The variance of successive peaks in synaptic amplitude histograms: effects of inter-site differences in quantal size

L. M. WAHL, K. J. STRATFORD, A. U. LARKMAN AND J. J. B. JACK

University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K.

SUMMARY

Variability in the measured amplitude of evoked synaptic events can arise from several factors, including: measurement noise, trial-to-trial variation in the amplitude of the response at a single release site, or variation between different release sites (inter-site variation) in the mean amplitude of the single quantal response. Classic (linear) models of variability include only the first two of these factors, although differences in the number of postsynaptic receptors or the degree of electrotonic attenuation for different release sites could cause substantial inter-site variations in quantal size. In this paper, the effect of inter-site variation on the variance of successive histogram peaks has been determined analytically and verified by computational studies. This effect is minimal at the edges of the histogram and contributes maximally to central peaks. Linear approximations to the variance of successive histogram peaks may therefore result in very poor fits to measured data if substantial inter-site variation in quantal size is involved. Our computational results indicate that for synaptic contacts with high release probabilities and substantial inter-site variation, the variance of histogram peaks will decrease with increasing quantal content.

1. INTRODUCTION

The classic studies of Katz and others (e.g. del Castillo & Katz 1954; Boyd & Martin 1956) at the vertebrate neuromuscular junction described small, spontaneously occurring potentials whose peak amplitudes varied unimodally about a mean size, with a coefficient of variation (CV, defined as standard deviation divided by mean) of approximately 30%. Histograms of the peak amplitude of evoked endplate potentials showed clear peaks at integer multiples of the mean amplitude of the spontaneous events. It was thus suggested that evoked endplate potentials are composed of a set of basic building blocks, quanta, whose amplitudes can be described by the amplitude distribution of the spontaneous miniature potentials.

For synapses of the central nervous system, however, the evidence in favour of this quantal hypothesis has been less clear. Difficulties in resolving distinct peaks in evoked amplitude histograms have undermined efforts to estimate both quantal size (the mean amplitude of a single quantal event) and quantal content (the number of quanta contributing to a given evoked response). It remains unclear whether the fundamental quantal unit represents the postsynaptic response to a packet of neurotransmitter, the response at a single synaptic apposition between the pre- and postsynaptic cells, the response of an isolated cluster of postsynaptic receptors, or some combination of these effects (see Korn & Faber 1991). For simplicity, we will use the term 'release site', to mean an independent point of contact between the pre- and postsynaptic cells, such that each release site either fails to respond or contributes a single quantum to an evoked response.

Variability in the measured amplitude of an evoked potential can arise from several different factors. The amplitude of the response at a given release site will have an intrinsic trial-to-trial variability, caused by such factors as random fluctuations in the number of neurotransmitter molecules released or stochastic channel opening. If the amplitude of the response at each site is independent and drawn from a given probability distribution (for example, normally distributed with the same mean, μ, and the same variance, σ²), the variance of the summed response will equal the sum of the variances of the individual quanta. Thus the intra-site variance associated with responses of quantal content k is given by kσ². This is the classic model of the variance of the kth peak in an amplitude histogram, used in studies such as del Castillo & Katz (1954) and Boyd & Martin (1956). We will refer to this variation in quantal responses as Type 1 quantal variance, following Walmsley (1993).

In central synapses, the contaminating noise of the system (instrumental and biological noise) will also make a significant contribution to variability. If the noise (measured in the same way as the response amplitude) is independent of the measured amplitude and has variance σ²n, the variance associated with responses of quantal content k can be described as:

\[ \sigma^2_k = \sigma^2_n + k\sigma^2. \]  

(1)

Here we have symbolized the intra-site variance as σ²n, to emphasize that this term represents the contribution...
of Type I quantal variance. This model of variance has been used in fitting sums of Gaussian distributions to histograms obtained from evoked events in central synapses (Kullmann 1989, 1993; Redman 1990; Voronin et al. 1992; Jonas et al. 1993; Stricker et al. 1994; Stricker & Redman 1994).

We have applied equation (1) to the analysis of amplitude histograms of evoked excitatory postsynaptic potentials (EPSPs) recorded from in vitro preparations of area CA1 of rat hippocampus. We found, however, that the variance associated with peaks of greater quantal content can decrease, rather than increase: ‘apparent negative quantal variance’ (Stratford et al. 1994; Jack et al. 1994). This implies that models of variability that incorporate only contaminating noise and the variance of the single quantal response (Cf. equation (1)) are inadequate to describe our measured data.

