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Approaches and tools for modeling signaling pathways and calcium dynamics in neurons

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ABSTRACT

Signaling pathways are cascades of intracellular biochemical reactions that are activated by transmembrane receptors, and ultimately lead to transcription in the nucleus. In neurons, both calcium permeable synaptic and ionic channels as well as G protein coupled receptors initiate activation of signaling pathway molecules that interact with electrical activity at multiple spatial and time scales. At small temporal and spatial scales, calcium modifies the properties of ionic channels, whereas at larger temporal and spatial scales, various kinases and phosphatases modify the properties of ionic channels, producing phenomena such as synaptic plasticity and homeostatic plasticity. The elongated structure of neuronal dendrites and the organization of multi-protein complexes by anchoring proteins imply that the spatial dimension must be explicit. Therefore, modeling signaling pathways in neurons utilizes algorithms for both diffusion and reactions. The small size of spines coupled with small concentrations of some molecules implies that some reactions occur stochastically. The need for stochastic simulation of many reaction and diffusion events coupled with the multiple temporal and spatial scales makes modeling of signaling pathways a difficult problem. Several different software programs have achieved different aspects of these capabilities. This review explains some of the mathematical formulas used for modeling reactions and diffusion. In addition, it briefly presents the simulators used for modeling reaction–diffusion systems in neurons, together with scientific problems addressed.

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1. Introduction

Signaling pathways are cascades of intracellular biochemical reactions and diffusion. They are typically activated by transmembrane receptors, and ultimately produce signaling to the nucleus, i.e., gene transcription. Many of these transmembrane receptors are G protein coupled receptors, where the bound receptor acts as an enzyme to catalyze the exchange of GDP for GTP, followed by dissociation of the Gα subunit from the Gβγ dimer (Premont and Gainetdinov, 2007). Both parts of the G protein can have downstream targets, either directly binding to and modifying activity of ion channels, or binding to enzymes such as

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adenylyl cyclase or phospholipase C to produce diffusible second messengers (Pierce et al., 2002). These pathways ultimately lead to phosphorylation or dephosphorylation events which change the state of key transmembrane receptors and ion channels, and also induce transcription and translation of new proteins (Hawk and Abel, 2011; Tanaka, 2001).

Calcium is a part of these signaling pathways in its role as a multi-functional, diffusible second messenger (Greer and Greenberg, 2008). Calcium can directly bind to and modulate ion channel activity; it can modulate the activity of G protein activated enzymes such as adenylyl cyclase and phospholipase C; through its binding to calmodulin, calcium can directly activate calcium calmodulin dependent protein kinase II (CaMKII) and protein phosphatase type 2B (calcineurin). What makes calcium unique from other second messengers is that it derives from influx through voltage dependent or synaptic ion channels. In addition, under some circumstances a major source of calcium is release from the smooth endoplasmic reticulum through calcium sensitive, calcium permeable channels (Hartmann and Konnerth, 2005).

Intense interest in the function of signaling pathways is due to their implication in disease. More than 50% of pharmaceuticals target G protein coupled receptors or downstream effectors (Howard et al., 2001). Signaling pathways are critically important for all cell types, from bacteria to mammalian systems. Modeling of signaling pathways has contributed to discovery in many areas of systems biology such as development (von Kriegsheim et al., 2009), cell cycle (Novak et al., 2007), cardiology (Jafri, 2012), immunology (Fallahi-Sichani et al., 2011), and cancer biology (Aksamitiene et al., 2011; Fey et al., 2012).

Although most research in computational neuroscience involves modeling or analysis of action potentials, understanding the signaling pathways underlying plasticity of intrinsic excitability or plasticity of synaptic responses is crucial for understanding learning and information storage. Signaling pathways interact with electrical activity at multiple spatial and time scales (Fig. 1), and the complexity of feedback loops and other pathway structures precludes a deep understanding of information processing without dynamical modeling. At the smallest time scales, calcium activates calcium dependent potassium channels (Berkefeld et al., 2006; Hirschberg et al., 1999) and inactivates voltage dependent calcium channels (Rankovic et al., 2011). The spatial scale for this temporal interaction tends to be small because tight regulation of calcium limits spread of the signal. At longer time scales, the phosphorylation or dephosphorylation of ionic and synaptic channels modulates neuron excitability (Daoudal and Debanne, 2003) and shapes synaptic integration (Lisman et al., 2002). The spatial scales for these longer time scales depend on whether the second messengers, kinases and phosphatases are highly diffusible, or are spatially confined by anchoring (Hulme et al., 2003) or rapid degradation (Tostevin et al., 2007). At the longest time scales, signaling pathways that lead to gene transcription and protein translation can produce changes that last the lifetime of the organism (Costa-Mattioli et al., 2009). Behavioral experiments have demonstrated the importance of various kinases and protein synthesis for long term memory (Abel et al., 1997; Shalin et al., 2006), but the interaction among all of these spatial and temporal scales implies that all of them are involved in learning, memory and information processing.

In summary, a complete understanding of neuronal function requires integration of electrical models of neurons with signaling pathway models of neurons. Both the spatial gradients produced by the elongated structure of neuronal dendrites and the organization of multi-protein complexes by anchoring proteins implies that the spatial structure must be explicit. Therefore, to model and simulate second messenger pathways in neurons requires algorithms for both diffusion and reactions, both deterministic and stochastic. This paper describes the various biochemical processes involved in signaling pathways, and also the modeling tools used to investigate signaling pathways. The first part of this review presents the biochemical reactions and mathematical equations used to describe and model signaling pathways. The second part of the review provides a brief description of several useful modeling tools and the models implemented using those tools.

