Appendix: Theoretical predictions for changes in calcium signals detected at the single pixel level

Joel R. Stiles

Mellon College of Science & Pittsburgh Supercomputing Center, Carnegie Mellon University, Mellon Institute Rm 220A, Pittsburgh, PA 15213 and Department of Neuroscience, University of Pittsburgh, Pittsburgh, Pennsylvania, 15260

There are three key experimental findings in the preceding paper: (1) high trial-to-trial variability of calcium signals detected at the single pixel level under normal conditions; (2) a graded reduction in pixel signal after a reduction in the driving force for calcium entry (reduced extracellular calcium concentration); and (3) a virtually all-or-none reduction of pixel signals after block of a subset of the voltage-gated calcium channels (VGCCs) present in the nerve terminal. The density of VGCCs in the active zone (AZ) is unknown, and therefore the number of VGCCs (n) that contribute to the signal detected by an overlying pixel is also unknown. In addition, the average probability that any given VGCC opens during a trial ($\bar{p}$) is unknown. We wish to determine what combination of possible values for n and $\bar{p}$ would be most likely to produce the key experimental results.

Anatomical evidence (discussed in preceding paper) suggests an upper limit of ~200 VGCCs per AZ. Because a linear array of 4 pixels is required to sample each AZ, each pixel would sample ~50 VGCCs. A lower limit cannot presently be established, but a common assumption is one VGCC per synaptic vesicle, leading to ~30 VGCCs per AZ, or 6-8 VGCCs per pixel. Possible limits on $\bar{p}$ are even more difficult to establish at present, and so here we illustrate predicted results for several values that cover a broad range.

Given n VGCCs sampled by each pixel, and given that each VGCC has a probability $\bar{p}$ of opening during a trial (action potential), the probability that r out of the n channels will open (p_r) is obtained from a binomial distribution:

$$p_r = \binom{n}{r} (\bar{p})^r (1-\bar{p})^{n-r} \quad ; \quad \binom{n}{r} = \frac{n!}{r!(n-r)!} \qquad \text{(Eq. 1)}$$

In principle, r may have any value from 0 (all channels fail to open) to n (all channels open), and the sum of all p_r values is equal to one $\left(\sum_0^n p_r = 1\right)$. The average value of r ($\bar{r}$) across multiple trials is given formally by the expectation of r $\left(E(r) = \sum_0^n (r \ p_r)\right)$, and, as intuition suggests, in this case simplifies to $\bar{r} = n \ \bar{p}$. The standard deviation (*SD*) of r is given by $\sqrt{n \ \bar{p}(1-\bar{p})}$, and a measure of relative variability across different sets of conditions is given by the coefficient of variation $(CV = SD/\bar{r})$.

Figure A1 shows distributions of p_r values computed from Eq. 1, using a low (0.1) or high (0.8) value for $\bar{p}$ and a range of values for n. Each panel also shows the corresponding values of $\bar{r}$ and *CV*. *CV* increases as n or $\bar{p}$ decreases, and thus a high degree of experimental variability argues for a relatively low value of n and/or $\bar{p}$. The probability of a failure (r = 0, probability p_0) also increases as n or $\bar{p}$ decreases, although for large values of $\bar{p}$ the actual magnitude of p_0 is very small even for small values of n.

For the case of a failure, Eq. 1 simplifies to:

$$p_0 = (1 - \bar{p})^n \qquad \text{(Eq. 2)}$$

The probability that a failure does not occur ($r > 0$, probability $p_{>0}$) therefore is given by:

$$p_{>0} = 1 - p_0 = 1 - (1 - \bar{p})^n \qquad \text{(Eq. 3)}$$

Assuming a linear detection system, the average calcium influx to be detected by fluorescence at the single pixel level is proportional to the average value (expectation) of r for all non-zero events $(E(r_{>0}))$. In other words, VGCCs that fail to open do not contribute to the signal, and so the proportional contributions of the remainder must be normalized to $p_{>0}$:

$$\bar{r}_{>0} = E(r_{>0}) = \sum_{1}^{n} \left(r \frac{p_r}{p_{>0}} \right) \qquad \text{(Eq. 4)}$$