A further source of variability, however, may occur if the mean amplitude of the single quantal response differs at different release sites. We will refer to this inter-site variation in the mean quantal size as Type II quantal variance (Walmsley 1993). Consider the simple case of a synaptic contact with two independent sites of contact between the pre- and postsynaptic cells, that on average have quantal sizes of 90 µV and 110 µV, respectively. If each site has no intrinsic variability (no Type I quantal variance), then when both sites contribute to the response the recorded potential will be 200 µV, with zero variance. If only one site contributes, however, the potential will be either 90 µV or 110 µV. Therefore, the variation in the single quantal response will be greater than the variation when both sites contribute; Type II quantal variance obviously does not always cause the variance of successive peaks to increase linearly with increasing quantal content.

Figure 1a shows one possible configuration of two independent release sites, illustrating the sources of Type I and Type II quantal variance. Figure 1b

---

Figure 1. Type I and Type II quantal variance. (a) Schematic diagram of a simple synaptic contact. Here two axonal boutons contact dendritic spines of the same postsynaptic cell. This illustrates one possible configuration of two independent ‘release sites’ which may, on average, have different quantal sizes. (b) A peaky amplitude histogram. Bars show an amplitude histogram of 248 EPSPs recorded from an in vitro preparation of a CA1 pyramidal cell of the rat hippocampus. Amplitudes have been binned at 40 µV. The solid line shows the same data smoothed using a digital Gaussian filter (standard deviation of the filter, 40 µV) and scaled for comparison to the original histogram. (c) Fit with Type I quantal variance. The smoothed histogram shown in (b) (circles) was fitted by a χ² minimization algorithm to the sum of three Gaussian distributions, with the constraint that the variance of successive Gaussians must increase linearly (equation (1)). Although the first and second peak could be well matched by Gaussians with standard deviations of 78 µV and 108 µV respectively, this constrained the standard deviation of the third Gaussian to be 131 µV, which obscured the third peak (solid line). (d) Fit with Type II quantal variance. The same fit was performed as in (c), except that the variance of the first and third Gaussians were constrained to be equal, as would be the case for Type II quantal variance alone (solid line). Note that there are no additional degrees of freedom. Circles as in (c).
shows a histogram of 248 amplitudes recorded from a hippocampal pyramidal cell binned at 40 μV; the solid line shows the same data smoothed using a digital Gaussian filter. It should be noted that this smoothing is strictly for illustrative purposes and that both the analytic and computational studies in the paper apply to unbinned, unsmoothed amplitudes. This histogram shows clear peaks separated by about 260 μV, and deconvolution indicates that the third peak has a smaller variance than the central peak. These data can be closely matched using a combination of Type I and Type II quantal variance, but are not well fitted under the assumption of Type I quantal variance alone (panels c and d).

Previous studies have not explicitly considered the effect of Type II quantal variance on the variance of successive peaks in an evoked amplitude histogram. The purpose of this study is to derive analytically the effects of Type II quantal variance on the variance of successive peaks in amplitude histograms, to determine the effects of various combinations of Type I and Type II quantal variance and to investigate the appropriateness of linear approximations to the variance of successive peaks across histograms.

2. ANALYTICAL FORMULATION

Consider a synaptic contact with \( n \) independent release sites, where the probability of neurotransmitter release (in response to an applied stimulus), \( p \), is the same at each site. Suppose that the quantal size at the \( i \)th site is given by \( q_i \), where \( i \) runs from one to \( n \), and that there is no trial-to-trial variation of the quantal size at each site (no Type I quantal variance). Further assume that the contaminating noise, \( \sigma^2_q = 0 \).

Let \( \bar{q} \) symbolize the mean quantal size, \( \Sigma q_i/n \), and let \( d_i = q_i - \bar{q} \) symbolize the difference between \( q_i \) and the mean quantal size. The variance of the quantal size is then

\[
\sigma^2_{q_i} = \frac{\sum_{i=1}^{n} (q_i - \bar{q})^2}{n} = \frac{\sum_{i=1}^{n} d_i^2}{n}
\]

Finally, for convenience, we will use \( D \) to represent the sum of squared differences, \( \Sigma d_i^2 \).