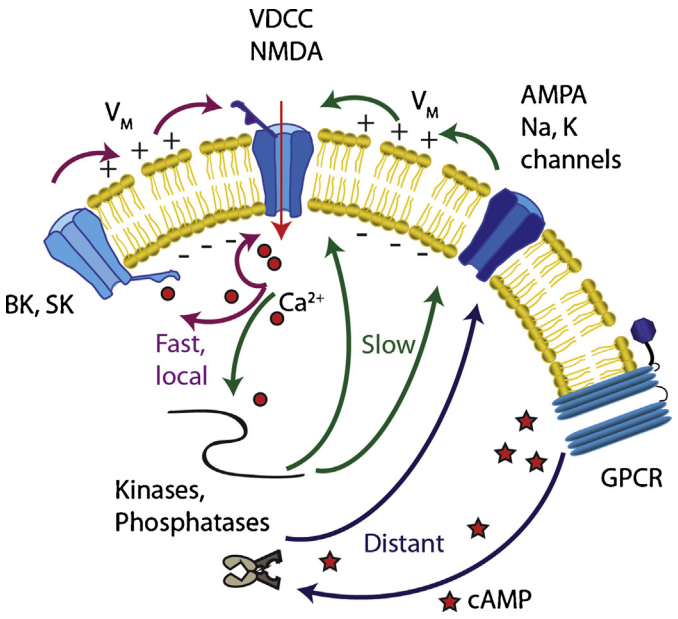

Fig. 1. Signaling pathways interact with electrical activity at multiple spatial and time scales. At the smallest spatial and temporal scales, calcium directly binds to and modulates ionic and synaptic channels. At longer time scales, calcium and second messengers produced by G protein coupled receptors (GPCR) lead to the phosphorylation or dephosphorylation of ionic channels and synaptic channels. The spatial scales for these longer time scales vary depending on the balance between diffusion and inactivation mechanisms. Feedback loops can be negative, as when phosphorylation enhances an outward current, or positive, as when phosphorylation enhances an inward current.

2. Biochemical reactions and diffusion

Both signaling pathways and mechanisms controlling calcium concentration are modeled as systems of uni- and bi-molecular reactions and diffusion. The rate at which reactions occur depends on factors such as frequency of collision between reacting molecules, energy of such collisions, and conformation of the collision (Stenesh, 1993). In order to simulate large systems of signaling pathways, various approximations are made in describing rates of reactions. Typically, the frequency of collision is captured by the concentration or density of reacting molecules, and the other factors governing the rate of reaction are captured in a single rate constant parameter. If the number of molecules is sufficiently large (e.g. in the cell body), the rate of change in molecule quantities can be described deterministically, whereas with small numbers of molecules (e.g. in spines) the reactions are more accurately simulated stochastically (Liu et al., 2012).

2.1. Uni- and bi-molecular reactions

Uni-molecular reactions are transformations of a molecule from one form to another. For example, a single sodium channel gate can transform from the closed state (m_c) to the open state (m_o) with forward rate k_f and backward rate k_b.

$$m_c \underset{k_b}{\overset{k_f}{\rightleftharpoons}} m_o \tag{1}$$

A bi-molecular reaction is a stoichiometric interaction between two (or more) substrate molecules to form one or two (or more) product molecules, and usually involves formation of a bond between the substrate molecules:

$$S_1 + S_2 \underset{k_b}{\overset{k_f}{\rightleftharpoons}} S_3 + S_4 \tag{2}$$

where S_1 and S_2 are substrates, and S_3 and S_4 are products. Though different types of bonds are possible, such details are excluded when modeling systems of reactions, and only the rate or propensity of a reaction occurring is important. A stoichiometric interaction implies that the reaction specifies the number of each molecule type required for the reaction, e.g. *one* hydroxy ion plus *one* hydrogen ion create *one* water molecule. This stoichiometry implies that molecules are consumed in order to make the product, i.e. mass is neither created nor destroyed.

When modeled deterministically, the change in molecule S_3 over time for bi-molecular reaction (2) is:

$$\frac{d[S_3]}{dt} = k_f \cdot [S_1][S_2] - k_b \cdot [S_3][S_4] \tag{3}$$

where $[S_i]$ is the concentration of molecule S_i, $i = 1, 4$. This formulation is often called mass action kinetics.

2.2. Enzyme reactions

An enzyme reaction is a special type of two step bi-molecular reaction in which the enzyme is regenerated in the second step. Thus, enzyme, E, is not consumed, and each enzyme molecule can make multiple product molecules, allowing amplification:

$$E + S_1 \underset{k_b}{\overset{k_f}{\rightleftharpoons}} ES_1 \overset{k_{cat}}{\rightleftharpoons} S_2 + E \tag{4}$$

ES_1 is the enzyme substrate complex, and typically the rate of the second backward reaction, from $E + S_2$ to S_1, is considered 0. Deriving the mathematical equations for an enzyme reaction, or indeed for any system of equations, involves (1) writing an equation for the time dependent concentration of each molecule, and (2) including terms describing each path (set of arrows) leading toward and away from each molecule, i.e., rates of reactions producing and consuming the molecule. Thus, the deterministic equations for the system of two enzyme reactions is:

$$\begin{aligned}
\frac{d[S_1]}{dt} &= -k_f[E]\cdot[S_1] + k_b[ES_1] \\
\frac{d[E]}{dt} &= -k_f[E]\cdot[S_1] + k_b[ES_1] + k_{cat}[ES_1] \\
\frac{d[ES_1]}{dt} &= k_f[E]\cdot[S_1] - k_b[ES_1] - k_{cat}[ES_1] \\
\frac{d[S_2]}{dt} &= k_{cat}[ES_1]
\end{aligned} \tag{5}$$

where $[S_1]$ is the concentration of the substrate, $[S_2]$ is concentration of the product, and $[E]$ is concentration of the enzyme. Note that if this was a closed system, two of the four molecules could be calculated by conservation of mass, e.g. $[E] = [E_{tot}] - [ES]$, where E_{tot} is the total enzyme in the system. Under some conditions, most notably that ES is in equilibrium and that substrate concentration is much greater than enzyme concentration (Tzafriri and Edelman, 2005), a single differential equation can be used to calculate product formation in an enzyme reaction:

$$\frac{d[S_2]}{dt} = \frac{k_{cat}[E]\cdot[S_1]}{[S_1] + K_M}, \quad \text{where} \quad K_M = \frac{k_b + k_{cat}}{k_f} \tag{6}$$

However, if the enzyme concentration varies rapidly, the assumption of ES in equilibrium is violated and this simplification is not valid. Other conditions which invalidate this approximation are discussed elsewhere (Chen et al., 2010).

