Analysis and Implications of Equivalent Uniform Approximations of Nonuniform Unitary Synaptic Systems

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ABSTRACT Real synaptic systems consist of a nonuniform population of synapses with a broad spectrum of probability and response distributions varying between synapses, and broad amplitude distributions of postsynaptic unitary responses within a given synapse. A common approach to such systems has been to assume identical synapses and recover *apparent* quantal parameters by deconvolution procedures from measured evoked (ePSC) and unitary evoked postsynaptic current (uePSC) distributions. Here we explicitly consider nonuniform synaptic systems with both intra (type I) and intersynaptic (type II) response variability and formally define an *equivalent* system of uniform synapses in which both uePSC and ePSC amplitude distributions best approximate those of the actual nonuniform synaptic system. This equivalent system has the advantage of being fully defined by just four quantal parameters: $\tilde{n}$, the number of equivalent synapses; $\tilde{p}$, the mean probability of quantal release; $\tilde{\mu}$, mean; and $\tilde{\sigma}^2$, variance of the uePSC distribution. We show that these equivalent parameters are weighted averages of intrinsic parameters and can be approximated by apparent quantal parameters, therefore establishing a useful analytical link between the apparent and *intrinsic* parameters. The present study extends previous work on compound binomial analysis of synaptic transmission by highlighting the importance of the product of p and μ, and the variance of that product. Conditions for a unique deconvolution of apparent uniform synaptic parameters have been derived and justified. Our approach does not require independence of synaptic parameters, such as p and μ from each other, therefore the approach will hold even if feedback (i.e., via retrograde transmission) exists between pre and postsynaptic signals. Using numerical simulations we demonstrate how equivalent parameters are meaningful even when there is considerable variation in intrinsic parameters, including systems where subpopulations of high- and low-release probability synapses are present, therefore even under such conditions the apparent parameters estimated from experiments would be informative.

INTRODUCTION

The complexity of synaptic transmission between central neurons can pose fundamental problems to its investigators. The number of synaptic contacts between given sets of pre and postsynaptic neurons is often unknown, as are the average probability of release upon activation at each site and the average size of the postsynaptic event generated by the release of a potentially variable quantum of transmitter packaged in presynaptic vesicles of potentially variable sizes. Furthermore, there is no a priori reason that average properties should be constant and uniform from synapse to synapse. The overall measured evoked postsynaptic current (ePSC) distribution will be a convolution of the distribution of unitary evoked synaptic currents (uePSCs) evoked at each synapse. By itself, the ePSC distribution provides minimal information about actual quantal properties of the underlying system of synapses. Here we examine whether methods that assume a simpler and more uniform underlying synaptic system provide physiologically meaningful approximations of the true, complex behavior of the native system; we conclude that they can. We place our analysis within a physiological context by considering fast glutamatergic synaptic transmission between mammalian CNS neurons mediated by punctate unitary synapses, as occurs on hippocampal pyramidal neurons in situ and in tissue culture. Although hippocampal synapses are not necessarily representative of all types of CNS synapses in terms of morphology and biophysical characteristics, the main steps in synaptic transmission and the methods of statistical analysis should be comparable.

Presynaptic interactions at individual glutamatergic contacts between hippocampal neurons appear to be small or absent (Murphy et al., 1995; Diamond and Jahr, 1995; Asztely et al., 1997) such that each synaptic site is "binary" or binomial; it either releases a single quantum of transmitter or fails to respond when invaded by a presynaptic action potential (Jack et al., 1981; Redman, 1990; Faber and Korn, 1991; Raastad et al., 1992; Kullmann and Siegelbaum, 1995; Stevens and Wang, 1995; Rosenmund and Stevens, 1996; Walmley, 1995). Although a small proportion of terminals associated with punctate synapses may release more than one vesicle in response to an action potential (see Prange and Murphy, 1999) these terminals may contain more than one active zone/postsynaptic density complex (see Edwards, 1995) and hence might be considered to represent a cluster of independent unitary synapses, especially if release is determined by highly localized signals of elevated calcium.

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There is evidence for two broad groups of synapses. Some synapses are high-output with probabilities of release >0.5, while others are low-output with release probabilities <0.2 (Rosenmund et al., 1993; Hessler et al., 1993). This distribution may well be a continuous one and is certainly influenced by activity (Markram et al., 1997). Within a given synapse, variability in release probability can arise due to interactions between the stimulation pattern and the state of depletion/refilling of the presynaptic vesicular pools (Murthy et al., 1997; Dobrunz and Stevens, 1997; Markram et al., 1997). Although the presynaptic active zone and postsynaptic density are closely matched in size and dimensions, these excitatory glutamate synapses do vary in shape. Moreover, release probability seems to be directly correlated with synaptic area (Schikorski and Stevens, 1997). It is also possible that the mean amplitude of uePSCs will, up to a point, be correlated with synaptic area, while the variance of uePSCs at a given synapse will be inversely correlated with synaptic area (see Uteshev and Pennefather, 1996, 1997). This, in turn, implies that variance and mean amplitude will be inversely correlated. In addition, because of intrinsic variability in vesicle size and content, the number of glutamate molecules released during a given fusion event can vary (see Bekkers et al., 1990). Thus, the observed variability in quantal size observed within and between these synapses may arise from a number of causes. This variance clearly complicates quantal analysis, but because of its structural origin may be tuned and exploited biologically.