Consider an evoked amplitude histogram of \( N_p \) amplitudes, where successive peaks in the histogram, including the failures peaks, are numbered from left to right as zero to \( n \). In general, for \( n \) release sites, there are \( n+1 \) possible peaks in the amplitude histogram, but some of these peaks (especially for extreme values of \( p \) or low \( N_p \)) may be very poorly sampled. We will assume for the moment, however, that the theoretical distribution of amplitudes has been perfectly sampled (i.e. that \( N_p \rightarrow \infty \)) and later consider the effects of finite sampling errors.

Because \( p \) is the same at all sites, the total number of amplitudes in the \( k \)th peak, \( N_k \), is given by the simple binomial distribution:

\[
N_k = \binom{n}{k} p^k(1-p)^{n-k}N_p.
\]

We know that peak \( k \) consists of amplitudes which result from combinations of \( k \) sites releasing simultaneously; each amplitude contributing to the \( k \)th peak will be the sum of a combination of \( k \) quantal sizes. We can see that there will be \( \binom{n}{k} \) of these different combinations, and that the relative frequency of each combination in the \( k \)th peak will be the same. Thus from equation (2) we deduce that the \( N_k \) amplitudes in the \( k \)th peak consist of \( \binom{n}{k} \) different combinations of \( k \) quantal sizes, with \( p^k(1-p)^{n-k}N_p \) amplitudes per combination. Furthermore, if one of these combinations contains a given \( q_i \), then the remaining \( n-1 \) amplitudes in the sum must be chosen from the remaining \( n-1 \) quantal sizes. Thus a given \( q_i \) will occur in exactly \( \frac{n-1}{k-1} \) of these combinations.

As an illustrative example, let \( n = 3 \), \( p = 0.5 \), \( N_p = 1200 \) and consider the amplitudes which contribute to peak 2. Here we have \( p^2(1-p)^{n-k} = 1/8 \), and thus by equation (2) peak 2 will contain \( N_k = 450 \) amplitudes. Each of these amplitudes will be one of the three possible combinations of two quantal sizes, i.e. \( p^2(1-p)^{n-k}N_p = 150 \) amplitudes equal to \( q_1 + q_2 \), 150 of \( q_2 + q_3 \), and 150 of \( q_1 + q_3 \). We can see that any given \( q_i \) occurs in just \( \frac{n-1}{k-1} = 2 \) of these combinations.

The mean of peak \( k \), \( \mu_k \), will be the sum of all of the amplitudes in the \( k \)th peak, divided by \( N_k \). To return to our example:

\[
\mu_k = \frac{150(q_1 + q_2) + 150(q_2 + q_3) + 150(q_1 + q_3)}{450} = \frac{150}{450} \sum_{i=1}^{3} 2q_i = \frac{2 \sum_{i=1}^{3} q_i}{3} = 2\bar{q}.
\]

In general, because we have \( p^k(1-p)^{n-k}N_p \) amplitudes of each combination and because a given \( q_i \) will appear in exactly \( \binom{n-1}{k-1} \) different combinations, we have:

\[
\mu_k = \sum_{i=1}^{n} \binom{n}{k} p^k(1-p)^{n-k}N_p \cdot \binom{n-1}{k-1} q_i = \frac{n}{k} \binom{n}{k} p^k(1-p)^{n-k}N_p q_i,
\]

which reduces to:

\[
\mu_k = \frac{k}{n} N_p \sum_{i=1}^{n} q_i = k\bar{q}.
\]

Now let \( s_k^2 \) symbolize the variance of peak \( k \) about its mean. In the example, the variance of peak 2 is then:

\[
\sigma^2_k = \frac{1}{n} \sum_{i=1}^{n} (q_i - k\bar{q})^2 = \frac{1}{n} \sum_{i=1}^{n} d_i^2
\]
\[ \sigma_k^2 = \frac{1}{450} [150(q_1 + q_2 - 2q)^2 + 150(q_1 + q_2 - 2q)^2 + 150(q_1 + q_2 - 2q)^2] \]
\[ = \frac{1}{450} [(q_1 - q) + (q_2 - q)^2 + (q_2 - q)^2 + (q_2 - q)] \]
\[ + (q_2 - q)^2 + (q_1 - q) + (q_1 - q)] \]
\[ + \frac{1}{3} [(d_3 + d_2)^2 + (d_4 + d_2)^2 + (d_4 + d_3)^2] \]
\[ = \frac{1}{3} [2d_1^2 + 2d_2^2 + 2d_3^2 + 2d_4^2 + 2d_5^2 + 2d_4 d_3 + 2d_4 d_3 + 2d_4 d_3] \]
\[ = \frac{1}{3} \left[ \sum_{i=1}^{3} d_i^2 + \sum_{i=1}^{2} \sum_{m=1}^{2} (2d_i d_m) \right]. \]

In general, just as the entries in the \( k \)th peak consist of all the possible combinations of \( k \) \( q \)s, so the terms which contribute to \( \sigma_k^2 \) consist of all the possible combinations of \( k \) \( d \)s. Once again there will be \( \binom{n}{k} \) combinations, each a sum of \( k \) \( d \)s, each occurring \( p^k(1-p)^{n-k} \) times. The variance of the peak will then be, as in the example, the sum of these sums squared and divided by the total number of amplitudes in the \( k \)th peak.

When these combinations are squared and the results summed, however, we find a \( d_i^2 \) term for every combination containing a specific \( d_i \), that is, \( \binom{n-1}{k-1} \) terms. Similarly, for a specific \( d_i \) and \( d_m \), we find a \( 2d_id_m \) term for every combination containing both \( d_i \) and \( d_m \), i.e. \( \binom{n-2}{k-2} \) terms. As illustrated in the example above, this yields:

\[ \sigma_k^2 = \frac{1}{n} \left[ \binom{n-1}{k-1} D + \binom{n-2}{k-2} \sum_{i=1}^{n-1} \sum_{m=1}^{n-1} (2d_i d_m) \right]. \]

Re-arranging, we can add \( \binom{n-2}{k-2} D \) to the second term in the sum and subtract it from the first:

\[ \sigma_k^2 = \frac{1}{n} \left[ \binom{n-1}{k-1} \right] D \]
\[ + \binom{n-2}{k-2} \left[ D + \sum_{i=1}^{n-1} \sum_{m=1}^{n-1} (2d_i d_m) \right]. \]

But note that by definition \( \Sigma d_i = 0 \), and thus \( (\Sigma d_i)^2 = 0 \). If we expand \( (\Sigma d_i)^2 \), we find a \( d_i^2 \) term for each \( d_i \) and a \( 2d_id_m \) term for every possible combination of different \( d_i \) and \( d_m \). We thus have:

\[ (\Sigma d_i)^2 = D + \sum_{i=1}^{n-1} \sum_{m=1}^{n-1} (2d_i d_m) = 0. \]

Substituting into equation (3), above, gives:

\[ s_k^2 = \frac{1}{n} \left[ \binom{n-1}{k-1} \right] D, \]

which simplifies to:

\[ s_k^2 = \frac{k(n-k)}{n(n-1)} D. \]

Because the variance of the quantal sizes, \( \sigma_q^2 \), is equal to \( D/n \), we have derived the contribution of Type II quantal variance to the variance of the \( k \)th peak of the amplitude histogram:

\[ s_k^2 = \frac{k(n-k)}{n(n-1)} \sigma_q^2, \]

and we re-write equation (1) as:

\[ \sigma_k^2 = \sigma_q^2 + k\sigma_q^2 + \frac{k(n-k)}{n(n-1)} \sigma_q^2. \]

Two interesting normalizations of \( s_k^2 \) follow from equation (4). First, for a synaptic contact with \( n \) independent release sites, up to \( n+1 \) peaks may appear in the amplitude histogram, and for \( n \) even, a central peak exists at \( k = n/2 \). If we let \( k = n/2 \) in equation (4),

\[ s_k^2 = \frac{n}{2(n-1)} - \frac{n}{4(n-1)} D. \]

Note that \( s_k^2 \) corresponds to the variance in the central peak of the histogram, for \( n \) even. So if we consider the variance of the \( k \)th peak, normalized by the variance in the central peak, we find that the dependence of the normalized variance on peak position (the proportion \( k/n \)) is parabolic:

\[ s_k^2 = \frac{4}{n^2} \left[ \frac{k}{n} \right] \left[ 1 - \frac{k}{n} \right]. \]

The variable \( (k/n) \) represents the peak position in the histogram, normalized by the total number of non-failure peaks.