When reactions are modeled stochastically, the rate constants and molecule concentrations are used to calculate the likelihood (propensity) that a reaction will occur. A set of molecules interacting in a system is specified with a state vector $\mathbf{x}$, in which x_i denotes the *number* of molecules of species S_i. $a_j(\mathbf{x})\cdot dt$ is the propensity of reaction j during the infinitesimal time interval dt (e.g. $a_j(\mathbf{x}) = k_f \cdot x_1 \cdot dt$ for a uni-molecular reaction involving species S_1 and $k_f \cdot x_1 \cdot x_2 \cdot dt$ for a bi-molecular reaction involving species S_1 and S_2). The stoichiometry matrix, $\mathbf{v}$, specifies the change in population i when the jth reaction occurs. Using this notation, the entire system of R reactions can be specified in a matrix format known as the chemical master equation (Gillespie, 1977):

$$\begin{aligned}
\frac{\partial P(\boldsymbol{x}, t|\boldsymbol{x}_0, t_0)}{\partial t} = \sum_{j=1}^{R} [&a_j(\boldsymbol{x} - \boldsymbol{v}_j)\cdot P(\boldsymbol{x} - \boldsymbol{v}_j, t|\boldsymbol{x}_0, t_0) \\
&- a_j(\boldsymbol{x})\cdot P(\boldsymbol{x}, t|\boldsymbol{x}_0, t_0)]
\end{aligned} \tag{7}$$

Because the chemical master equation cannot be solved for anything but trivial problems, solutions typically are obtained using Monte Carlo simulations.

2.3. Diffusion

Several characteristics of neurons require consideration of molecule movement along concentration gradients. Both the extremely elongated dendrites and the thin necked dendritic spines support different concentrations between various dendritic locations, or between spine head and dendrite (Harvey et al., 2008; Lee et al., 2009). The concentration gradients created by these spatial features imply that diffusion – the movement of molecules from an area of high concentration to an area of low concentration via Brownian motion – significantly contributes to signaling pathway dynamics (Schmidt et al., 2012). As is the case with reactions, diffusion can be described quantitatively either deterministically or stochastically. When considered deterministically, diffusion is described using a partial differential equation, here given for one spatial dimension, y:

$$\frac{\partial C(y, t)}{\partial t} = D \cdot \frac{\partial C(y, t)}{\partial y^2} \tag{8}$$

Derivation of the deterministic diffusion equation (which is identical to the heat equation) gives an intuitive feel for the geometrical factors, such as spine neck radius and length, influencing the rate of movement of molecules, and can be found in many sources (Haberman, 1997).

The stochastic diffusion equation gives the probability that stochastic process $Z(t)$, which describes the position of a particle in space, will transition from state z_0 at time t_0 to state z at time t:

$$\frac{\partial P(z, t|z_0, t_0)}{\partial t} = D \cdot \frac{\partial P(z, t|z_0, t_0)}{\partial z^2} \tag{9}$$

Approaches to simulating both the stochastic diffusion equation and the continuous diffusion equation are described in the section on simulation.

3. Calcium

Calcium is a component of many signaling pathways, and also provides an interaction between the biochemical and electrical aspects of neurons. Calcium diffuses and reacts with other molecules as described above. Most of the calcium reacts with "buffer" molecules, such as calbindin and parvalbumin (Schmidt

Table 1
Several characteristics of biochemical reaction diffusion simulators. Up-to-date list of properties are available from the simulator specific websites listed in the text.

Simulator	Electrical activity?	Deterministic algorithm	Stochastic algorithm	Other properties
NeuroRD	No	Dufort–Frankel	Lattice, leap*	Model specification resembles experimental workflow
Steps	No	None	Lattice, spatial next	Tetrahedral mesh, python interface
Smoldyn	No	None	Particle, fixed *dt*	Anisotropic diffusion and drift
MCell	No	None	Particle, adaptive *dt*	Image based morphology
CDS	No	None	Particle	Molecular crowding
Moose	Yes	5th order Runge–Kutta–Fehlberg	Particle (smoldyn), lattice (Gillespie)	Implicit Crank–Nicolson for electrical activity
VCell	No	Stiff ODE solvers, semi-implicit and fully implicit spatial solvers	Particle (smoldyn)	Image based morphology
Genesis/Kinetikit	Yes	Exponential Euler for reactions and 1D diffusion	Lattice, leap*, hybrid stochastic	Implicit Crank–Nicolson for calcium dynamics
Genesis/Chemesis	Yes	Exponential or forward Euler	None	2D diffusion, calcium release

* Leap = multiple reaction/diffusion events can occur within single *dt*.

et al., 2003), which serve to prevent too high a calcium concentration. Calcium also binds to calmodulin (Faas et al., 2011), which then activates diverse enzymes, such as adenylyl cyclase, calcineurin, and CaMKII (Xia and Storm, 2005). Another mechanism for controlling the elevation in calcium concentration is transport across membranes. Several plasma membrane proteins, such as the sodium calcium exchanger and the calcium ATPase, transport calcium from the cytosol to the extracellular space (Blaustein et al., 2002). Other transporters are located on the membranes of intracellular organelles, such as the smooth endoplasmic reticulum calcium ATPase (Solovyova and Verkhratsky, 2003). The smooth endoplasmic reticulum is itself a source for calcium: calcium is released from the smooth endoplasmic reticulum through the IP_3 (and calcium sensitive) receptor channels (De Young and Keizer, 1992), and the calcium sensitive ryanodine receptor channels (Tang and Othmer, 1994).