A deconvolution theory for nonuniform synaptic systems, where all intrinsic synaptic parameters simultaneously exhibit intra (type I), inter (type II), and temporal synaptic variability, is the most general but the least developed to date. To deal with the problem of nonuniform synaptic systems, theoretical studies typically consider partial nonuniformity, i.e., type I, type II, or temporal variability (Brown et al., 1976; McLachlan, 1975; Bennett and Lavidis, 1979; Walmsley, 1995; Stricker et al., 1994; Wahl et al., 1995; Frerking and Wilson, 1996; Quastel, 1997). Experimental conditions are therefore sought to separate these types of variabilities from each other and to study one type at a time (Bekkers et al., 1990; Raastad et al., 1992; Jack et al., 1994; Liu and Tsien, 1995a,b; Forti et al., 1997; Murthy et al., 1997; Dobrunz and Stevens, 1997; Liu et al., 1999). Here we consider in detail the general case where all quantal parameters can vary and demonstrate useful relationships between parameters derived for an equivalent uniform system and those describing the actual underlying synaptic system containing mixtures of binomial synapses of variable "loudness" (see Kullmann, 1999).

In the present study we ignored synaptic noise; however, the approach will be valid in the case of "noisy" synaptic systems as well. Addition of noise would have contributed one or more Gaussian amplitude distributions that would have convoluted with the response amplitude distributions to produce an overall "noisy" uePSC and/or ePSC amplitude distributions. Accordingly, this Gaussian noise amplitude distribution could have been deconvoluted from the overall distribution to reveal noise-free uePSC and ePSC amplitude distributions (for a review, see Redman, 1990).

THEORY

Equivalent uniform distributions: basic principles

It will be shown that an infinite variety of nonuniform synaptic systems can give rise to any given unimodal ePSC distribution. This impossibility of a unique solution does not, however, mean that such systems cannot be meaningfully quantified provided additional information is considered. We postulate that the simplest, most convenient, and most meaningful approximation to such nonuniform synaptic systems is the unique uniform synaptic system that best describes the experimentally recorded distribution. We then demonstrate how such an *equivalent* uniform system can be formally defined in terms of the underlying, *intrinsic* parameters of the nonuniform system. A specific set of *apparent* parameters can then be estimated from observation of such a system. Although the question of compound binomial systems has been discussed extensively (Brown et al., 1976; Mclachlan, 1975; Bennett and Lavadis, 1979; Korn and Faber, 1991; Quastel, 1997) our present analysis is more general and not only provides a useful extension of the previous work, but also gives rise to new insights into the stochastic nature of synaptic transmission.

Equivalent uePSC distributions

A hypothetical uniform synaptic system is defined as "equivalent" to a nonuniform system if it produces ePSC and uePSC amplitude distributions that closely approximate the actual (observed) distributions. Let $\alpha_j = p_j/\Sigma_{j=1}^{n} p_j$ denote a weighting factor, where $p_j = 1 - q_j$ is the probability of the evoked release at the jth synapse upon the arrival of an action potential. An equivalent uniform system will then be described by the normalized uePSC amplitude distribution function,

$$\tilde{f}^* \approx \sum_{j=1}^{n} \alpha_j f_j^* = \frac{\sum_{j=1}^{n} p_j f_j^*}{\sum_{j=1}^{n} p_j}.$$

Here, f_j^* is the normalized uePSC amplitude distribution of the jth synapse of the nonuniform system and $\tilde{f}^*$ is the normalized uePSC amplitude distribution of the equivalent synapse. (The asterisk indicates that the integral of the distribution function over the area where it is defined is normalized to unity, i.e., $\int_{-\infty}^{\infty} f_j^* dx = \int_{-\infty}^{\infty} \tilde{f}^* dx = 1$.)

Now, consider a system of n nonuniform synapses that produce a total of M uePSCs upon arrival of a train of K action potentials to the presynaptic terminals. If $p_j = 1$ for all synapses, then there are no failures and $M = nK$, otherwise $M \leq nK$. The jth synapse would contribute $M_j = p_j K$ events on average, such that $M = \Sigma_{j=1}^{n} M_j = K \Sigma_{j=1}^{n} p_j$. Therefore,

$$\frac{M_j}{p_j} = \frac{M}{\sum_{j=1}^{n} p_j} = K \tag{1}$$

The same Eq. 1 can be rewritten for the equivalent system, $\tilde{M}/\tilde{p} \approx M/\Sigma_{j=1}^{n} p_j$, where $\tilde{M}$ is the number of responses produced by each of the equivalent uniform synapses during a train of K action potentials and $\tilde{p}$ is the mean probability of release of each equivalent synapse. The number of responses, however, should remain constant whether equivalent or real synapses are considered; therefore, the following is true: $\tilde{n}\tilde{M} = M$. Hence,

$$\tilde{n}\tilde{p} \approx \sum_{j=1}^{n} p_j = n\langle p \rangle. \tag{2}$$

Here $\langle p \rangle$ is defined as the actual mean release probability. Equation 2 is the same as that derived previously for a simpler nonuniform system (Bennett and Lavadis, 1979).

Let us now represent a uePSC amplitude distribution produced by the jth synapse of the nonuniform system by a Gaussian distribution function, $f_j^* = (1/\sigma_j\sqrt{2\pi})\exp[-(x-\mu_j)^2/2\sigma_j^2]$. The overall uePSC amplitude distribution, f_Σ, (Fig. 1 *B*), which is a sum of Gaussian functions with different weighting factors, must also be a Gaussian function such that $f_\Sigma \approx \tilde{n}\tilde{M}\tilde{f}^*$, where $\tilde{f}^* = (1/\tilde{\sigma}\sqrt{2\pi})\exp[-(x-\tilde{\mu})^2/2\tilde{\sigma}^2]$. Because the mean and variance by definition will be approximately the same for both the equivalent and the actual uePSC distributions, we can equate actual parameters to the equivalent parameters using a moment-generating function. The first two moments around the origin of the characteristic function, $\tilde{\Phi}(t)$, will be, $\tilde{\Phi}'(t=0) = \tilde{\mu}$, and $\tilde{\Phi}''(t=0) = \tilde{\sigma}^2 + \tilde{\mu}^2$ (see Kendall, 1977). Thus,

$$\tilde{\Phi}(t) \overset{\text{def}}{=} \int_{-\infty}^{\infty} \tilde{f}^* e^{\text{xit}} dx = \sum_{j=1}^{n} \left\{ \frac{p_j}{\sum_{j=1}^{n} p_j} \exp[\mu_j it - (t^2\sigma_j^2/2)] \right\}, \tag{3}$$

$$\tilde{\mu}_1' = \tilde{\Phi}'(t=0) = \frac{\sum_{j=1}^{n} p_j\mu_j}{\sum_{j=1}^{n} p_j} = \tilde{\mu} \tag{4}$$

$$\tilde{\mu}_2' = \tilde{\Phi}''(t=0) = \frac{\sum_{j=1}^{n} p_j(\sigma_j^2 + \mu_j^2)}{\sum_{j=1}^{n} p_j} = \tilde{\sigma}^2 + \tilde{\mu}^2, \tag{5}$$

