NMJ stained with PNA-FITC (green) to outline the nerve terminal and BTX-Alexa 594 (red) to stain postsynaptic ACh receptors and predict AZ position (banded pattern).

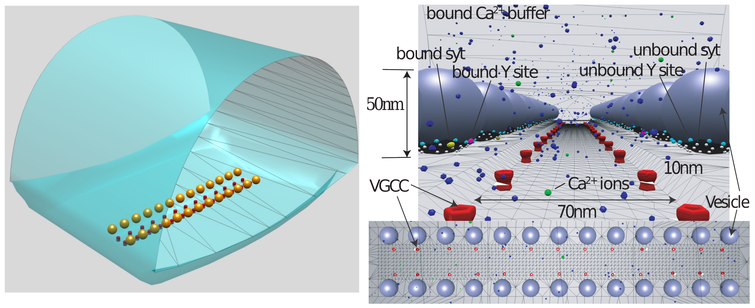

Figure 2. MCell model of the frog NMJ active zone. The 3-D model was created via CellBlender based on ultrastructural data from the adult frog NMJ and contains 26 docked synaptic vesicles arranged in 2 parallel rows. 26 VGCCs were positioned in a 1:1 stoichiometry, with ~38 nm distance from the neighboring docked synaptic vesicle.

A

$$C_1 \underset{X_2\beta}{\overset{X_1\alpha}{\rightleftharpoons}} C_2 \underset{Y_2\beta}{\overset{Y_1\alpha}{\rightleftharpoons}} C_3 \underset{Z_2\beta}{\overset{Z_1\alpha}{\rightleftharpoons}} O \quad (Ca^{2+}, k)$$

B

$$O_1 \quad O_2 \qquad X_1\alpha \uparrow\downarrow X_2\beta \qquad Y_1\alpha \uparrow\downarrow Y_2\beta$$

$$C_1 \underset{15}{\overset{4x1.5e7}{\rightleftharpoons}} C_B \underset{2x15}{\overset{3x1.5e7}{\rightleftharpoons}} C_{BB}$$

$$k(t) = -\gamma \frac{G}{2e}(V_m(t) - E_{Ca}) \quad \alpha = 0.06e^{(V_m+24)/14.5} \quad \beta = \frac{1.7}{e^{(V_m+34)/16.9}+1}$$

Figure 3. Gating scheme of VGCC and BK Channel. Based on previous literature, we predicted gating schemes for the VGCC and BK channel binding reactions. The VGCC gating scheme consists of three closed states and one open state (Figure 3A) and the BK channel model uses three closed states and two open states (Figure 3B). X_1, X_2, Y_1, Y_2, Z_1, and Z_2 are unknown coefficients. α, β, and k are voltage dependent rates.

Results

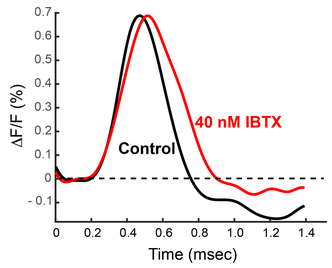

Figure 4. Newly recorded AP using BeRST voltage dependent imaging dye in frog NMJ. Neuromuscular junction action potentials are much briefer than previously recorded action potentials in the cell body (1-2 milliseconds in duration).

$$C_1 \underset{\beta}{\overset{7\alpha}{\rightleftharpoons}} C_2 \underset{2\beta}{\overset{6\alpha}{\rightleftharpoons}} C_3 \underset{3\beta}{\overset{5\alpha}{\rightleftharpoons}} O \quad (Ca^{2+}, k)$$

Figure 5. Updated VGCC gating scheme. With BeRST dye imaging data, we constrained the AP waveform in the MCell model and determined accurate kinetic rates for the opening and closing of VGCCs. Based on previous literature, the desired probability of opening is between 20-25% based on calcium imaging data.