The interaction with electrical activity includes both influx from calcium permeable ion channels and modulation of calcium dependent potassium channels. The influx of calcium through voltage dependent channels is typically modeled with an equation describing the voltage dependent gating of the calcium channel:

$$f_o = m(V,t)^P \cdot h(V,t)$$
$$\frac{dm}{dt} = \frac{m - m_{ss}}{\tau_m}, \quad \frac{dh}{dt} = \frac{h - h_{ss}}{\tau_h} \tag{10}$$

The time constants (τ_h, τ_m) and steady state values (m_{ss}, h_{ss}) of the gating variables are voltage dependent, though calcium channels also exhibit calcium dependent inactivation (Tuckwell, 2012), implying that h_{ss} also depends on calcium. Given the open fraction, f_o, the current through the channel can be modeled as if ohmic:

$$I(V,t) = G_{\max} \cdot f_o \cdot (V - E_{rev}) \tag{11}$$

where E_{rev} is the reversal potential for calcium and G_{max} is the maximal conductance, or using the Goldman–Hodgkin–Katz formula, which captures the observation that calcium channels do not reverse:

$$I(V,t) = P_{\mathrm{Ca}} \cdot f_o \cdot \frac{z^2F^2V}{RT} \frac{[\mathrm{Ca}_i] - [\mathrm{Ca}_o]e^{\frac{-zFV}{RT}}}{1 - e^{\frac{-zFV}{RT}}} \tag{12}$$

where F is Faraday's constant, R is the ideal gas constant, T is temperature in Kelvin, and P_{Ca} is permeability of the channel to calcium. Glutamate receptor channels, such as NMDA and some AMPA subtypes, also are calcium sources because they have some degree of calcium permeability (Jahr and Stevens, 1993). Calcium influx through these channels is determined by multiplying the synaptic conductance by the fractional calcium permeability. Calcium influx, Φ_{Ca} is calculated from calcium current by dividing by Faraday's constant and the valence, z, of calcium ions:

$$\Phi_{\mathrm{Ca}} = \frac{I_{\mathrm{Ca}}}{zF} \tag{13}$$

Modulation of calcium dependent potassium channels can be described as a separate binding reaction to the channel, e.g. when a Markov kinetic model formulation is used to calculate f_o (Rothberg and Magleby, 1999), or using calcium dependent rate constants (analogous to the voltage dependent rate constants), when using Eq. (10). The exact form of the gating variables depends on the type of potassium channel, in that SK (voltage independent) potassium channels have a calcium term but no voltage term (Hirschberg et al., 1999):

$$m_{ss}(\mathrm{Ca}) = \frac{1}{1 + \left(\mathrm{EC50}/[\mathrm{Ca}^{2+}]\right)^H}, \quad \tau_m = K \tag{14}$$

whereas gating variables of the BK channels depend on both calcium and voltage (Berkefeld et al., 2006).

4. Simulation

Many different software simulation tools are available to model signaling pathways in neurons. One class of approaches is to use a general purpose equation solver. XPPAUT is one such software package that is freely available and can be learned easily from the accompanying tutorial (http://www.math.pitt.edu/~bard/bardware/tut/start.html). MATLAB and Python are other commonly used software tools for simulation. Another approach is to use software specially written to simulate signaling pathways within neurons. The next part of the paper reviews many of these simulators that have been used for reaction–diffusion systems, both explaining some of the distinguishing characteristics of the simulators (Table 1 summarizes a few of their properties), and also explaining a particular question in neuroscience addressed using the simulator. The numerous models of signaling pathways that exclude diffusion are not discussed here; nor are models of calcium dynamics alone, which are reviewed elsewhere (Blackwell, 2013).

4.1. Deterministic simulators

The first group of simulators employs deterministic calculations of reaction and diffusion for signaling pathways. Both Kinetikit (Vayttaden and Bhalla, 2004) and Chemesis (Blackwell and Hellgren-Kotaleski, 2002) are extensions of the GENESIS (Bower and Beeman, 1998) neural simulation software, adding capabilities

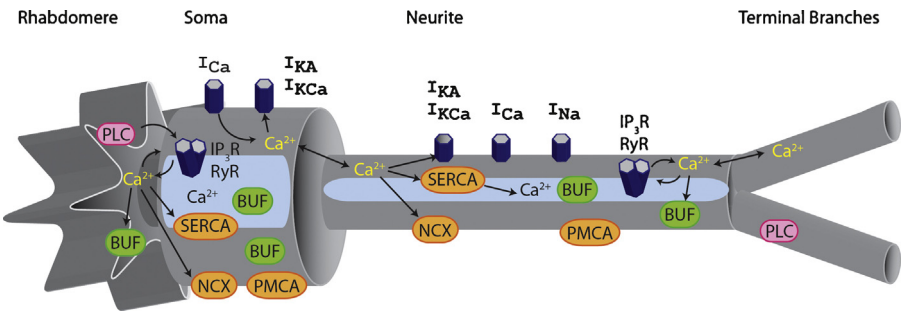

Fig. 2. Model of phototransduction and calcium dynamics in *Hermissenda*. The morphology included a rhabdomere, connected to the soma with a thin neck, a neurite and two terminal branches. Calcium and potassium permeable voltage dependent ion channels were included throughout the model, and IP_3 sensitive sodium channels were the light sensitive channels. The biochemical cascade for phototransduction included rhodopsin activation of phospholipase C, which produced diffusible IP_3. Calcium dynamics included release of calcium through both the IP_3 and ryanodine receptor channels, a calcium buffer in the cytosol and in the ER, a plasma membrane calcium ATPase pump (PMCA), and a smooth endoplasmic reticulum calcium ATPase pump (SERCA), and the sodium calcium exchanger (NCX).

of modeling reaction–diffusion systems to software extensively used for modeling electrical activity. VCell (Moraru et al., 2008) was designed for simulating signaling pathways in neurons. Neuron is used less frequently for modeling signaling pathways in neurons, though the kinetic scheme for reactions can be specified in the nmodl language, and capabilities for calcium diffusion exist. Consequently, most signaling pathway models using NEURON implement the biochemical pathways in a different simulator and then interact with the NEURON electrical model (Allam et al., 2012; Djurfeldt et al., 2010). Both Kinetikit and VCell have both stochastic and deterministic capabilities, but are described in this section because most of their published neuroscience models use deterministic simulation.

Chemesis has been used for modeling signaling pathways and calcium dynamics underlying classical conditioning in the marine mollusc *Hermissenda crassicornis* (Blackwell, 2004). Chemesis simulates both one and two-dimensional diffusion in addition to bi-molecular and enzyme reactions. The numerical methods for both reactions and diffusion use the GENESIS solvers, and thus typically employ the exponential Euler. Models of calcium concentration can include Markov kinetic models of calcium release from intracellular stores, both the eight state model of the inositol triphosphate (IP_3) receptor channel (De Young and Keizer, 1992), or the four state model of the ryanodine receptor (Dupont and Goldbeter, 1994), as well as ATPase pumps and the sodium-calcium exchanger. The software is freely available: http://krasnow1.gmu.edu/CENlab/software.html, and a tutorial on using Chemesis is available (Blackwell and Hellgren-Kotaleski, 2002).