The equivalent values $\tilde{\mu}$ and $\tilde{\sigma}^2$ are thus related to the intrinsic parameters, essentially as weighted averages of the nonuniform synaptic values. The use of gamma distribution functions instead of Gaussian functions, such that

$$f_j^* = \frac{\beta^{\alpha_j} x_j^{\alpha_j - 1} e^{-\beta_j x_j}}{\Gamma(\alpha_j)}$$

and

$$\tilde{f}^* = \frac{\tilde{\beta}^{\tilde{\alpha}} x^{\tilde{\alpha}-1} e^{-\tilde{\beta}x}}{\Gamma(\tilde{\alpha})}$$

(where $\tilde{\mu} = \tilde{\alpha}/\tilde{\beta}$ and $\tilde{\sigma}^2 = \tilde{\alpha}/\tilde{\beta}^2$), leads to equations identical to Eqs. 4 and 5.

Let us introduce two components of the uePSC variance: variance within a given synapse (type I) such that $CV_{\text{I},j}^2 = (\langle x_j^2 \rangle - \mu_j^2)/\mu_j^2$ and variance between a given synapse and the mean for the system (type II), such that $CV_{\text{II},j}^2 = (\mu_j^2 - \tilde{\mu}^2)/\mu_j^2$. Therefore, from Eq. 5 we conclude,

$$\tilde{\sigma}^2 = \frac{\sum_{j=1}^{n} p_j\mu_j^2\left(\frac{\sigma_j^2}{\mu_j^2} + \frac{\mu_j^2 - \tilde{\mu}^2}{\mu_j^2}\right)}{\sum_{j=1}^{n} p_j} = \frac{\sum_{j=1}^{n} p_j\mu_j^2(CV_{\text{I},j}^2 + CV_{\text{II},j}^2)}{\sum_{j=1}^{n} p_j} \tag{6}$$

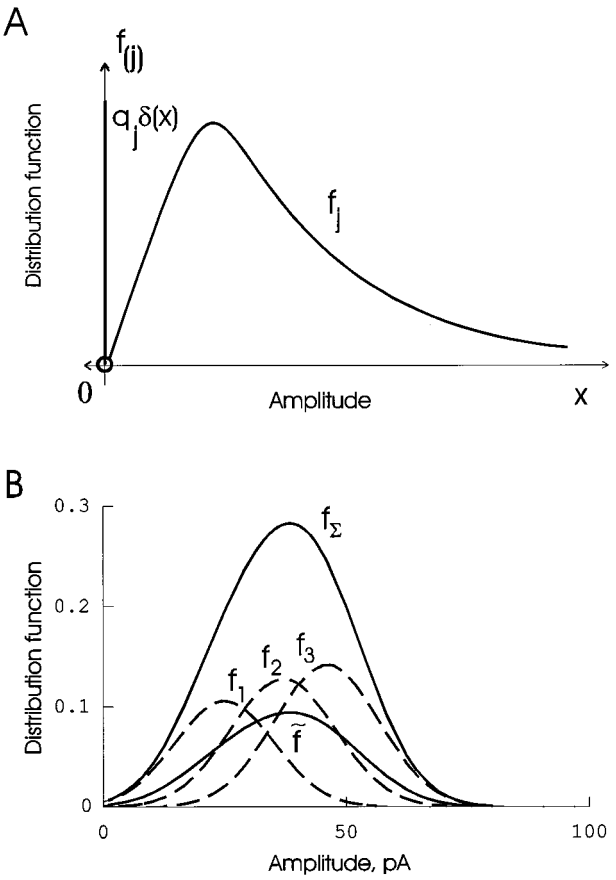

FIGURE 1 Definition of amplitude distributions and their components. (*A*) The complete amplitude distribution function of a unitary synapse. A complete amplitude distribution function, $f_{(j)}(x)$, is the sum of the delta function describing the amplitude distribution of release failures, $q_j\delta(x)$, and the partial amplitude distribution function, $f_j(x)$. The latter contains no information regarding failures. These two components reflect presynaptic and postsynaptic influences, respectively, and are of a fundamentally different nature. Note that the partial amplitude distribution function is not determined at zero amplitude, owing to the assumption that the source of transmission failures is strictly presynaptic and not due to any failure in detection. (*B*) An overall partial uePSC amplitude distribution function of unitary evoked events. A compound partial uePSC amplitude distribution function, f_Σ, is normalized to M and obtained as a sum of components, f_j, each normalized to M_j (*long dashes*), that correspond to amplitude distributions of unitary evoked synapses involved in transmission. An amplitude distribution function for an equivalent uniform system can be defined as, $\tilde{f} = f_\Sigma/\tilde{n}$ and is normalized to $\tilde{M}$.

