Spatially Realistic Monte Carlo Simulations Predict Calcium Dynamics Underlying Transmitter Release at a Neuromuscular Active Zone

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The effect of active zone (AZ) ultrastructure on neurotransmitter release remains poorly understood. A supralinear (~4th order) relationship (*CRR*) between extracellular Ca^{2+} ($[Ca^{2+}]_o$) and transmitter release indicates that multiple Ca^{2+} ions are required for fusion of a synaptic vesicle (SV), but how this empirical observation relates to the stoichiometry and architecture of voltage-gated Ca^{2+} channels (VGCCs), Ca^{2+} binding sites, and SVs is unclear. We created a spatially realistic model of a frog neuromuscular AZ, and used MCell (www.mcell.psc.edu) to simulate action potential (AP)-induced Ca^{2+} influx through VGCCs, Ca^{2+} binding to SVs, and several models of Ca^{2+}-dependent SV fusion. We varied spatial parameters to simultaneously reproduce 3 experimental observations: (1) average release probability (p_r) per trial per AZ at physiological $[Ca^{2+}]_o$; (2) the distribution of release latencies (*Ldis*); and (3) the 4th order CRR. Also, a 4-state VGCC model reproduced macroscopic Ca^{2+} current kinetics, and the on and off rates for Ca^{2+} binding were based on the synaptotagmin-1 C2A domain. Given all these constraints, we obtained a surprisingly unique set of model parameters and several counter-intuitive predictions. With a VGCC:SV stoichiometry of 1:1 (supported by other experimental data), each SV contains ~20 Ca^{2+} binding sites, and 6 sites must be bound simultaneously to induce fusion. Alternative models were either much too Ca^{2+}-insensitive to reproduce p_r or could not simultaneously reproduce *Ldis* and *CRR*. These results demonstrate the dramatic sensitivity of *CRR*, p_r, and *Ldis* to presynaptic architecture, and suggest that vesicle fusion may require a variety of SNARE protein and membrane lipid binding sites for Ca^{2+}. Supported by NIH R01 GM068630 (JRS), R01 NS43396 (SDM), P20 GM065805 (JRS), P41 RR06009 (JRS).