# Calcium diffusion models and transmitter release in neurons<sup>1,2</sup>

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As a first approximation, one may think of neuron cytoplasm as a single compartment. In that case, a constituent of cytoplasm with regulatory functions, such as calcium or cyclic nucleotides, can be treated as a simple variable controlling a cell function, such as transmitter release or membrane permeability. It is sufficient to relate cell function to regulator concentration sampled at one point in cytoplasm or to an average concentration.

Although this approximation greatly simplifies both the measurements and the calculations, it may lead to serious errors and even false conclusions. Nowhere is this more evident than in studies of intracellular calcium and its regulation of cell function. Only by considering the spatial distribution of cytoplasmic calcium during nervous activity can one make sense of the relation between calcium concentration and its effects on cell function.

# CALCIUM DIFFUSION IN SOMATA

The importance of nonuniform calcium concentration was first made clear to me during studies of calcium indicator responses in neuronal cell bodies. Absorbance changes in neurons filled with the calcium indicator dye arsenazo III reflect increases in cytoplasmic calcium during depolarizing pulses. The signals rise rapidly during a pulse and recover slowly afterward. Photoemissions from neurons filled with acquorin also indicate free cytoplasmic calcium increases in depolarized cells, but rising and falling phases of these signals are both rapid. Arsenazo is a linear calcium indicator that reveals the total number of cal-

#### ABSTRACT

Calcium enters neurons or nerve terminals through the surface membrane, and then diffuses inwardly and is diluted. Calcium acts at membrane sites to release transmitter or modulate channels, or at organelles or the nucleus to regulate metabolism. Thus the average cytoplasmic calcium concentration is a poor predictor of calcium-dependent processes. Solutions of the diffusion equation with appropriate geometries and boundary conditions help considerably in our quantitative understanding of calcium-dependent processes in neurons.—Zucker, **R. S.** Calcium diffusion models and transmitter release in neurons. *Federation Proc.* 44: 2950-2952; 1985.

cium ions in the light path that traverses the neuron. Aequorin, on the other hand, is a nonlinear indicator that responds preferentially to local high-calcium concentrations. Thus as calcium diffuses away from the membrane where it entered, arsenazo senses it without change; aequorin's photoemissions reflect calcium's dilution. Thus the indicator responses have markedly different shapes for the same time-dependent changes in calcium distribution.

To prove this requires simulations of calcium movements in cells, with diffusion from the surface where it enters, binding to large cytoplasmic and membrane-bound proteins internally, and removal by extrusion into organelles and at the surface. Solving the diffusion equation in spherical coordinates with these boundary conditions provides the time-dependent spatial distribution of calcium in cytoplasm. A simulation that includes first-order and higher-order reactions with arsenazo and aequorin, respectively, accurately replicates the indicator responses measured physiologically (7).

Another difference between arsenazo and aequorin indicator responses is that although arsenazo signals to successive pulses are identical or even

get smaller because of calcium channel inactivation, successive aequorin signals facilitate, or grow to, repeated pulses. The behavior of aequorin photoemissions was originally taken as evidence for facilitated calcium entry or for saturation of cytoplasmic calcium buffering. However, simulations show that aequorin response facilitation results from the nonlinear dependence of photoemission intensity on calcium concentration and from the presence of residual calcium under the membrane after activity (7). A given influx of calcium will have a greater effect if there is some calcium left from previous activity.

### RESIDUAL CALCIUM AND SYNAPTIC FACILITATION

This led me to consider whether a similar nonlinear dependence of trans-

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mitter release on calcium concentration at nerve terminals leads to synaptic facilitation where successive action potentials release increasing amounts of transmitter. Turning to the squid giant synapse, Charlton et al. found that presynaptic calcium currents to repeated depolarizing pulses are constant, as are the intracellular calcium concentration changes that occur directly under the membrane during the pulses, as measured with arsenazo (1). Injecting calcium presynaptically facilitates transmitter release to action potentials, so residual calcium might well underlie synaptic facilitation.

It had already been shown that calcium is required in the medium not only for transmitter release but for synaptic facilitation at neuromuscular junctions (4). Pharmacological manipulations that purportedly increase presynaptic calcium also sometimes facilitate release, but their effects on presynaptic action potentials are unknown. Besides evoked transmitter release, synapses spontaneously release quanta of transmitter in the absence of action potentials, and these quanta are easily detected at neuromuscular junctions. Their frequency of release is thought to be regulated by presynaptic calcium, so it too should increase after presynaptic action potentials. In fact, Lara-Estrella and I found that after a tetanus, quantal frequency and evoked release are both increased (9). However, quantal frequency facilitation is larger and decays faster than facilitation of evoked transmitter release. A simple model of the calcium dependence of transmitter release shows that this is exactly the result expected. Spontaneous quantal release should be more sensitive to changes in residual calcium than evoked release, which is governed by the sum of a small residual calcium and the large constant calcium transient accompanying an action potential (9).