Equivalent ePSC distributions

An ePSC consists of the summed responses of n independent but synchronous unitary synaptic events, each of which can vary in amplitude from one event to the next, or fail altogether. Therefore, the responses of each synaptic contact j measured over many stimulations can be described by an amplitude distribution consisting of the sum of two components (Eq. 7; Fig. 1 *A*): 1) the response failures, $q_j\delta(x)$, where $q_j = 1 - p_j$ and $\delta(x)$ is the Dirac delta function; and 2) the normalized failure-free uePSC amplitude distribution, $f_j(x) = (1 - q_j)f_j^*(x)$. The normalized complete amplitude distribution for the synapse j (containing both failures and responses and designated by the bracketed subscript) can thus be defined as,

$$f_{(j)}^*(x) = q_j\delta(x) + f_j(x) = q_j\delta(x) + (1 - q_j)f_j^*(x) \tag{7}$$

If a synaptic system contains n independent synaptic units, the resulting complete ePSC amplitude distribution $E_{(z)}$ will be expressed as a convolution

of the n individual complete uePSC amplitude distributions, such that

$$E_{(z)} = f_{(1)} * f_{(2)} * f_{(3)} * \cdots * f_{(n)} \equiv \left[\underset{j=1}{\overset{n}{*}} f_{(j)} \right] \tag{8}$$

The characteristic function, $\Phi_{(E)}(t)$ for Eq. 8, assuming that each failure-free distribution f_j is Gaussian, is given by,

$$\Phi_{(E)}(t) \overset{\text{def}}{=} \prod_{j=1}^{n} \int_{-\infty}^{\infty} f^{*}_{(j)}(x) e^{xit} dx \tag{9}$$

$$= \prod_{j=1}^{n} \{q_j + p_j \exp[\mu_j it + ((it)^2 \sigma_j^2)/2]\}.$$

We solve Eq. 9 using the Fourier convolution theorem and considering a logarithmic transformation. Hence,

$$\ln \Phi_{(E)}(t) = \cdots = (it) \sum_{j=1}^{n} \{p_j \mu_j\}$$

$$+ \frac{(it)^2}{2} \sum_{j=1}^{n} \{p_j \sigma_j^2 + p_j q_j \mu_j^2\} + \cdots. \tag{10}$$

Therefore,

$$\mu_{(E)} = \sum_{j=1}^{n} p_j \mu_j \tag{11}$$

$$\sigma^2_{(E)} = \sum_{j=1}^{n} p_j \sigma_j^2 + p_j q_j \mu_j^2 = \sum_{j=1}^{n} p_j \mu_j^2 (CV^2_{i,j} + q_j)$$

$$= \sum_{j=1}^{n} p_j (\sigma_j^2 + \mu_j^2) - \sum_{j=1}^{n} p_j^2 \mu_j^2 \tag{12}$$

Where $\mu_{(E)}$ is the mean and $\sigma^2_{(E)}$ is the variance of the complete ePSC amplitude distribution. Equation 12 is the same as that derived by Quastel (1997) using a different approach.

Equivalent ePSC amplitude distributions with uniform synapses

Equation 8 can be further simplified if n identical synapses are considered ("uniform synapses"). The function $E_{(z)}$ will simplify to,

$$E_{(z)} = q^n \delta(z) + \sum_{k=0}^{n-1} C_n^k q^k \left(\underset{}{\overset{n-k}{*}} f \right)$$

$$= q^n \delta(z) + E_{(z)}(z), \tag{13a}$$

where q is the probability of failure for all the synapses, $f \equiv f_j$ is their failure-free amplitude distribution, and C_n^k is the binomial coefficient (i.e., $C_n^k = n!/k!(n-k)!$). Equation 13a can be rewritten as follows for Gaussian and gamma distributions (see McLachlan, 1975, 1978):

$$E_{(z)} = q^n \delta(z) + \sum_{k=0}^{n-1} C_n^k q^k$$

$$(1-q)^{n-k} \exp[-(z-(n-k)\mu)^2/2(n-k)\sigma^2] \quad \text{(Gauss)} \tag{13b}$$

$$E_{(z)} = q^n \delta(z) + \sum_{k=0}^{n-1} C_n^k q^k$$

$$(1-q)^{n-k} \frac{\beta(\beta z)^{(n-k)\alpha-1} e^{-\beta z}}{\Gamma[(n-k)\alpha]} \quad \text{(Gamma)} \tag{13c}$$

Both gamma and Gaussian distributions are members of a class of unimodal distribution functions that convolute regularly and thus can be used for defining the uniform equivalent synapse systems. The failure-free ePSC amplitude distribution function of members of this class can be introduced as $E_z(z) = \Sigma_{k=0}^{n-1} C_n^k q^k (1-q)^{n-k} V_n^k(z)$. Based on the physical understanding of the failure-free distribution function $E_z(z)$, the kernel, $V_n^k(z)$, must be determined as the kth component of this distribution, i.e., as a convolution of k equivalent uePSC amplitude distributions with each other selected from a total of n distributions.

Because ePSC amplitude distributions represent sums of different components, i.e., a convolution of uePSC amplitude distributions, appropriate distribution functions for an equivalent uniform system should convolute analytically with each other, giving rise to a new function of the same class. Therefore, useful distribution functions must be *stable* with respect to convolution. This will minimize the number of parameters that is involved in describing the response distribution. Furthermore, the statistical moments generated by these equivalent distribution functions will be expressed in simplistic mathematical forms, readily permitting comparisons and physical interpretations.

