Modeling hair cell exocytosis using a hybrid deterministic-stochastic implementation

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Introduction

Modeling Aims

We present a novel computational modeling approach to simulate the synaptic details of a bullfrog saccular hair cell. Our model focuses on a single pre-synaptic active zone, and addresses calcium influx, buffered calcium diffusion, and exocytosis. Our aims are to create a **comprehensive** and **detailed** computational model of hair cell exocytosis that:

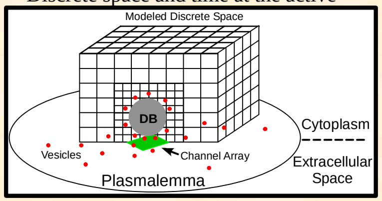
Unifies disparate experimental paradigms:
•Voltage clamp exocytosis (Parsons et al 1994, Moser and Beutner 2000)
•Flash photolysis of caged calcium (Beutner et al 2001)
•Spontaneous rates of VIIIth nerve
•Evoked response of VIIIth nerve

Considers *anatomical* details of the synapse:
•Distribution of vesicles about the dense body (Lenzi et al 1999, 2002)
•Possible distributions of calcium channels (Roberts et al 1990, Roberts 1994 , Wu et al 1996, Rodrigeuz-Contreras and Yamoah 2001)

Considers *physiological* details of the synapse:
•Gating and single channel conductance of calcium channels (Rodrigeuz-Contreras and Yamoah 2001, 2003)
•Movement of vesicles toward the active zone (Zenisek et al 2002)

Remains founded in whole cell kinetic analysis:
•Activation of calcium current (Hudspeth and Lewis 1988)
•Calcium buffering (Roberts 1993, Tucker and Fettiplace 1996, Ricci et al 1998, Moser and Beutner 2001)
•Calcium dependent exocytosis (Beutner et al 2001)

Discrete space and time at the active



Methods Overview

Hybrid Deterministic-Stochastic Implementation (HDSI)

Deterministic Components:
•Whole cell calcium current activation (Hudspeth and Lewis 1988)
•Buffered Diffusion (Roberts 1994)

Stochastic Components:
•Calcium channel gating
•Conversion of deterministic [Ca^{++}] to number of calcium ions
•Calcium binding and vesicle fusion

Program Structure – 25 msec v-clamp simulation (run time on 1.8 GHz P4):
•*Deterministic*– whole cell, voltage dependent calcium current activation (<1 sec)
•*Stochastic* – channel gating (<1 sec)
•*Deterministic*– buffered calcium diffusion computation (~4 hours)
– save results to disk (~40 MBytes)
•*Stochastic* – calcium binding and vesicle fusion (~10 min)
– output results to text file for graphing and further analysis

Methods

Modeling Details

Geometrical considerations
We considered a cytoplasmic "active zone volume" of 3.2 x 3.2 x 1.6 mm. This volume was bounded against a planar plasma membrane surface, with one complete active zone at its center. We discretized the active zone volume into thousands of cubical volume elements (voxels), for numerical evaluation purposes. We used multiple voxel sizes to efficiently model the active zone volume, with small voxels closer to the active zone center and large voxels further away. When modeling exocytosis, where the diameter of vesicles is approximately 40 nm (Lenzi et al 1999), we used a minimum voxel size of (40 nm)^3 closest to the active zone center, and up to (320 nm)^3 at the edges of the array (left schematic). When modeling the activation of BK channels we used a minimum voxel size of (10 nm)^3, based on the dimension of the channel mouth (Doyle et al 1998).

Deterministic Components
Both deterministic components were solved by solving differential equations defined by the respective kinetic equations. Solutions to whole cell current activation and buffered diffusion of calcium have been described in detail by others (Hudspeth and Lewis 1988, Roberts 1994), following is a recapitulation of the kinetic equations solved (Eq 1-4).