Another similar normalization can be made, which is applicable for both even and odd \( n \). The variance in the \( k \)th peak, normalized by the variance in the first peak, \( s_1^2 \), gives:

\[ s_k^2 = \frac{k(n-k)}{n-1}. \]

Note that as long as the release probability is the same at all sites, this solution is completely general with respect to the distribution of quantal sizes, that is, equations (4), (5) and (6) hold for any distribution of the \( q \) values.

3. Computational Studies

To test these theoretical predictions, computational studies of evoked amplitudes from a multi-site synaptic contact were conducted. Here any number of release sites could be defined, and the probability of release, \( p \), was constrained to be the same at all sites, unless otherwise noted. At each site, a mean quantal size, \( q \),
Effects of quantal variance on amplitude histograms  L. M. Wahl and others

Figure 2. Comparison of Type I and Type II quantal variance. Histograms of 10000 amplitudes were generated for $n = 8$, $p = 0.67$, $q = 100$ μV, and $\sigma_q = 0$; the histograms were smoothed by a Gaussian filter with a standard deviation of 60 μV. Dotted lines show the resulting histogram for $cv_q = cv_{q1} = 0$. (a) Type I quantal variance. The solid line shows the result for $cv_q = 10\%$, $cv_{q1} = 0$. (b) Type II quantal variance. The solid line shows the result for $cv_q = 0$, $cv_{q1} = 15\%$. Type II quantal variance contributes predominantly to the central peaks in the histogram, leaving outer peaks relatively sharp. Note that $cv_q$ and $cv_{q1}$ affect the heights of the peaks as well.

Figure 3. Effects of combinations of Type I and Type II quantal variance on the variance of successive peaks in an amplitude histogram. The solid lines show the variance of histogram peaks, normalized by the variance in the initial peak, plotted against peak number over $n$. An 8-release-site model was used, with $p = 0.67$, $cv_{q1} = 10\%$, and $cv_q = 0$, 5, 10 and 15%. As the contribution of Type I quantal variance increases, the parabolic effect of Type II quantal variance is dominated by the linear effect of Type I. The dotted line shows the case for $cv_{q1} = 0$. Note that the effect of Type II quantal variance alone, or for a mix of Types I and II, is very close to linear for the first quarter of the histogram ($k/n < 0.25$).

was defined. The expected trial-to-trial variation of this quantal size was also defined for each site; we use $cv_q$ to represent the coefficient of variation of this Type I quantal variance, expressed as a percentage. Note that the mean quantal sizes were not constrained to be the same at every site. We use $cv_{q1}$ to represent the coefficient of variation of Type II quantal variance, that is, the ratio of the standard deviation to the mean of the $n$ mean quantal sizes, expressed as a percentage.

To compute the amplitude of an evoked potential, for each site a random number between zero and one was drawn from a uniform distribution and compared with $p$. If the random number was less than $p$ (the site ‘fires’), a second random number was drawn from a normal distribution with mean $q$ and standard deviation given by $q_i/(cv_q/100)$, thus sampling the distribution of quantal sizes for that site. Note that the standard deviation of this distribution is thus scaled by $q_i$, such that the $cv$ is the same at all sites. The responses of all the sites, computed in this way, were then summed. Although the quantal content (the number of sites contributing to the total response) of synaptic potentials evoked during experiments on central synapses is often unclear, each computed amplitude had a known quantal content, and thus the variance of successive peaks of the amplitude histogram could be calculated with precision.

Figure 2 offers a simple comparison of the effects of Type I or Type II quantal variance alone, for an 8-site synaptic contact, and $p = 0.67$. The distribution of quantal sizes between the sites was modelled as Gaussian. As predicted, Type II quantal variance increases the variance of the central peaks in the histogram to the greatest extent, leaving the outer peaks sharp. Note especially the different effects of Type I and Type II quantal variance on the sharpness of peaks on the right-hand side of the histogram and that both $cv_q$ and $cv_{q1}$ affect the heights of the peaks as well.