# SIMULATIONS OF PRESYNAPTIC CALCIUM AND RELEASE

Given this evidence for a residual calcium mechanism of synaptic facilitation at squid synapses and crayfish neuromuscular junctions, it seemed worthwhile to attempt to simulate synaptic facilitation by using a calcium diffusion model like that developed for cell bodies. Stockbridge and I solved

the diffusion equation in cylindrical coordinates, with dimensions and cytoplasmic calcium binding, extrusion, and influx boundary conditions determined by experimental measurements on souid preparations (10). Taking the square law relationship between calcium influx under presynaptic voltage clamp and transmitter release (1) as a measure of the stoichiometry of transmitter release, we predicted a rapid phasic transmitter release to an action potential, a two-component synaptic facilitation, and a slow decline in arsenazo spectrophotometric signals that were remarkably similar to the behavior of the giant synapse (10).

Recently I have been investigating extensions of this model to more demanding stimulus regimens (8). For example, during a tetanus of 100 action potentials at 20 Hz, synaptic facilitation grows and decays with the same components as for a single spike, plus a new, slow, seconds-long component. This is remarkably similar to the accumulation of facilitation and the appearance of augmentation described at neuromuscular junctions (5). If calcium extrusion in the model occurs primarily at the surface rather than into organelles, an even slower component of potentiation appears as a result of the extra time it takes for calcium that has diffused away from the membrane to be pulled back to the surface before it can be pumped out. This, too, resembles the behavior of neuromuscular junctions (5).

However, these simulations display a serious defect: transmitter release is much too prolonged after action potentials late in the tetanus. This is because submembrane residual calcium at the end of a tetanus reaches a level similar to the peak calcium concentration at the end of a single action potential in the simulations. Thus there is something wrong with either the model or our ideas of how synaptic transmission is regulated.

One possible fault in the model is that it does not adequately represent the events at transmitter release sites in the immediate neighborhood of calcium channels. In the present simulation, calcium enters uniformly through the membrane surface and diffuses radially in only one dimension. However, in reality calcium enters through discrete channels and initially diffuses rapidly in three dimensions until it is equilibrated beneath the sur-

face. Only then does it diffuse radially, essentially in one dimension. Thus the peak calcium concentration at release sites near channel mouths at the end of a spike is really much higher than it would be if calcium entered uniformly across the membrane. In such a discrete channel model, residual calcium should remain a small fraction of peak calcium in a spike, and transmitter release should terminate rapidly even after a tetanus. However, to generate levels of facilitation similar to those observed experimentally, a power law with an exponent greater than 2 will be needed for the calcium stoichiometry of release.

Transmitter release does depend on a high power of external calcium in squid (3). Why, then, do we observe only an approximately square law dependence between calcium influx and release for different size depolarizations? It is probably because increasing depolarization increases influx by opening more calcium channels and thus recruiting additional domains of transmitter release. It is helpful to recall that increasing transmitter release occurs by the release of more quanta that recruit additional nonoverlapping postsynaptic receptor domains (2). Just as quantal effects add linearly despite the nonlinear relation between transmitter concentration and postsynaptic response, so should transmitter release increase linearly with depolarization as additional channels are opened, regardless of the calcium-release stoichiometry. The calcium concentration in each domain would not increase with depolarization. In fact, bigger depolarizations approaching the calcium equilibrium potential will admit less calcium per channel, which will lead to release with a less-than-linear dependence on influx (6). However, if calcium channels that open during an action potential are close enough to each other, the calcium domains surrounding separate channels will overlap, and there will be partial summation of calcium entering through discrete channels. Preliminary simulations of this sort of model reveal that the relation between transmitter release and calcium current has a rough power law form whose exponent lies between the less-than-one power of completely independent channels and the full power of the stoichiometric dependence of release on calcium concentration (R. Zucker and A. Fo-

gelson, manuscript in preparation). The latter would be seen only if local submembrane calcium concentration were directly related to total calcium influx, which requires complete overlap of all calcium domains.

These considerations lead to the

idea that transmitter release can be understood only in terms of the interactions of calcium entering through neighboring discrete calcium channels and diffusing in three dimensions away from channel mouths. The residual calcium after activity would still diffuse

radially and be subject to buffering and extrusion. A new diffusion model is being developed to take these ideas into account, and it is hoped that it will be even more successful in helping us to understand how synapses work. Б

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