Simulation of Catecholamine Release and Voltage Detection using Patch Clamp Amperometry

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Abstract

A popular method of voltage detection across a cell membrane involves patch clamp amperometry. With the assistance of Blender, a 3D visualization may be constructed to model the patch clamp, with the carbon fibre electrode (CFE), connecting to the cell membrane, with corresponding cations. Simulating the model in DreaMM allows for a 3D visualization of how ions are detected on the CFE and how different variables may affect the release rate of ions through the channel.[1] Neurotransmitters such as catecholamines are released through the fusion pore by a process of exocytosis. Efflux of catecholamines hitting the carbon fibre electrode after they have been released from the vesicle produce what is known as an amperometric current.[2] The position of the CFE within the patch clamp, as well as vesicle and pore size is varied to determine the rate of efflux across the pore.

Introduction

The main focuses include the construction of the models, simulations and validations, and the time step, by modeling the voltage dependent pore. Previous experiments led to the assumption of two detectable currents, an amperometric current, and a cell membrane current detected by the patch electrode. Although a cell membrane current was indeed detected, it was not visible and proposed to be non-existent. Therefore, another source of current must be present to balance the charge. Sodium ions may be the resulting molecules that enter the cell as a catecholamine molecule is released, making up for the loss of positive charge and balancing the net charge.[3] Tests have been run with the compound sodium chloride, yet not with sodium or chloride ions separately. Simulations will first be run disregarding the charge of the ion, to simply demonstrate the mechanisms of catecholamine release and CFE positioning; charges will added after a basic understanding has been obtained. Data obtained is displayed graphically by an amperometric foot signal, followed by an amperometric spike. The spike indicates the release of catecholamines, and the foot signal demonstrates the initial flux exiting the pore. As molecules exit the pore, they become oxidized when they reach the CFE. Two electrons are transferred for every one molecule, thus resulting in what is called the amperometric current.[3]

Methods

Blender is a program used to create 3D graphical models, and will be used to demonstrate the geometry of the patch clamp and vesicles. Each of the models will be constructed in Blender, including a cell with various ions inside, a fusion pore, patch clamp, CFE, and any additional structures. After charges the catecholamines and sodium have been taken into account, details may be added to the inside of the cellular area. For

example, a matrix may be added inside the cytoplasm of the cell. With the addition of a matrix, the effect of affinity of binding sites for catecholamines on the matrix may be demonstrated. If there exists a high affinity, the molecules will stay inside the cell longer and result in a slower release through the fusion pore.

To convert the models into files to be interpreted for simulation, a program called MCell must be used. A set of Monte Carlo algorithms serve as the backbone of MCell, which has its very own Model Description Language (MDL). Simulation is run in MCell by the interpretation of MDL files.[4] DreaMM is a program which interprets the MCell information to produce visualizations. Data may be imported to DreaMM through mesh objects which are in the form of OpenDX. Once in DreaMM, procedures such as data clipping, depth cueing, and image clipping may be performed to facilitate the rendering process and improve the images' quality.[1]

Predictions

A more detailed look at the sodium chloride concentration effects will be achieved by making predictions regarding the several models created. If the CFE is farther away from the pore, one may expect a low-pass filtering affect, which indicates that the further away a molecule is, the heavier the filter. Similarly, if the vesicle size is changed, the rate of diffusion is also altered.

Within the models, the fusion pore site will be replaced with a transporter that behaves as a normal fusion pore. The transporter serves to mimic the combination of the pore and voltage across the cell. From this adaptation, probabilities may now be calculated across the transporter using the Nernst Plank Equation. The Nernst Equation

Potential will also be used to observe if there is an increase in the net movement of sodium ions across the transporter, resulting in a change in voltage.

References

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