To explicitly define the class of suitable equivalent distribution functions, let us consider an arbitrary (unimodal or multimodal) failure-free ePSC amplitude distribution consisting of n components, where n is the number of uniform synapses involved in transmission. An immediate consequence of the synaptic uniformity is that the mean of the $(n-k)$th individual component of the failure-free ePSC amplitude distribution must be equal to $(n-k)\mu$, where μ is the mean of the unitary component, which will be the same as the uePSC amplitude distribution (see above). Thus,

$$\int z V_n^k(z) dz \overset{\text{def}}{=} \mu(n-k), \tag{14a}$$

$$\int z^2 V_n^k(z) dz \overset{\text{def}}{=} (n-k)\sigma^2 + (n-k)^2 \mu^2 \tag{14b}$$

The kernel $V_n^k(z)$ denotes a class of failure-free distribution functions such that the first two statistical moments of $E_z(z)$ about the origin are given by Eqs. 15a and b, respectively.

$$\mu_1' = \mu_E = \int z E_z(z) dz$$

$$= \frac{\mu}{1-q^n} \sum_{k=0}^{n-1} \{C_n^k q^n (1-q)^{n-k} (n-k)\}$$

$$= \frac{n\mu(1-q)}{1-q^n} \tag{15a}$$

$$\mu_2' = \int z^2 E_z(z)dz$$

$$= \frac{1}{1-q^n}\sum_{k=0}^{n-1}\{C_n^k q^k(1-q)^{n-k}((n-k)\sigma^2 + (n-k)^2\mu^2)\}$$

$$= \frac{n(1-q)}{1-q^n}(\sigma^2 + \mu^2(q+n-nq))$$

$$= \frac{np}{1-q^n}(\sigma^2 + \mu^2 q) + \mu_E^2 \qquad (15b)$$

In Eqs. 15a and b we used the equalities

$$\sum_{k=0}^{n-1} C_n^k q^k(1-q)^{n-k}(n-k) = n(1-q)$$

and

$$\sum_{k=0}^{n-1} C_n^k q^k(1-q)^{n-k}(n-k)^2 = n(n-1)(1-q)^2 + n(1-q),$$

which we provide here without proofs. Although potentially the kernel $V_n^k(z)$ can have arbitrary shapes, we will consider only unimodal kernels.

Equations 15a and b or 14a and b comprise a formal definition for the class of distribution functions that are appropriate for defining equivalent uniform systems. In particular, it can be readily verified that Gaussian and gamma distributions belong to the above class.

The relationships between parameters describing complete and failure-free portions of the ePSC distribution function (identified by the subscripts (*E*) and *E*, respectively) for uniform systems are known, but can be obtained from Eqs. 11 and 12 by making all synaptic parameters identical, and combining with Eqs. 15a and b. Thus,

$$\tilde{\mu}_E(1-\tilde{q}^n) = \tilde{\mu}_{(E)} = \tilde{n}\tilde{\mu}\tilde{p}, \qquad (16)$$

$$\tilde{\sigma}^2_{(E)} = \tilde{n}\tilde{p}(\tilde{\sigma}^2 + \tilde{q}\tilde{\mu}^2) = \frac{\tilde{\mu}_{(E)}}{\tilde{\mu}}(\tilde{\sigma}^2 + \tilde{q}\tilde{\mu}^2), \qquad (17a)$$

or

$$\tilde{\sigma}^2_{(E)} = (1-\tilde{q}^n)(\tilde{\sigma}^2_E + \tilde{q}^n\tilde{\mu}^2_E) \qquad (17b)$$

Unique modeling of unimodal ePSC amplitude distributions

Using Eqs. 16 and 17a it is easily shown that there are an infinite number of sets of uniform quantal parameters that can describe a given unimodal ePSC amplitude distribution [i.e., (μ_1, σ_1, q_1, n_1), (μ_2, σ_2, q_2, n_2), . . . , (μ_i, σ_i, q_i, n_i), . . .]. Each such sets of parameters lead to identical values of $\mu_{(E)}$ and $\sigma^2_{(E)}$. Combining Eqs. 16 and 17a we obtain, for arbitrary *i* and *j*, $\mu_{(E)i} = \mu_{(E)j}$ and $\sigma^2_{(E)i} = \sigma^2_{(E)j}$. The latter leads to Eq. 18.

$$\frac{(\sigma_i^2 + q_i\mu_i^2)}{\mu_i} = \frac{(\sigma_j^2 + q_j\mu_j^2)}{\mu_j} \qquad (18)$$

and reduce the number of parameters involved to three. Thus, two of the four parameters need to be specified before a unique set of equivalent parameters can be estimated. For example, if μ and σ^2 are determined from the uePSC distribution and fixed at predicted values, such that $\mu_i = \mu_j = \mu$ and $\sigma_i^2 = \sigma_j^2 = \sigma^2$ for any *i* and *j*, then Eq. 18 will give $q_i = q_j = q$ and Eq. 16 will give $n_i = n_j = n$. In fact, a thorough analysis of Eqs. 16–18 determines that the uniqueness of the deconvolution will hold when the following pairs of parameters are known and fixed at correct values during the deconvolution: (μ and σ^2) or (q and n), or (μ and n), or (μ and q). In contrast, the knowledge of the pairs (σ^2 and q) or (σ^2 and n) is insufficient for a unique deconvolution.

Relation between actual and equivalent synaptic systems

For an equivalent complete ePSC amplitude distribution to approximate a nonuniform ePSC distribution, the equivalent complete mean, $\tilde{\mu}_{(E)}$, and variance, $\tilde{\sigma}^2_{(E)}$, must by definition be identical to values defined in Eqs. 11 and 12, respectively (because Gaussian functions are defined completely by their means and variances). Therefore, using Eqs. 11–17 we arrive at Eqs. 19 and 20.

$$\mu_{(E)} = \tilde{\mu}_{(E)} = \tilde{n}\tilde{p}\tilde{\mu} = \sum_{j=1}^{n} p_j\mu_j, \qquad (19)$$

$$\sigma^2_{(E)} = \tilde{\sigma}^2_{(E)} = \tilde{n}\tilde{p}(\tilde{\sigma}^2 + \tilde{\mu}^2) - \tilde{n}\tilde{p}^2\tilde{\mu}^2$$

$$= \sum_{j=1}^{n} p_j(\sigma_j^2 + \mu_j^2) - \sum_{j=1}^{n} p_j^2\mu_j^2 \qquad (20)$$

Solving Eqs. 2, 4, 5, 19, and 20 simultaneously we arrive at Eqs. 21 and 22.

$$\tilde{n}\tilde{p}^2\tilde{\mu}^2 = \sum_{j=1}^{n} p_j^2\mu_j^2; \qquad (21)$$

$$\tilde{n} = \frac{\left(\sum_{j=1}^{n} p_j\mu_j\right)^2}{\sum_{j=1}^{n} p_j^2\mu_j^2}; \qquad (22)$$

Equation 22 is somewhat similar to the equation derived by Bennett and Lavadis (1979) and Quastel (1997), but more general, as it now includes variability in $p_j\mu_j$.