Computational studies were also conducted to determine the effects of various combinations of Type I and Type II quantal variance. In these cases, both the distributions of quantal size at and between sites were modelled as Gaussian. Figure 3 shows the variance of successive peaks across an amplitude histogram ($n = 8$, $p = 0.67$), for $cv_{q1}$ of 10%, and for $cv_q$ ranging from 0 to 15%. As Type I quantal variance increases, the contribution of $cv_{q1}$ becomes negligible and the variance of successive peaks increases nearly linearly; the asymptote on the figure illustrates the case of Type I quantal variance alone.
Figure 4. Effects of linear fits to the variance of successive histogram peaks. (a) \( p \) constant, \( cv_x \) varying. 10,000 trials histograms were generated for \( cv_x \) of 0, 2.5, 5, 7.5 and 10%, with \( cv_{y} \) ranging from 0 to 50%. Again, an 8-site model was used with \( p = 0.67 \). The variance of successive peaks in the histogram was fitted to the equation \( \sigma_{x}^{2} = \sigma_{y}^{2} + k \sigma_{x}^{2} \), using a linear regression, and ignoring those peaks whose area was less than 3% of the total area under the histogram. The resulting best-fit slope, \( \sigma_{y}^{2} \), is plotted against \( cv_{y} \). Note that increasing amounts of Type II quantal variance cause increasingly negative solutions to the linear fit. (b) \( p \) varying, \( cv_{x} \) constant. Histograms were generated computationally and the variance of successive peaks fitted to a straight line as in (a), except that \( cv_{x} \) was fixed at 7.5%, and \( p \) was varied from 0.2 to 0.8 (as marked on the right edge of the figure). For values of \( p \) greater than 0.5, increasing \( cv_{y} \) or \( p \) resulted in increasingly negative solutions in the linear fit.

To test the effect of using equation (1) (which ignores any contributions of Type II quantal variance) to model quantal variance when \( cv_{y} \) is non-zero, we fitted straight lines to the variances of successive peaks in our 8-site model, using a conventional weighted least squares algorithm (Press et al. 1988) and ignoring peaks whose area was less than 5% of the total area under the histogram. A Gaussian distribution of quantal sizes was used in each case, with \( cv_{y} \) varying between 0–50%. The results of these regression analyses are shown in figure 4a, for \( cv_{x} \) ranging from 0 to 10%, with \( p = 0.67 \). This figure illustrates that increasing amounts of Type II quantal variance may result in increasingly negative slopes in linear fits to the variance of successive histogram peaks, i.e. the optimum solution of equation (1) gives \( \sigma_{y}^{2} \) less than zero.

Similarly, figure 4b shows the effect of a linear fit to the variance of successive peaks, with \( cv_{y} \) fixed at 7.5%, but with \( p \) ranging from 0.2 to 0.8. For \( p \) greater than 0.5, higher values of \( p \) or \( cv_{y} \) result in increasingly negative slopes in the linear fit. For \( p \) less than 0.5, the optimal solution of equation (1) always yielded a positive value for \( \sigma_{y}^{2} \). In these cases, increasing amounts of Type II quantal variance resulted in increasingly positive slopes in the linear solution; \( cv_{y} \) was always over-estimated in these cases.

In the analytic formulation, and in each of the computational studies reported above, the probability of release, \( p \), was constrained to be the same at all sites. Figure 5a illustrates the results of similar computational studies incorporating non-uniform release probabilities. Again we used an 8-site model, with \( cv_{y} \) fixed at 10% (\( cv_{y} = 0 \)). We chose eight values of \( p \) so that they represented equal areas under a normal distribution, that is, we divided a normal distribution with mean 0.5 and standard deviation \( \sigma_{y} \) into eight segments of equal area, and used the midpoint, in area, of each of these segments as one value for \( p \). (We use the shorthand notation \( p \sim N(0.5, \sigma_{y}) \) to represent this set of \( p \) values.) The eight values of \( p \) were randomly assigned to the eight quantal sizes and a histogram of 10,000 amplitudes was generated. The mean amplitude of each peak (the peak position) and variance of each peak in the amplitude histogram were recorded. This random assignment was then repeated 10,000 times and the average position and average variance of each peak were calculated. Note that the variance of this variance (because of random assignment of different release probabilities to different sites) could also be determined in this way.