The software capabilities of Chemesis were employed to investigate how paired presentation of light (the conditioned stimulus) and turbulence (the unconditioned stimulus) lead to enhanced calcium elevation, and down regulation of potassium channels in the *Hermissenda* photoreceptor. The photoreceptor model (Fig. 2) included voltage dependent ion channels, and also the biochemical cascade of rhodopsin activation of phospholipase C, which produced IP_3 in the rhabdomere. The model also included synaptic input to the photoreceptor from the turbulence sensing hair cells which led to production of IP_3 in the terminal branches of the neurite. Both sources of IP_3 initiated calcium release which propagated as waves toward each other. The main result was that paired turbulence and light did ***not*** produce a supralinear elevation in calcium, instead the two waves destructively interfered, indicating that some other mechanism caused learning in these neurons in response to paired stimuli.

Kinetikit was designed for large scale modeling of signaling pathways in neurons and has capabilities for both stochastic and deterministic simulation. The exponential Euler is used for deterministic calculations of reactions and one-dimensional diffusion. The stochastic algorithm is lattice based and allows multiple reaction and diffusion events per time step. Because the adaptive stochastic algorithm switches to deterministic for large numbers of molecules, it is classified as a hybrid algorithm. The database of pathways (Sivakumaran et al., 2003) allows re-use and combination of selected pathways into novel models, and the graphical user interface facilitates development of completely novel models. Kinetikit is distributed as part of the GENESIS software (http://www.genesis-sim.org/GENESIS/), and a tutorial on using Kinetikit is found in (Bower and Beeman, 1998; Vayttaden and Bhalla, 2004).

Kinetikit has been employed in numerous publications, most notably investigating emergent properties such as gates and switches (Bhalla, 2002; Bhalla and Iyengar, 1999). More recently Kinetikit has been used in a spatial model integrated with electrical activity to investigate mechanisms underlying the all or none phosphorylation of mitogen activated protein kinase (MAPK) in a subset of dendrites in hippocampal area CA1 after induction of long term potentiation (LTP) (Ajay and Bhalla, 2007). A series of models evaluated whether diffusion from a single point of calcium influx could account for the >100 μm long phosphorylated MAPK observed experimentally, or whether calcium influx at multiple points along the dendrite were required. Simulations showed that diffusion was not sufficient to explain the spatially extended phosphorylated MAPK, and that either distributed synaptic input or a dendritic action potential was required. This biochemical model was activated by input from the electrical model, but there was no feedback from the biochemical to the electrical model. Therefore, to further develop this multi-scale model with bi-directional interactions, the biochemical reactions were simplified, while still retaining their essential dynamical properties such as bi-stability and switching (Bhalla, 2011). Not only did calcium from the electrical model activate biochemical reactions, but also phosphorylated MAPK controlled conductance of the transient potassium channel and AMPAR trafficking in the biochemical model controlled the AMPAR conductance in the electrical model. Simulations, which used MOOSE/kinetikit12 (http://moose.ncbs.res.in/), demonstrated that LTP induction potentiates some spines and enhances dendritic excitability. The latter combined with background activity resulted in depression of some spines. Thus, this model, perhaps the largest and best integrated in terms of electrical–biochemical interactions, suggests a mechanism for homeostatic plasticity.

VCell has been used to develop signaling pathway models in a large variety of cell types in addition to neurons. VCell includes multiple numerical methods, both for stochastic (described below) and deterministic simulation. VCell has long employed various stiff solvers for reaction systems, and a semi-implicit, fixed time step method for diffusion. For more efficient simulation of diffusion the developers recently incorporated a finite volume method

for spatial discretization coupled with a fully implicit solver and adaptive time step control. A users guide and several tutorials explain model development and simulation using VCell: http://www.nrcam.uchc.edu/vcell_software/user_guide.html.

In neuroscience the VCell software has been used to investigate how coincident activation of parallel fibers and climbing fibers in Purkinje cells of the cerebellum leads to long term depression (LTD). The model included calcium activation of phospholipase C and production of IP_3 and diacylgylcerol from the membrane phospholipid phosphoinositol bisphosphate. The original models (Brown et al., 2008; Hernjak et al., 2005) showed that spine geometry influences the ability to generate a supralinear calcium signal in response to paired parallel fiber and climbing fiber stimulation. In addition, the decrease in IP_3 as it diffused away from the activated spine conveyed spatial specificity – adjacent spines exhibited no calcium release. Though these models did not include explicit electrical activity, a more recent model employed a simplified morphology which exhibits similar electrical activity to a full NEURON model, and also exhibits supralinear calcium elevation in response to paired stimulation (Brown et al., 2011).

Another VCell model investigated the control of micro-domains of signaling pathways downstream of the β-adrenergic receptor in the soma and dendrite of a hippocampal pyramidal neuron (Neves et al., 2008). The model included activation of adenylyl cyclase, production of cAMP, and activation of protein kinase A (PKA), which led to activation of the MAPK cascade. Phosphodiesterases inactivated the cAMP, and two phosphatases turned off several kinases in the model. Simulations revealed two main factors controlling the spatial gradients: dendritic diameter, and negative regulators such as phosphodiesterases and phosphatases.

