Stochastic model of calcium initiated reactions in a dendritic spine

W.R. Holmes and S. Zeng

Introduction. Theoretical studies have conjectured that CaMKII, because of its autophosphorylation properties, may act as a bistable molecular switch that forms the basis of learning and memory (Lisman 1985; Lisman and Goldring 1988). Models have shown that CaMKII activation is a nonlinear function of tetanus frequency, but what causes this nonlinearity and how steep is it? While properties of CaMKII alone can cause switch-like behavior, the steepness of this switch may depend on relative rates of phosphorylation and dephosphorylation of CaMKII subunits.

Models have explored parameter space to determine factors that can cause switch-like behavior in CaMKII activation. Coomber (1998a,b) states that to get switch-like behavior in his model, the ratio of phosphatase concentration to CaMKII concentration must be small. Okamoto and Ichiwara (2000) report that switch-like behavior occurs because phosphatase concentration is limited. In their model dephosphorylation is modeled as a Michaelis-Menten reaction and with a low phosphatase concentration the maximum velocity of the dephosphorylation reaction is low. In addition, switch-like behavior depends on CaMKII concentration being large compared to the Michaelis-Menten constant of the dephosphorylation reaction. Switch-like behavior becomes more gradual as these restrictions are relaxed. In their simulations, calcium was constant, but as calcium concentration was stepped to higher levels, the probability that CaMKII would bind $CaMCa_4$ eventually reached a threshold beyond which almost all CaMKII entered the *trapped* state. Zhabotinsky (2000) used a much more realistic calcium signal and included phosphatase dynamics in his model and found bistability over a more realistic range of calcium concentrations than Okamoto and Ichiwara (2000). Zhabotinsky (2000) also concluded that bistability was the result of the concentration of CaMKII subunits being much greater than the Michaelis-Menten constant of dephosphorylation. Bistability was found to be a robust phenomenon that occurred over a wide range of modeled parameter values.

Some experimental confirmation of this switch-like behavior was recently provided by Bradshaw et al. (2003). They found that CaMKII autophosphorylation had a steep dependence on calcium concentration (Hill coefficient ~ 5) with an even steeper calcium dependence when

autophosphorylation was balanced by PP1 dephosphorylation (Hill coefficient ~ 8). These experiments were done with bath concentrations of 50 μM calmodulin, 2 mM ATP, 0.2-25 μM CaMKII and varying concentrations of calcium and PP1 (0-2.5 μM). In addition the temperature for these experiments was 0° C to prevent phosphorylation at the $T^{305,306}$ sites.

While these modeling and experimental results are intriguing, the conditions for bistability within an actual dendritic spine receiving physiological calcium signals in which autophosphorylation of $T^{305,306}$ is permitted have not been explored. As a first step towards doing this, we have developed a stochastic model of calcium entry and reaction and diffusion within a dendritic spine.

Methods. Eight pulse tetani of varying frequency are applied to 300 synapses in a model of a dentate granule cell to determine the voltage change at the synapses. At each synapse each pulse in the tetanus is modeled as the release of 2000 glutamate molecules. Openings of AMPA and NMDA channels at synapses are modeled deterministically except at the synapse of interest where openings are modeled stochastically with MCell (Stiles and Bartol 2001). The voltage from the neuron level model is used to determine if individual NMDA channels are blocked by magnesium or not. The proportion of the NMDA current due to calcium is computed. This calcium current is the input to the stochastic spine model.

In the stochastic spine model, the spine is divided into a number of homogeneous voxels. Calcium influx occurs in voxels occupying the PSD region. Given the number of reactions that can take place within a voxel and diffusions that can take place between voxels, we use the Gillespie algorithm (Gillespie 1976, 1977) first, to determine the time of the next reaction or diffusion event and second, to determine the specific reaction or diffusion event and the voxel of this event that takes place. To verify the model is working properly, model results from an average of 10 stochastic runs were compared to results reported with a mixed stochastic-deterministic model by Holmes (2000) for specific cases.

Results. Two major results are apparent to date. First, small variations in the number of NMDA receptors can make 20 and 50 Hz input have CaMKII activation levels similar to that with 100-400 Hz tetani. This result was discovered by accident. When a receptor density is specified in MCell, MCell will randomly determine the number of receptors to place in the

model. Different random seeds will produce different numbers of receptors and with our models this meant that the number of NMDA receptors varied from 15 to 23. With the higher number of receptors, CaMKII activation with 20 and 50 Hz input was similar to that with 100-400 Hz input.

We compiled a list of MCell seeds that would produce 19 NMDA and 63 AMPA receptors at our synapse and use of seeds from this list led to our second result. We found that calcium influx and CaMKII activation varied 2-3 fold among different stochastic runs.

Discussion. If CaMKII activation is switch-like, then stochastic variations might allow low frequency tetani to produce potentiation that otherwise might not occur. We would hope that potentiation might be a more robust phenomenon than this. Perhaps we need to model phosphatase activation more carefully than at present. Then isolated forays into highly activated CaMKII regions might be corrected. Alternatively, perhaps the probability of release at synapses needs to be taken into account. We are looking into both of these possibilities.

REFERENCES

Bradshaw JM, Kubota Y, Meyer T, Schulman H. 2003. *Proc. Natl. Acad. Sci.* 100(18):10512-10517.

Coomber C. 1998a. *Computers and Chemistry* 22:251-263.

Coomber, CJ. 1998b. *Neural. Comput*. 10:1653-1678.

Gillespie, DT. 1976. *J. Comput. Physics* 22:403-434.

Gillespie, DT. 1977. *J. Stat. Physics* 16(3) 331-338.

Holmes, WR. 2000. *J. Computat. Neurosci.* 8:65-85.

Lisman, J. 1985. *Proc. Natl. Acad. Sci.* 82:3055–3057.

Lisman, J. E., and M. A. Goldring. 1988. *Proc. Natl. Acad. Sci.* 85:5320–5324.

Okamoto H, Ichikawa K. 2000. *Biol. Cybern.*, 82: 35-47.

Stiles JR and Bartol TM. 2001. In: *Computational Neuroscience: Realistic Modeling for Experimentalists.* E. DeSchutter (ed.), CRC Press: Boca Raton.

Zhabotinsky AM. 2000. *Biophys J* 79: 2211-2221.