Thus, all binomial quantal parameters needed to define an equivalent uniform system can be expressed in terms of weighted averages of the intrinsic quantal parameters of a nonuniform system. These relations are summarized in Table 1 and represent generalizations of some of the equations derived by Brown et al. (1976).

The meaning of $CV_{p\mu}$

If we define the following means:

$$\langle\mu\rangle \overset{\text{def}}{=} \frac{1}{n}\sum_{j=1}^{n}\mu_j, \qquad \langle p\rangle \overset{\text{def}}{=} \frac{1}{n}\sum_{j=1}^{n} p_j,$$

and

$$\langle p\mu\rangle \overset{\text{def}}{=} \frac{1}{n}\sum_{j=1}^{n} p_j\mu_j, \qquad \langle p^2\mu^2\rangle \overset{\text{def}}{=} \frac{1}{n}\sum_{j=1}^{n} p_j^2\mu_j^2,$$

TABLE 1 Relation between weighted averages of intrinsic quantal parameters and quantal parameters describing the equivalent uniform system

$\tilde{n} = \frac{(\Sigma_{j=1}^{n} p_j\mu_j)^2}{\Sigma_{j=1}^{n} p_j^2\mu_j^2}$;	$\tilde{p} = \frac{1}{\tilde{n}}\Sigma_{j=1}^{n} p_j = \frac{\Sigma_{j=1}^{n} p_j^2\mu_j^2}{\tilde{\mu}\,\Sigma_{j=1}^{n} p_j\mu_j}$;
$\tilde{\mu} = \frac{\Sigma_{j=1}^{n} p_j\mu_j}{\Sigma_{j=1}^{n} p_j}$;	$\tilde{\sigma}^2 = \frac{\Sigma_{j=1}^{n} p_j\mu_j^2\left(\frac{\sigma_j^2}{\mu_j^2} + \frac{\mu_j^2 - \tilde{\mu}^2}{\mu_j^2}\right)}{\Sigma_{j=1}^{n} p_j} = \frac{\Sigma_{j=1}^{n} p_j\mu_j^2(CV_{I,j}^2 + CV_{II,j}^2)}{\Sigma_{j=1}^{n} p_j}$

then Eq. 22 can be rewritten as

$$\tilde{n} = \frac{n^2\langle p\mu\rangle^2}{n\langle p^2\mu^2\rangle} = \frac{n\langle p\mu\rangle^2}{\langle p^2\mu^2\rangle} = n\frac{\langle p\mu\rangle^2}{\langle p\mu\rangle^2 + \sigma_{p\mu}^2} = \frac{n}{1 + CV_{p\mu}^2} \tag{23}$$

where $CV_{p\mu}$ is a coefficient of variation of the values $p_j\mu_j$. In contrast to the case considered by Brown et al. (1976), in the present case of continuous distributions, the means and variances are also variable. Therefore, the value CV_p used by Brown et al. (1976) is upgraded in our theory by a more general value of $CV_{p\mu}$. Similarly, Eq. 2 can be combined with Eq. 23 to give,

$$\tilde{p} = \frac{1}{\tilde{n}}\sum_{j=1}^{n} p_j = \frac{n}{\tilde{n}}\langle p\rangle = \langle p\rangle\frac{\langle p^2\mu^2\rangle}{\langle p\mu\rangle^2} = \langle p\rangle(1 + CV_{p\mu}^2) \tag{24}$$

Eqs. 23 and 24 lead to Eq. 25a,

$$(1 + CV_{p\mu}^2) = \frac{\langle p^2\mu^2\rangle}{\langle p\mu\rangle^2} \approx \frac{\langle p^2\rangle\langle\mu^2\rangle\langle p^2\mu^2\rangle}{\langle p\rangle^2\langle\mu\rangle^2\langle p^2\rangle\langle\mu^2\rangle} = (1 + CV_p^2)(1 + CV_\mu^2)\frac{\langle p^2\mu^2\rangle}{\langle p^2\rangle\langle\mu^2\rangle} \tag{25a}$$

The approximation $\langle p^2\mu^2\rangle \approx \langle p^2\rangle\langle\mu^2\rangle$ and the approximations $\langle p\mu\rangle = \langle p\rangle\tilde{\mu} \approx \langle p\rangle\langle\mu\rangle$ and $\langle p\mu^2\rangle \approx \langle p\rangle\langle\mu^2\rangle$ hold relatively well for sufficiently smooth distributions of the parameters p^2 and μ^2. Moreover, these equalities do not require synaptic parameters to be independent from each other under our definitions of means (the derivation and the computer simulations are not shown). Therefore, under these conditions,

$$(1 + CV_{p\mu}^2) \approx (1 + CV_p^2)(1 + CV_\mu^2), \tag{25b}$$

$$CV_{p\mu}^2 \approx CV_p^2 + CV_\mu^2 + CV_p^2CV_\mu^2, \tag{25c}$$

If synapses are uniform such that $\mu_j = \tilde{\mu}$ and $\sigma_j = \tilde{\sigma}$, then $CV_\mu = 0$. Under this condition $CV_{p\mu}$ becomes CV_p, such that Eq. 24 reduces to that derived by Brown et al. (1976).