4.2. Stochastic simulators

The second group of simulators use stochastic simulation of signaling pathways to investigate fundamental questions in neuroscience. Stochastic simulation of reaction–diffusion systems is required whenever the number of molecules is small, e.g. <10 (Liu et al., 2012), a situation that occurs frequently in dendritic spines due to their small size. There are several approaches to modeling diffusion stochastically, with the two most common approaches used in neuroscience being particle based and lattice-based. Yet a third approach encompasses hybrid solvers that integrate lattice-based-stochastic methods with deterministic methods into a single solver. For example, some simulators, such as the hybrid tau-leaping method (Rossinelli et al., 2008) simulates diffusion deterministically and reactions stochastically. An alternative method, such as the spatial partitioned leaping algorithm (Iyengar et al., 2010) categorizes each reaction and diffusion based on propensity and/or number of molecules, and then simulates one set of reactions (and diffusions) stochastically and the other set deterministically. After briefly describing the general numerical approach used by these two different classes of stochastic simulations (hybrid simulators are included in the section on lattice-based simulators), I present several simulators in each of these two classes that have been used to address questions in neuroscience, and also describe a few of the unique characteristics of each simulator.

In the particle based methods (Andrews et al., 2010), the subcellular space is represented as membrane surfaces together with the surrounding or enclosed space, and the state of the system is the location of each molecule within the space. The distance a molecule moves, Δr, during a time step Δt is determined from the probability distribution:

$$p(\Delta r, \Delta t) = \frac{1}{(4\pi D\Delta t)^{3/2}} e^{[-\Delta r^2/4D\Delta t]} \quad (15)$$

and the direction of movement is uniformly distributed. The location of each particle is traced as it diffuses through the space and reactions occur when two particles pass sufficiently near each other.

MCell is the oldest (Bartol et al., 1991) and most well known particle simulator used to investigate cell signaling in neuroscience. The numerical algorithm employs several different heuristics to determine reaction occurrence, e.g., whether the reaction is between two surface molecules, two volume molecules, or one surface and one volume molecule (Kerr et al., 2008). In all cases the reaction probability depends on the probability of collision and the probability of a reaction given a collision. To optimize speed and accuracy of the random walk, look-up tables store radial distance and direction of movement, which are then selected using uniform random numbers. A tutorial on using Mcell is found in http://www.mcell.org/tutorials/.

A fascinating question about synaptic plasticity addressed using MCell is how coincidence detection by NMDA receptors can control calcium elevation in the spine. To address this question, an MCell model of calcium dynamics was coupled with a NEURON simulation of a pyramidal neuron to obtain the dendritic voltage and voltage dependent open probability of calcium channels (Keller et al., 2008). The MCell model included calcium buffers and pumps, and calcium permeable channels, including NMDA receptors. Simulations using these coupled models revealed that calcium concentration in the post-synaptic density (PSD) was enhanced by an appropriately timed action potential much more so than the average spine calcium, revealing the importance of calcium microdomains. Most recently, MCell has been used for highly accurate 3D reconstructions of neuropil from electron-microscope tomography (Kinney et al., 2013).

Two other particle based simulators have become available recently for neuroscience modeling. Smoldyn (Andrews et al., 2010) is a stand alone software system that also has been incorporated into MOOSE (an update of the GENESIS simulation software: http://moose.ncbs.res.in/) and VCell (Cowan et al., 2012). Models with complicated morphology can be constructed in Smoldyn, though most models employ regular geometry, such as cuboid and ellipsoids. Diffusion in a membrane is simulated using Gaussian-distributed random displacements in three dimensions, followed by a projection back onto the membrane surface. Second order reactions occur with probability one when diffusing particles pass within a distance specified by the binding radius of both particles. The binding radius depends on the reaction rate, diffusion constant and the simulation step size. A users guide explains how to create and simulate models http://www.smoldyn.org/Smoldyn_doc1.pdf.

In addition to various systems biology publications (Lipkow et al., 2005), Smoldyn has been used to study CaMKII aggregation in dendritic spines (Khan et al., 2012, 2011). Close coupling of imaging experiments that employ fluorescence recovery after photoactivation with simulations of CaMKII diffusion and binding demonstrate that CaMKIIα diffuses out of a mushroom spine more slowly than CaMKIIβ, which is consistent with the known binding to F-actin of CaMKIIβ. Smoldyn also has been employed to investigate the role of NMDAR subunit composition (Singh et al., 2011). The model consisted of a synaptic cleft in which glutamate diffused, and a post-synaptic part with AMPA receptors and various di- and tri-heteromeric NMDA receptors. Simulations demonstrated that the activation of different NMDAR subtypes depended on the stimulation frequency and number of glutamate vesicles released.

An even more recent particle based simulator for stochastic reaction–diffusion modeling is CDS (Byrne et al., 2010), which is an event driven algorithm that approximates the first passage scheme. An advantage of the CDS algorithm compared to VCell and Smoldyn is that it takes into account volume exclusion, and thus can simulate the effect of molecular crowding which may affect both diffusion

and reactions in dendritic spines which have a high density of proteins. The software and a users guide are available for download: http://nba.uth.tmc.edu/cds/content/download.htm. The CDS software was used to model the activation and accumulation of CaMKII in dendritic spines (Byrne et al., 2011). The narrowness of spine necks impedes diffusion of CaMKII out of the spine, but this factor alone does not account for the long retention time of CaMKII. Simulations demonstrate that binding targets in the PSD help to retain CaMKII, and also bundled F-actin at the base of the spine impeded diffusion out of the spine. All three mechanisms are required to explain CaMKII accumulation in spines in response to calcium influx through NMDA receptors.

In the lattice-based methods, the subcellular space is subdivided into lattice points or voxels (Takahashi et al., 2005) using a mesh generation algorithm, and molecules are represented as populations within each voxel. The location of molecules is less precise than in the particle based simulators, and is limited by the voxel size. The probability that a molecule moves from one voxel to an adjacent one depends on distance between the center of the voxels, Δy, and the area, A of the shared surface between the two voxels:

$$p = \frac{D \cdot A \cdot dt}{\Delta y} \tag{16}$$

Reactions can occur between molecules within the same voxel. The propensity of reactions occurring is calculated within each voxel, and in some cases, the likelihood that a molecule moves from one lattice point to another is included as if it was another reaction (Elf and Ehrenberg, 2004; Koh and Blackwell, 2011). The state of the system, $\mathbf{Z}$, is a matrix composed of SV vectors $\mathbf{z}$, in which z_{ki} denotes the *number* of molecules of species S_i in voxel k. This notation is used to derive the equation called the reaction–diffusion master equation (Drawert et al., 2010):