$$(1)\quad \frac{d[Ca]_{total}}{dt} = \frac{d[Ca]_{Ca\ influx}}{dt} + \frac{d[Ca]_{diffusion}}{dt} + \frac{d[Ca]_{fixed\ buffer}}{dt} + \frac{d[Ca]_{mobile\ buffer}}{dt}$$

$$(2)\quad \frac{d[Ca]_{Ca\ influx}}{dt} = N_{open\text{-}Vox}(t) \bullet G_{unit}\,[V_{mem}(t) - E_{rev\text{-}Ca}] = -\frac{I_{Ca-Vox}(t)}{(Z_{ca} \bullet F \bullet Volume_{Vox})}$$

$$(3)\quad \frac{d[Ca]_{diffusion}}{dt} = D_{Ca}\nabla^2[Ca] = D_{Ca}\left(\frac{\partial^2[Ca]}{\partial x^2} + \frac{\partial^2[Ca]}{\partial y^2} + \frac{\partial^2[Ca]}{\partial z^2}\right)$$

$$(4)\quad \frac{d[Ca]_{buffers}}{dt} = \overbrace{k_{off}^{FB}[FB\text{-}Ca] - k_{on}^{FB}[FB][Ca]}^{\text{Fixed Buffer Contribution}} + \overbrace{k_{off}^{MB}[MB\text{-}Ca] - k_{on}^{MB}[MB][Ca]}^{\text{Mobile Buffer Contribution}}$$

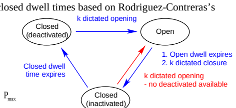
Calcium Channel Gating
L-type calcium channels in the hair cell have not been completely characterized at the single channel level. Rodriguez-Contreras and Yamoah (2001) have described the distribution of open and closed dwell times at a single holding potential and the maximum open probability P_{max}. Therefore we use our deterministic solution of whole cell Ica activation to calculate single channel open probability, then randomly choose which channels undergo transitions based on Rodriguez-Contreras's dwell time distributions.
•Probabilistically find number of open channels, *k*, every 250μsec based on deterministic activation parameter, *m* (Eq 5-8)
•Randomly choose which channels to transition based on their states (depicted below)
•Probabilistically find new open/closed dwell times based on Rodriguez-Contreras's distributions

$$(5)\quad \text{Whole Cell Ica} = G_{max} \times m^3 (V_{mem} - E_{Ca})$$

$$(6)\quad \frac{dm}{dt} = \alpha(1-m) - \beta m$$

$$(7)\quad \text{Open Probability} = P_o(t) = (m(t))^3 \times P_{max}$$

$$(8)\quad \Pr[\#\text{open channel} = k] = \binom{N}{k} \times (P_o(t))^k \times (1 - P_o(t))^{N-k}$$



Number of calcium ions
When considering small volumes, deterministic calcium concentrations translate to fractions of calcium ions per voxel. A single calcium ion is predicted when deterministic concentrations are 25.95 μM for a (40 nm)^3 voxel, yet 13.28 mM for a (5 nm)^3 voxel! The quanta of calcium ion associated with a deterministic concentration fluctuates stochastically as calcium undergoes Brownian motion. We iteratively find the quantal number of calcium ions in each voxel at each calcium diffusion time step, which is half the voxel dimension squared divided by the calcium diffusion coefficient (1.3 μsec (40 nm)^3, 2 nsec (5 nm)^3).
•Find deterministic number of Ca ions = *n* = [Ca] x Avagadro's number / voxel volume
•Scale up volume to make *n* large, so it can be approximated by an integer number *N*
•Use binomial to find the number of ions expected in the pre-scaled volume

Calcium binding and exocytosis
We stochastically model calcium dependent exocytosis based on the six state model of Beutner et al 2001. Their model includes five calcium binding steps, and one irreversible fusion step (Eq 9). We convert the kinetic model to a stochastic model based on the law of mass action. Example probabilities are shown for transitions from a single state (Eq 10-11)
•Quantal number of calcium ions are found based on deterministic solution (see above)
•Calcium dependent forward and backward rates are converted to probabilities
•Time step is reduced so no probably exceeds 0.1 (Gil et al 2000).
•Binomial deviates are drawn based on the respective transition probabilities and number of vesicles in the state under consideration