Correlation between parameters of inter-synaptic (Type II) variability

In Eq. 25a we have separated the two components of the $CV_{p\mu}$. Here we take one further step to establish dependence among p-, μ-, and $p\mu$-variabilities. Consider three definitions: $\sigma_{p\mu}^2 = \langle p^2\mu^2\rangle - \langle p\mu\rangle^2$; $\sigma_p^2 = \langle p^2\rangle - \langle p\rangle^2$; $\sigma_\mu^2 = \langle\mu^2\rangle - \langle\mu\rangle^2$. From Table 1 we have

$$\tilde{\mu} = \frac{\sum_j^n p_j\mu_j}{\sum_j^n p_j} = \frac{\langle p\mu\rangle}{\langle p\rangle} \approx \langle\mu\rangle$$

and thus, $\tilde{\mu}^2\langle p\rangle^2 = \langle p\mu\rangle^2$. Solving all the above equations together, we obtain $\tilde{\mu}^2\langle p\rangle^2 = \tilde{\mu}^2(\langle p^2\rangle - \sigma_p^2) \approx \langle p\rangle^2(\langle\mu^2\rangle - \sigma_\mu^2)$. Therefore, for the relationship among $\sigma_{p\mu}^2$, σ_p^2, and σ_μ^2, we obtain:

$$\sigma_{p\mu}^2 = \tilde{\mu}^2\sigma_p^2 + \langle p^2\mu^2\rangle - \tilde{\mu}^2\langle p^2\rangle \approx \langle p\rangle^2\sigma_\mu^2 + \langle p^2\mu^2\rangle - \langle p\rangle^2\langle\mu^2\rangle \tag{26a}$$

Recalling that $\langle p^2\mu^2\rangle \approx \langle p^2\rangle\langle\mu^2\rangle$ for even distributions of p and μ, we further obtain

$$\sigma_{p\mu}^2 = \tilde{\mu}^2\sigma_p^2 + \langle p^2\rangle\sigma_\mu^2 = \langle p\rangle^2\sigma_\mu^2 + \langle\mu^2\rangle\sigma_p^2 = \tilde{\mu}^2\sigma_p^2 + \langle p\rangle^2\sigma_\mu^2 + \sigma_\mu^2\sigma_p^2 \tag{26b}$$

Equations 26a and b provide an additional link among parameters of p- μ-, and $p\mu$-variability and the averaged synaptic parameters.

Coefficient of variation and variance to mean analysis of ePSCs

Using the definition of $\sigma_{(E)}^2$, given in Eq. 17a, one can further show that for a fully equivalent system,

$$CV_{(E)}^2 = \frac{1}{\tilde{n}\tilde{p}}(C\tilde{V}^2 + \tilde{q}) = \frac{1}{\langle m\rangle}(1 + C\tilde{V}^2 - \langle p\rangle(1 + CV_{p\mu}^2)), \tag{27}$$

where $\tilde{n}\tilde{p} = n\langle p\rangle = \langle m\rangle$ is the mean quantal content and $C\tilde{V}$ is the equivalent coefficient of variation for the uePSC amplitude distribution, which by definition approximates the actual CV. The form of this equation is well known for compound binomial systems; however, the contribution of $CV_{p\mu}$ has not previously been explicitly included and analyzed. Equation 27 shows that the inverse relation between $CV_{(E)}^2$ and quantal content allows $CV_{(E)}^2$ to give an accurate indication of presynaptic changes, even for nonuniform systems where values of p, μ, and σ^2 vary between synapses (see also Quastel, 1997) provided $\langle p\rangle$ is low and $C\tilde{V}$ can be estimated from experimental observations.

Recently, there has been renewed interest in analyzing the expected parabolic relation between ePSC variance and ePSC means. From Eqs. 17a and 27, we obtain:

$$\sigma_{(E)}^2 = \mu_{(E)}\tilde{\mu}(1 + C\tilde{V}^2 - \langle p\rangle(1 + CV_{p\mu}^2)) \tag{28a}$$

or

$$\sigma_{(E)}^2 = \mu_{(E)}\tilde{\mu}(1 + C\tilde{V}^2) - \frac{\mu_{(E)}^2}{n}(1 + CV_{p\mu}^2)$$

Using the equations from Table 1, we can rewrite Eq. 28a in the form of

Eq. 28b,

$$\sigma^2_{(E)} = \frac{\mu_{(E)} \sum_{j=1}^{n} p_j \mu_j^2 (1 + CV_j^2)}{\tilde{\mu} \sum_{j=1}^{n} p_j} - \frac{\mu^2_{(E)}(1 + CV^2_{p\mu})}{n} \quad (28b)$$

Eq. 28b is just as general as Eq. 28a, and represents both intra and intersynaptic variability. It can be reduced to Eq. 28c if $\langle p^2\mu^2\rangle \approx \langle p^2\rangle\langle\mu^2\rangle$ (see above and Eq. 25a).

$$\sigma^2_{(E)} = \mu_{(E)}\langle\mu\rangle(1 + \widetilde{CV}^2_{\mu}) - \frac{\mu^2_{(E)}}{n}(1 + CV_p^2)(1 + CV_{\mu}^2) \quad (28c)$$

Using a different approach, Frerking and Wilson (1996) and Silver et al. (1998) have derived an identical equation for the specific condition of no intrasynaptic variability.

A similar equation has been derived by Reid and Clements (1999) with the assumption that $CV_j = CV = \text{const}$ for all synapses. In contrast, Eq. 28a presents a general form of such a relationship defined in terms of equivalent parameters.