$$\begin{aligned}\frac{\partial P(\boldsymbol{z}, t|\boldsymbol{z}_0, t_0)}{\partial t} = &\sum_{k=1}^{SV}\sum_{j=1}^{R}[a_j(\boldsymbol{z}_k - \boldsymbol{v}_j)\cdot P(\boldsymbol{z}_k - \boldsymbol{v}_j, t|\boldsymbol{z}_{0k}, t_0) \\ &- a_j(\boldsymbol{z}_k)\cdot P(\boldsymbol{z}_k, t|\boldsymbol{z}_{0k}, t_0)] + \sum_{i=1}^{Sp}\sum_{k=1}^{SV}\sum_{m=1}^{adj} \\ &[d_{i,k,m}(\boldsymbol{z} - \boldsymbol{G}_i^{k,m})\cdot P(\boldsymbol{z} - \boldsymbol{G}_i^{k,m}, t|\boldsymbol{z}_0, t_0) \\ &- d_{i,k,m}(\boldsymbol{z})\cdot P(\boldsymbol{z}, t|\boldsymbol{z}_0, t_0)]\end{aligned} \tag{17}$$

where $d_{i,k,m}$ is the rate of diffusion of species S_i from voxel k to one of the adjacent voxels m, Sp is the number of diffusible species, and $G_i^{k,m}$ is the stoichiometry matrix. As with the chemical master equation, the reaction–diffusion master equation is solved using Monte Carlo simulations.

STEPS is a spatial extension of the exact stochastic simulation algorithm, similar to the spatial next reaction method (Hattne et al., 2005) that is implemented in mesoRD; the most significant difference from mesoRD is in the use of tetrahedral instead of cuboidal meshes, which allows STEPS to simulate more realistic geometries. To facilitate model development, simulations can be specified using Python (Wils and De Schutter, 2009), and the software is available for download: http://steps.sourceforge.net/STEPS/Home.html.

STEPS has been used to investigate cerebellar LTD in a single spine (Antunes and De Schutter, 2012). The positive feedback loop of protein kinase C – MAPK – phospholipase A_2, was updated from the version in the hippocampal model, and was simulated with the addition of AMPA receptor phosphorylation and trafficking. This model demonstrated that due to the small size of spines and the stochastic nature of reactions, the number of synaptic AMPA receptors exhibited a bimodal distribution, suggesting that LTD was an all-or-none phenomena in single spines. Although single small spines did not exhibit graded responses to calcium influx, large spines (i.e. in the deterministic regime) and populations of spines did indeed exhibit graded responses. This result provides an outstanding example of how the dynamical behavior of a system depends on whether simulations are performed stochastically or deterministically.

NeuroRD is a spatial extension of the Gillespie tau-leap algorithm (Gillespie, 2001). The computational efficiency in a tau-leap algorithm results from allowing multiple reaction and diffusion events to occur during each time step. The morphology, reactions, stimulation, and initial conditions are specified in separate files to facilitate performing control simulations, analogous to experimental work flow. The software is available for download with an explanation on developing and simulating models: http://krasnow1.gmu.edu/CENlab/software.html.

NeuroRD has been used to investigate two spatial aspects of long term synaptic plasticity in hippocampal CA1 pyramidal neurons (Kim et al., 2011): the role of multi-protein complexes, and the extent of spatial specificity. Proteins are not randomly distributed in a neuron, but are organized into multi-protein complexes, by molecules such as A kinase anchoring proteins (AKAPs). Both AKAP12 and AKAP5 are highly expressed in the hippocampus and striatum, and have binding sites for protein kinase A as well as adenylyl cyclase, calcineurin, protein kinase C, L type calcium channels, or β adrenergic receptors. Experiments show that disruption of anchoring produces deficits in protein kinase A dependent forms of LTP in the hippocampus, but it is unknown whether anchoring near activator molecules, such as adenylyl cyclase, or targets, such as AMPA receptors, is more important. To address this question, a model was created in NeuroRD of calcium and Gs coupled signaling pathways in a dendrite with spines. Simulations were performed using four trains of 100 Hz stimulation as input, in model variants in which protein kinase A is colocalized either with its activator adenylyl cyclase, or targets, such as AMPA receptors, or both. Simulations showed that protein kinase A activity was higher when localized with adenylyl cyclase, due to strong inactivation mechanisms for cAMP. This model also was used to investigate the spatial specificity of synaptic activation. Though cAMP exhibits spatial gradients with a spatial decay constant of ~3.5 μm, protein kinase A activity did not exhibit spatial specificity. A similar result obtained in a striatal model (Oliveira et al., 2012), which further showed that lack of spatial specificity was due to diffusion of DARPP-32. These models are exemplary for investigating spatial aspects of signaling pathways using stochastic approaches.

Outside of synaptic plasticity, phototransduction is the most common subject of signaling pathway modeling, because it is the best characterized G protein signaling pathway. Indeed, the history of computational models that investigate the role of calcium, as well as activation, inactivation and recovery of the light response is as extensive as that of synaptic plasticity. Most of these models use Michaelis–Menton kinetics to simplify the biochemical reaction system, use deterministic simulation techniques and exclude diffusion, e.g. (Korenbrot, 2012; Lamb and Pugh, 1992; Tranchina et al., 1991); however, a subset of models do include diffusion. One of the first models implemented the rhodopsin, transducin, phosphodiesterase cascade, using custom written software for diffusion via random walk (Lamb, 1994). Simulations demonstrated that coupling efficiency of transducin (the G protein) to the phosphodiesterase (the enzyme) depended on both the diffusion coefficients as well as concentration of the enzyme. In addition, when reactions were not diffusion limited, i.e., they occurred after collision with a probability less than one, then a delay in enzyme activation appeared, consistent with experiments. This model did not include inactivation mechanisms, but these were added to later models as they became better characterized. For example, a hybrid deterministic–stochastic model, programmed in MATLAB, was developed to investigate the source of variability in the single

photon photocurrent (Bisegna et al., 2008). The diffusion calculations and some first order reactions were performed deterministically, whereas the state transitions for rhodopsin inactivation were drawn from an exponentially distributed random variable. Simulations demonstrated that multi-step inactivation of isomerized rhodopsin is the major source of variability. Whereas previous models claimed that abrupt turn off of rhodopsin limits the variability to the experimentally measured values (Hamer et al., 2005), this model revealed that diffusion of cGMP and calcium serves to reduce that variability, instead of contributing to variability.