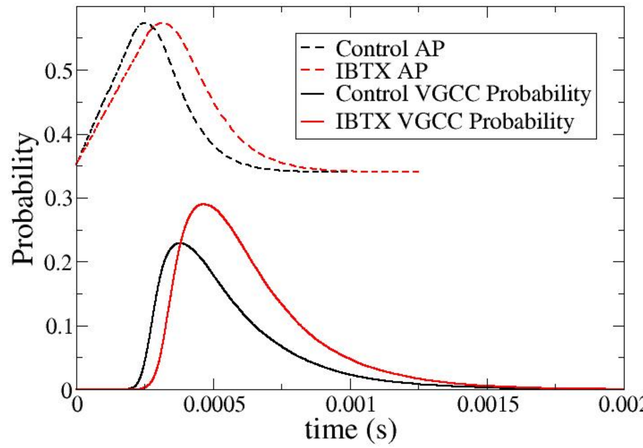

Figure 6. Probability of VGCC opening. Under the new kinetic rates (Figure 5), the probability of voltage-gated calcium channels opening under normal conditions was controlled around 23%. The addition of Iberiotoxin (IBTX), a BK channel blocker, increased to the probability of opening to roughly 30%.

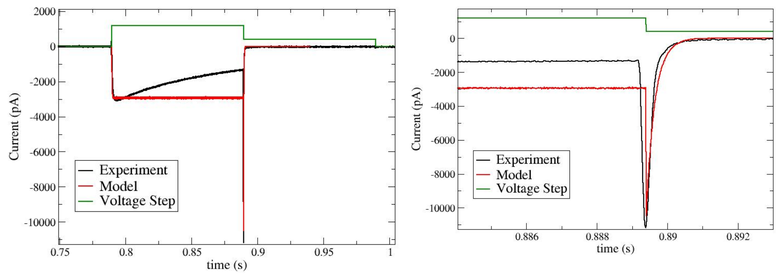

Figure 7. Calcium current during a 100ms voltage-step from -100 mV to 20 mV. Black traces: calcium current recorded from a cultured cell. Red traces: modeled calcium current. The reduction in the recorded current during the voltage step is due to voltage-dependent inactivation (not modeled because it does not occur during physiological action potential activity).

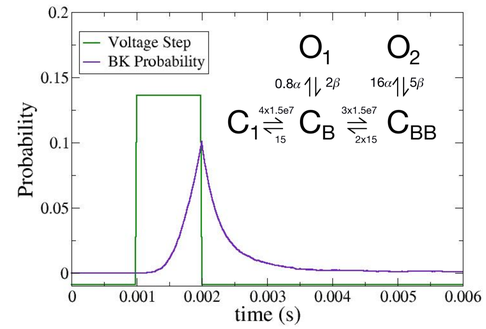

Figure 8. Probability of BK channel opening during voltage step. The BK channel gating scheme (Figure 3B) was added and the rate coefficients were determined by fitting the probability of opening to 10% during a 1ms voltage-step from -60 to 20 mV.

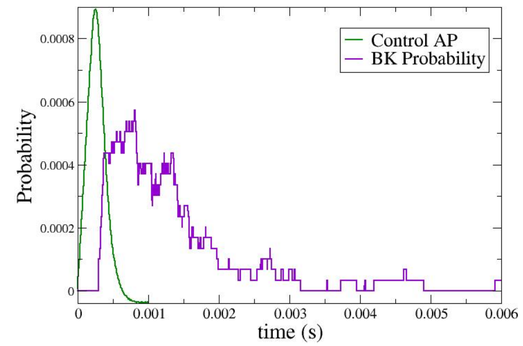

Figure 9. Probability of BK channel opening during the control AP. Using our current gating scheme, very few BK channels open in the AZ during an AP.

Discussion/Future Directions

According to the model, IBTX is predicted to nearly double the probability of neurotransmitter release compared to the control AP waveform. The control calcium current produced from the model also closely matches the current produced from physiological data, indicating that the chosen kinetic coefficients model the channel well. In addition, our new MCell model was modified to incorporate the impact of BK channels on voltage waveforms. The BK channel gating scheme and kinetic rates were modified to roughly match voltage-step physiological data produced by different research groups. However, the probability of opening during an AP was found to be too low so the gating scheme needs to be further modified. With additional imaging techniques, we will be able to accurately model the BK channel's probability of opening, distribution, and effects on repolarization. In addition, we will combine MCell with NEURON to predict changes in AP shape due to BK channel activation and the resulting effects on transmitter release. These data will be compared and constrained by physiological recording.

References

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Acknowledgements

This work was funded by the Undergraduate Research Fellowship in Computation Neuroscience, provided by CNBC. Special thanks to Dr. Stephen Meriney and Dr. Rozita Laghaei for their mentorship and support this past year.