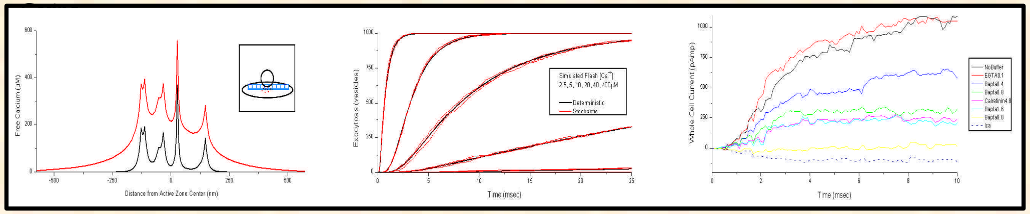
$$(9)\quad B_0 \underset{k_{off}}{\overset{5k_{on}[Ca]}{\rightleftarrows}} B_1(Ca) \underset{2k_{off}}{\overset{4k_{on}[Ca]}{\rightleftarrows}} B_2(Ca)_2 \underset{3k_{off}}{\overset{3k_{on}[Ca]}{\rightleftarrows}} B_3(Ca)_3 \underset{4k_{off}}{\overset{2k_{on}[Ca]}{\rightleftarrows}} B_4(Ca)_4 \underset{5k_{off}}{\overset{k_{on}[Ca]}{\rightleftarrows}} B_5(Ca)_5 \xrightarrow{\gamma} \text{Fusion}$$

$$(10)\quad \Pr[B_1 \text{ transitions left to } B_0] = P_L = \frac{\Delta B_{1\text{-left}}}{[B_1]} = k_{off}\Delta t$$

$$(11)\quad \Pr[B_1 \text{ transitions right to } B_2] = P_R = \frac{\Delta B_{1\text{-right}}}{[B_1]} = 4k_{on}[Ca]\Delta t$$

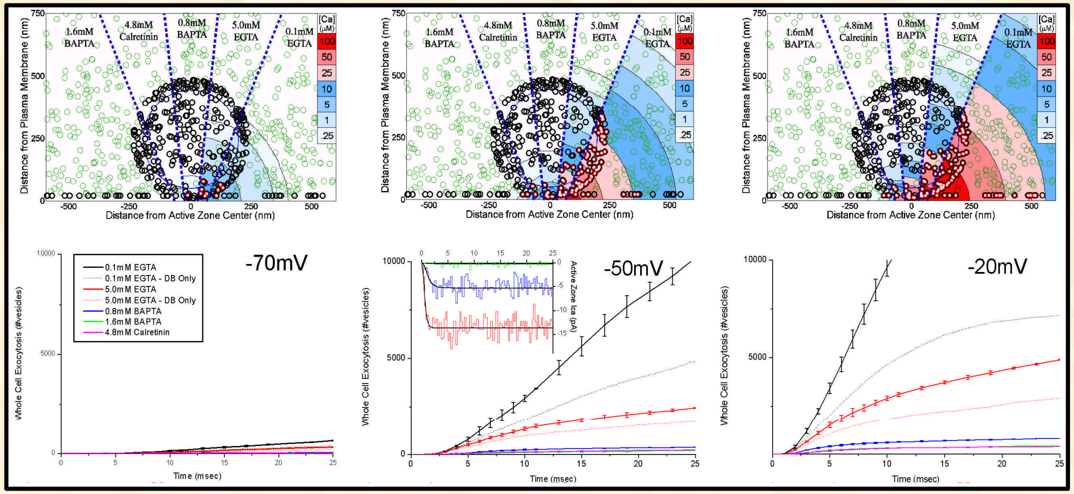
Technique Verification

Deterministic Buffered Diffusion — *Stochastic* Exocytosis — *HDSI* Buffered Diffusion and BK



Exocytosis Predictions

[Ca^{++}] profiles and time course of exocytosis elicited by voltage clamp steps from -75mV



Summary

Deterministic vs Stochastic
•Deterministic modeling simulates continuous processes and seeds stochastic processes – buffered diffusion and I_{ca} activation
•Stochastic modeling simulates discrete processes - channel gating, calcium binding, and vesicle fusion

Exocytosis Buffering Conditions
•Proposed endogenous buffer (4.8mM Calretinin, Edmonds et al 2000) mediates exocytic response between 0.8 mM and 1.6 mM BAPTA, as predicted by Roberts (1993)
•Weak buffering conditions reported by Moser and Beuter (2000) yields radically different time course and absolute levels of exocytosis than proposed endogenous buffer

Features of the HDSI Technique
•Generally applicable technique that can be used to simulate wide range of conditions
•Stochastic components required for simulations with low probability (i.e. spontaneous release, or central synapses with few vesicles)
•Stochastic components required for modeling non-stationary [Ca^{++}] (i.e. from sinusoidal or sound evoked depolarizations)
•Uses deterministic solutions as basis for stochastic process modeling when sufficiently detailed data is not available