From the figure, it is clear that non-uniform distributions of release probabilities reduce the variance of the peaks which is attributable to Type II quantal variance, but the general effect of \( cv_{y} \) to preferentially increase the variance in central peaks, is unchanged. It is worth noting that the release probabilities must have a fairly large standard deviation before this effect is apparent (only a 10% decrease in the variance of the central peak was found for \( p \sim N(0.5, 0.0225) \); this distribution corresponds to values of \( p \) ranging from 0.269 to 0.731). Figure 5a also shows results of the modelling when \( p \) is correlated with \( q \) (open circles), that is, when sites with higher quantal sizes are assigned higher release probabilities. In this case, the peak position is shifted slightly to the right and the variance reduced, but again the general shape of the curve is preserved.

To investigate the effects of a non-Gaussian distribution of \( p \), we reproduced these models for a set of release probabilities which had mean 0.5 and standard deviation 0.25, but were distributed bimodally (see the legend to figure 5 for details of the distribution). The results for the random assignment and for the correlation with quantal size are shown in figure 5a (filled and open squares, respectively) Once again, the
variance of the peaks in reduced, but the general shape of the curve is unchanged.

As mentioned above, the variance of the variance of histogram peaks because of the random assignment of release probabilities was calculated; figure 5b shows this result for the 8-site model, for 10000 random assignments of the release probabilities, and with $\rho \sim N(0.5, 0.0225)$ ($\rho$ ranges from 0.269 to 0.731). In these computations, 10000 amplitudes were included in each histogram to adequately sample each peak. To investigate the effects of finite sampling, however, the same computation was repeated, again with 10000 random assignments of $\rho$, but with only 500 amplitudes generated for each histogram. The additional variation produced by finite sampling is also shown in figure 5b. The variance of successive peaks for $\rho$ uniformly equal to 0.5 is also shown for comparison; note that these values are well within one standard deviation of the results for non-uniform $\rho$.

4. DISCUSSION

The trial-to-trial variation in the postsynaptic response at a single site, Type I quantal variance, is usually attributed to two main factors: variations in the number of transmitter molecules released, and the variation resulting from probabilistic channel opening. For $N$ independent channels, each with probability $p_o$ of opening, the predicted coefficient of variation caused by this latter factor is:

$$CV_p = \sqrt{1 - \frac{p_o}{Np_o}}.$$  (7)

Jonas et al. (1993) have shown experimentally that for fast application of high concentrations of glutamate to extrasynaptic glutamatergic AMPA receptors, the coefficient of variation of the peak conductance is well fitted by this expression. The relation between the two sources of Type I quantal variance, however, is complex. For large numbers of receptors (such as in the neuromuscular junction), variations in the number of molecules released will dominate; for small numbers of available postsynaptic receptors, variations resulting from probabilistic channel opening will produce the main effect (see Jack et al. 1994). Our computational studies, in which the standard deviation of the distribution of measured amplitudes is proportional to the quantal size at each site (such that $cv_i$ is the same for all sites) effectively assume that the latter effect is dominant, however we have also constrained the absolute value of Type I quantal variance to be the same at all sites in our computational studies, and found the results virtually indistinguishable (data not shown).

Two main factors could contribute to Type II quantal variance: differences between the numbers of postsynaptic receptor channels in each postsynaptic density and differences in electrotonic attenuation. Electron micrograph studies in the CA1 region of rat hippocampus (Harris & Landis 1986) reveal large variations in the density of particles in postsynaptic aggregates ($cv$ of 25%). Likewise, cable filtering is likely to have marked effects on the peak amplitudes.
recorded at the soma for both postsynaptic potentials and currents recorded under voltage clamp (Spruston et al. 1993; Major 1993; Major et al. 1994; Spruston et al. 1994). In studies of the mossy fibre-CAL synapse in rat hippocampus, Jonas et al. (1993) suggest that the variation associated with Type II quantal variance is between two and four times (cv) that associated with Type I. It is therefore highly probable that measures of the variation of single quantal events in central synapses could contain very large contributions from inter-site variations in quantal response.

Throughout the computational studies in this paper, cv, has been modelled as ranging between 0–15%. Although much larger values of cv, (50%) have been deduced from the study of miniature events in CAL cells (Bekkers et al. 1990; Raastad et al. 1992), the qualitative features of our results will still hold, but will be less evident under conditions of high cv,.

