Variance analysis of action potential-evoked calcium influx reveals low opening probability of presynaptic calcium channels at the frog neuromuscular junction

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Neurotransmitter release is a stochastic event that is tightly regulated by calcium entry via presynaptic voltage-gated calcium channels (VGCCs). Knowledge of the number of VGCCs at active zones (AZs) and VGCC opening probability in response to a single action potential is critical to understanding the calcium regulation of transmitter release. We have previously shown in frog motor nerve terminals, which contain a series of large AZs (~1µm long), that overall VGCC opening probability appears to be very low following a single nerve stimulation (Wachman et al., 2004, J. Neurosci 24:2877). To test this hypothesis, we performed calcium imaging experiments on single nerve terminals using Calcium Green-1, a high speed imaging acquisition system, and repeated stimulation of the motor nerve at low frequency (0.5 Hz). Successive fluorescence images before and after stimulation were collected from the terminal within well-focused AZ regions as identified by labeling and monitoring the postsynaptic acetylcholine receptors with rhodamine-conjugated  $\alpha$ -bungarotoxin. Binomial analysis then was performed on single pixels (0.275 µm resolution) that detected a calcium signal above noise in a sub-region of an AZ after nerve stimulation. Such analysis indicated an average of 7.5 VGCCs within each sampled AZ region, and that the mean opening probability was only ~0.12. Interpretation of our data was strengthened by treatment with 50 µM 3,4-DAP to block a fraction of presynaptic  $K^{\dagger}$  channels, which, as expected, increased the probability of opening. Thus, our data strongly support the idea that a single action potential normally opens a very small fraction of VGCCs within each AZ, which could explain the characteristic low probability of transmitter release at single AZs of the frog neuromuscular junction.

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