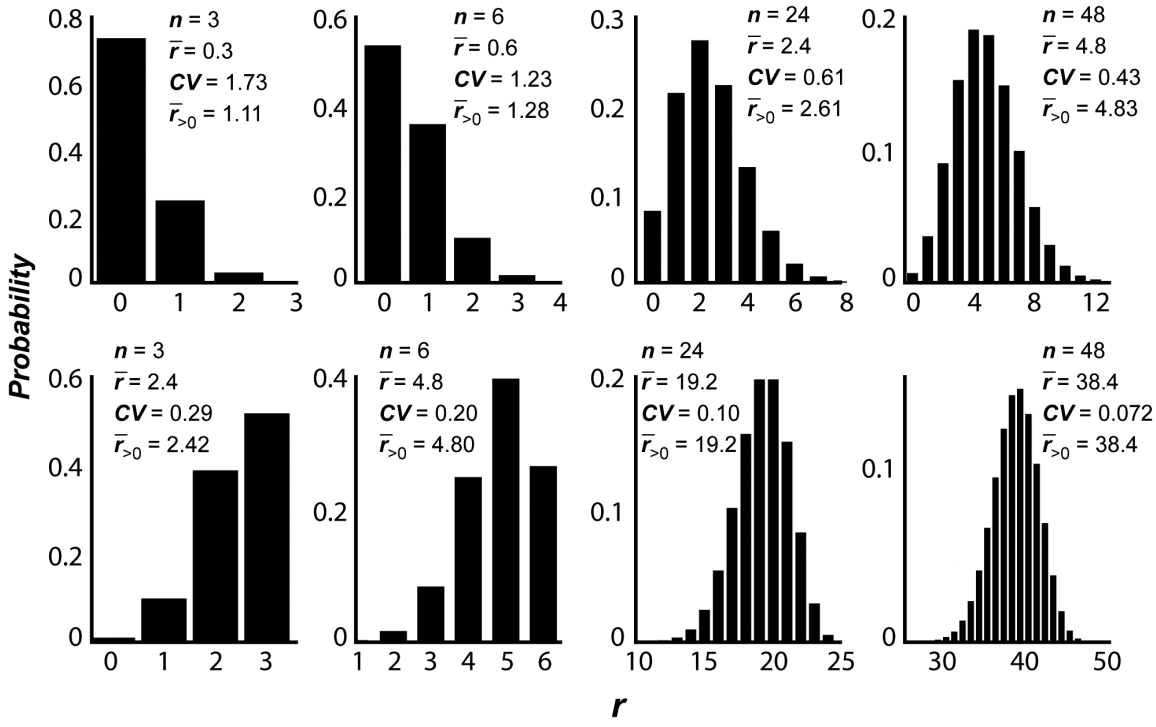
Using Eqs. 2-4, the values of p_0 and $\bar{r}_{>0}$ can be computed for different assumed values of n and $\bar{p}$. In Fig. A1, p_0 is shown by the first bar in each panel, and $\bar{r}_{>0}$ is indicated. By computing these values after a reduction in n, the proportional effect of blocking a fraction of VGCCs then can be predicted. For example, Fig. A1 shows a 2-fold reduction in n from 48 to 24 for the assumed upper extreme of VGCC density, and from 6 to 3 for the lower extreme. Table A1 summarizes the effects of such a 2-fold reduction in n for $\bar{p}$ values of 0.1, 0.2, and 0.8.

In the preceding paper, an experimental reduction in *n* of about 2-fold produced a similar decrease in total calcium signal, but the intensity of remaining single pixel signals did not decrease significantly. To observe this all-or-nothing effect at the single pixel level, the value of $\bar{r}_{>0}$ must be approximately the same before and after blocking half of the VGCCs, i.e., $\bar{r}_{>0}^{\,n} \approx \bar{r}_{>0}^{\,n/2}$. Table A1 shows that this is predicted only when *n* is at the low extreme and $\bar{p}$ is very small, on the scale of 0.1 ($\bar{r}_{>0}^{\,n/2} \approx 0.9\ \bar{r}_{>0}^{\,n}$). Under these conditions, the predicted increase in the proportion of failures is relatively small (less than 2-fold). In contrast, large values of $\bar{p}$ and/or large values of *n* predict single pixel changes that scale nearly in direct proportion to the change in *n* ($\bar{r}_{>0}^{\,n/2} \approx 0.5\ \bar{r}_{>0}^{\,n}$), i.e., a graded response is expected. At the same time, the predicted change in proportion of failures increases enormously, although this is simply a consequence of the very small overall frequency of failures. In summary, the experimental data presented in the preceding paper argue very strongly and specifically for both sparse density (about one per vesicle) and low opening probability (on the scale of 0.1) for VGCCs at the frog neuromuscular junction.

As $\bar{p}$ approaches 0, Eq. 2 can be approximated by $p_0 = (1-\bar{p})^n \approx 1 - n\ \bar{p}$. The right-hand side of this expression shows that a decrease in *n* will cause a relatively small increase in p_0, because *n* simply modifies the already small value of $\bar{p}$. On the other hand, as $\bar{p}$ becomes appreciably larger than 0, this simplified expression for Eq. 2 does not apply. A decrease in *n* will cause a very large relative increase in p_0 because *n* is an exponential modifier of the small quantity $(1-\bar{p})$.

Fig. A1. Binomial probability distributions for an assumed average opening probability ($\bar{p}$) of 0.1 (top row) or 0.8 (bottom row). See text for explanation of additional computed values.

Table A1. Predicted effect of a 2-fold reduction in n on the probability of failure (p_0) and average single pixel signal intensity ($\bar{r}_{>0}$). Values are shown as ratios, where the superscript n refers to the indicated values of 6 or 48, and the superscript $n/2$ refers to a reduction to 3 or 24, respectively.


Probability
n = 3
r̄ = 0.3
CV = 1.73
r̄>0 = 1.11
n = 6
r̄ = 0.6
CV = 1.23
r̄>0 = 1.28
n = 24
r̄ = 2.4
CV = 0.61
r̄>0 = 2.61
n = 48
r̄ = 4.8
CV = 0.43
r̄>0 = 4.83
n = 3
r̄ = 2.4
CV = 0.29
r̄>0 = 2.42
n = 6
r̄ = 4.8
CV = 0.20
r̄>0 = 4.80
n = 24
r̄ = 19.2
CV = 0.10
r̄>0 = 19.2
n = 48
r̄ = 38.4
CV = 0.072
r̄>0 = 38.4
r

	n	$\bar{p} = 0.1$	$\bar{p} = 0.2$	$\bar{p} = 0.8$
$p_0^{n/2} / p_0^n$	**6**	1.37	1.95	125
	48	12.5	212	6×10^{16}
$\bar{r}_{>0}^{n/2} / \bar{r}_{>0}^{n}$	**6**	0.864	0.756	0.504
	48	0.540	0.502	0.500