4.3. Rate constants and parameter sensitivity

As with all modeling, constraining the rate constants with experimental measurements is important for simulations of reaction diffusion systems to provide meaningful results. Measurements of enzyme kinetics typically provide K_M from Eq. (6) (Bardsley et al., 1980), and often will provide a reaction velocity, equivalent to k_{cat} [E]. Thus, using Eq. (6) is common not only because it decreases the number of reactions in a system, but also because the measures of k_f and k_b needed for Eq. (5) are rarely available. A common assumption when using Eq. (5) is that $k_b = 4k_{cat}$ (Bhalla and Iyengar, 1999). Similar to the case for enzyme reactions, measures of k_f and k_b often are not available for bi-molecular reactions; instead measures of dissociation constant ($K_D = k_b/k_f$) are more commonly available (Desdouits et al., 1995; Herberg et al., 2000). Occasionally, experiments using stopped-flow techniques (Putkey et al., 2003; Waas et al., 2003) are used to measure k_f, also known as the on-rate. The advent of live cell imaging (Giepmans et al., 2006) which shows the time course of activation of molecules coupled to fluorescent indicators provides an alternative approach to estimating kinetics of reactions. For example, the rate of protein kinase C activation by diacylglycerol can be inferred from the rate of translocation to the membrane in response to application of a diacylgylcerol analog (Oancea and Meyer, 1998).

The diffusion constant depends on the radius of the diffusing molecule as well as viscosity of the fluid environment, η, and temperature, T in Kelvin. Sometimes diffusion constants are directly measured, e.g. fluorescent imaging experiments (Allbritton et al., 1992). More commonly, they are estimated using molecular weight, N, as an estimate of radius (Young et al., 1980):

$$D = \frac{8.34e^{-8T}}{\eta N^{1/3}} \tag{18}$$

The viscosity of cytosol has been estimated by comparing diffusion of molecules in water with diffusion in cytosol (Khan et al., 2011; Oliveira et al., 2010).

Molecule location and quantity is often the most difficult parameter to constrain. This difficulty is similar to the lack of constraints for the maximal conductance of various ion channels in multicompartmental modeling. The quantities of some proteins, such as CaMKII or DARPP-32, have been published (Hemmings and Greengard, 1986), and basal concentrations of other molecules, such as calcium and cAMP, also have been measured (Maravall et al., 2000; Mironov et al., 2009). The location of various molecules can be determined from subcellular fractionation techniques (Xie et al., 2006) or microscopy. Immunogold labeling coupled with electron microscopy has been used to count the number of molecules in spines versus dendrites, in the cytosol versus membrane-associated (Uchigashima et al., 2007). Electron microscopy coupled with PSD isolation and gel electrophoresis has been used to measure the numbers of several proteins in the PSD (Chen et al., 2005). Confocal fluorescence microscopy with double or triple labeling can identify whether molecules colocalize with pre- or post-synaptic proteins (Micheva et al., 2010). Calcium imaging has been used to estimate the number of NMDA receptors functioning during glutamatergic stimulation (Nimchinsky et al., 2004). Thus, the techniques used to measure molecule quantity are quite diverse.

Since many rate constants and molecule quantities are not available, it is necessary to adjust these unconstrained parameters to reproduce experimental results that represent the output of cascades of reactions and diffusion. For this reason and also due to uncertainty in parameter values due to experimental variability, validating a model requires demonstrating that simulation results are somewhat robust to changes in parameters. Most types of parameter sensitivity analysis are classified into one of two types: local and global (Marino et al., 2008; Zi, 2011). Local sensitivity analysis measures the response of a system to changes in parameters about a specific operating point. However, these results are valid only for small perturbations around that operating point and different sets of parameters may have a greater influence around different operating points. Global sensitivity analysis calculates the sensitivities in a widely sampled range of operating points, either regularly spaced or randomly selected; however, this approach tends to be more computationally intensive than local sensitivity analysis. One software tool that can perform both local and global sensitivity analysis is SBM-SAT (Zi et al., 2008), which can analyze SBML models developed using any of the SBML compliant simulators. Yet a third approach, used by the reaction simulator Copasi (http://www.copasi.org/), combines parameter optimization with sensitivity analysis (Sahle et al., 2008). Several optimization routines are offered, such as genetic algorithm, particle swarm and simulated annealing. All of these approaches become more difficult as parameter space is enlarged, and thus one work-around is to subdivide the system into modules and optimize each module independently (Jain and Bhalla, 2009).

5. Challenges

Most signaling pathway models address questions related to synaptic plasticity and learning, or phototransduction. These publications demonstrate that several software tools can be used for developing integrated electrical–biochemical models of neurons. Nonetheless, only a subset of signaling pathway models has been integrated with electrical activity, and typically such models use a reduced number of electrical and/or biochemical compartments due to the computational complexity. Integration of electrical activity with signaling pathways is the goal of most scientists in this field; thus, the lack of bi-directional integration highlights the computational difficulty in achieving this goal. One challenge is the different temporal and spatial scales involved in synaptic plasticity. Electrical activity such as action potentials occur with sub-millisecond dynamics, whereas activation of some kinases, such as MAPK, occurs with a time scale of minutes, and transcription and translation of plasticity related proteins has a time scale of hours. The spatial scale of kinase activation related to synaptic plasticity is similarly diverse, with elevations in calcium being confined to single spines, yet initiation of gene transcription requiring signals traveling to the nucleus quite distant from the dendritic spines. Another challenge to developing integrated models relates to software development. Though there are many simulators available for biochemical systems, most do not include electrical activity. Conversely, the simulators for neuronal electrical activity do not have all the features, such as parameter optimization and sensitivity analysis, available in the biochemical simulators. Though simulators continue to include additional features, an alternative approach is develop interfaces for different simulators (Allam et al., 2012; Djurfeldt et al., 2010). The increase in computational power as well as active development of novel algorithms signifies that integrated simulators and models are likely to become more common in the near future.

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