Our results indicate that the effect of Type II quantal variance is to increase the variance of successive histogram peaks such that no effect is seen at the first and last peaks, and maximum effect is seen in the central peaks. In the special case of the release probability being the same at all sites, the dependence of variance on peak number in the histogram can be described analytically as a parabola. Therefore linear approximations to the change in variance across amplitude histograms are likely to be inappropriate if Type II quantal variance is involved. Jonas et al. (1993) fit the variance of successive histogram peaks to equation (1) and argue that the resulting linear fit contains contributions from the variability of the number of transmitter molecules available, the probabilistic opening of channels and the number of receptor molecules available: the latter being the dominant effect. While we have shown that, in general, cv, will not scale linearly with peak number, in situations where release probability is low (such as conditions of reduced Ca++ concentration used by Jonas et al.) this assumption may be quite reasonable.

Specifically, it may be very difficult to distinguish the effects of Type I and Type II quantal variance at low release probabilities. Because the relative areas under the peaks of an evoked amplitude histogram depend on p, a finite sample obtained in an experimental situation may represent some of the theoretical peaks in the amplitude distribution very poorly, if at all. In fitting a straight line through the variance of successive peaks in the modelled amplitude histograms, we have ignored those peaks which contribute less than 5% to the total area, because these peaks might be very poorly defined in experimental data. Thus, for low p, early peaks are primarily sampled; as illustrated in figure 3, the effect of Type II quantal variance for peaks in the first quarter or third of the histogram is roughly linear. In the classic experiments in the neuromuscular junction (del Castillo & Katz 1954; Boyd & Martin 1956), a linear fit to the change in variance across successive peaks in the histogram would be appropriate, because the average release probability is considerably depressed. The slope of this line, however, will contain indistinguishable contributions from both Type I and Type II quantal variance.

Figure 2 illustrates that both the heights and areas of the peaks in amplitude histograms are sensitive to changes in Type I and Type II quantal variance. It will thus be very difficult to determine the distribution of inter-site variations in the probability of release based on peak height or area, unless the contributions of Type I and Type II quantal variance are known. In addition, our computational results suggest that even wide inter-site variations in p (see figure 5b) may be indistinguishable from the case of uniform release probability when finite sampling is considered.

It is clear from our results that Type II quantal variance could contribute to the ‘apparent negative quantal variance’ that has been reported. This phenomenon, however, has also been observed for histograms with estimated release probabilities less than 0.5 (Stratford et al. 1994; Larkman et al. 1992). We therefore speculate that some other factor, such as the non-independence of signal and noise, must be involved. We have previously made the observation that the variance of independently recorded noise was greater than the optimum variance associated with each peak entry, when amplitude histograms were fitted by sums of Gaussian distributions (Jack et al. 1990; Larkman et al. 1991); this phenomenon has been reported by other researchers (Liao et al. 1992; Voronin et al. 1992). Some of this discrepancy may be explained by biased sampling, that is, that histograms were selected from larger data sets partly on the basis of clarity of peaks. Biased sampling would not contribute, however, to apparent negative quantal variance.

If a significant proportion of the baseline noise arises from release sites on the stimulated fibre, however, it may be that a ‘refractory’ period following release at each site will cause variance reductions for larger amplitude peaks (Larkman et al. 1991). Consider the simple example of two release sites, each of which contribute significantly to the measured noise through spontaneous release of neurotransmitter. If both sites release in response to the applied stimulus, then neither were in a refractory state at the stimulus time and therefore neither could have contributed contaminated noise through release immediately before the stimulus. Experimentally, it is difficult to quantify the magnitude of this ‘noise reduction’, but it presents another possible factor affecting the variance of amplitude histogram peaks.

This work was supported by the Natural Science and Engineering Research Council of Canada, and by the Wellcome Trust. We thank Christophe Pouzet and Dr Martin Nowak for their comments on the mathematics.

REFERENCES

Effects of quantal variance on amplitude histograms  L. M. Wahl and others  85


Jonas, P., Major, G. & Sakmann, B. 1993 Quantal components of unitary EPSCs at the mossy fibre synapse on CA3 pyramidal cells of rat hippocampus. J. Physiol. 472, 615–663.


Received 10 July 1995; accepted 27 July 1995