As pointed out by Silver et al. (1998) and Reid and Clements (1999) (see also Clements and Silver, 2000) useful information can be obtained by measuring ePSC variance and means at different levels of release probability (controlled, for example, by reducing Ca^{2+} influx). This allows $\tilde{n}$, $\tilde{\mu}$ to be determined from the ePSC data set alone, and therefore allows for unique deconvolution of equivalent parameters (see above). When release probability is low, the limiting slope of the relation will be $\tilde{\mu}(1 + C\tilde{V}^2)$. The maximal ePSC amplitude will give information about n, since $\mu_{(E,max)} = n\langle p_{max}\rangle\tilde{\mu}$, while the degree of curvature will give information about $\langle p\rangle(1 + CV_{p\mu})$ and how this parameter varies with $\langle p\rangle$. However, estimated equivalent quantal parameters are still model-dependent, especially if actual quantal parameters such as p_j, μ_j, and σ_j are not independent. Equivalent parameters will depend on both pre and postsynaptic factors. For example, if low-output synapses also had, on average, larger values of μ_j and were somehow selectively potentiated, a presynaptic change can appear to be associated with an increase in $\tilde{\mu}$, a "postsynaptic" parameter.

RESULTS

Our strategy for studying the relationship among *intrinsic*, *equivalent*, and *apparent* parameters was to simulate a large number of different synaptic systems, each with substantial nonuniformity in the intrinsic parameters defining each synapse in the system. For each of these simulated nonuniform systems we calculated the equivalent parameters and compared them to the average intrinsic parameters for that system. We then analyzed the ePSC and uePSC amplitude distributions generated numerically to determine apparent quantal parameters.

Statistical relationships between equivalent and intrinsic parameters

We generated a set of N synaptic systems, $j = 1 \ldots N$, by randomly varying the number of synapses n_j and the values of intrinsic synaptic parameters of the ith individual synapse (for $i = 1 \ldots n_j$), i.e., p_{ji}, μ_{ji} and σ^2_{ji} or CV_{ji} were varied randomly. Each intrinsic synaptic parameter was allowed to vary uniformly between two limiting values, ξ_{min} and ξ_{max}. Once the intrinsic synaptic parameters for each nonuniform synaptic system were chosen, the fully equivalent synaptic parameters were calculated using the relations shown in Table 1.

n versus ñ

Fig. 2 *A* shows the relationship between the actual number of synapses and the number of synapses in the equivalent system for $n = 500$ simulated systems. The intrinsic number

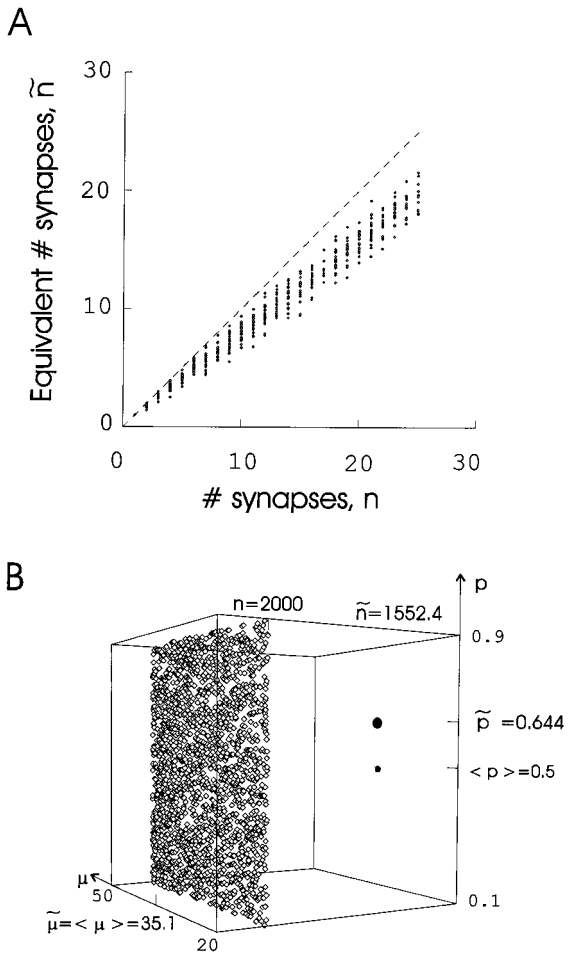

FIGURE 2 Simulations of the effect of synaptic variability on the relationships between intrinsic and equivalent parameters. (*A*) A typical relationship between the actual number of synapses used in simulation, n, and the equivalent estimate, $\tilde{n}$, built for 500 nonuniform systems. The intrinsic synaptic parameters for each system varied within the following ranges: $p_{min} = 0.1$, $p_{max} = 0.9$; $\mu_{min} = 20$ pA, $\mu_{max} = 50$ pA; $CV_{min} = 0.2$, $CV_{max} = 0.9$, and the number of synapses varied between 1 and 25. The dashed line forms a 45° angle to either of the axes. (*B*) In a different simulation, values of intrinsic parameters of 2000 synapses have been generated with the same ranges of variability as in (*A*). The graph shows relative positions of the intrinsic, average, and equivalent parameters in a 3D parametric space.

of synapses in each system, n_j, could be any number between 1 and 25, while the other intrinsic parameters were chosen from the broadly distributed ranges given in the figure legend. The expected estimate for the number of synapses in the equivalent system, $\tilde{n}_j$, is shown as a function of the actual intrinsic value for n_j.

Note that in each case the number of synapses in the fully equivalent uniform system, $\tilde{n}_j$, is always less than or equal to the actual number of nonuniform synapses, n_j, regardless of the values of other synaptic parameters used. This is a known feature of compound binomial systems (see Brown et al., 1976; Quastel, 1997) and is predicted from Eq. 23. It is notable that under these conditions $CV_{p\mu}$ is independent of n. The latter is obvious from Eq. 24, as $CV_{p\mu}$ is not an explicit function of n, but a function of the averages of $\langle p\mu\rangle^2$ and $\langle p^2\mu^2\rangle$ by definition.

n, p, μ versus ñ, p̃, μ̃

We next simulated six different synaptic systems with a fixed value of n_j, but widely varying ranges for p_j, μ_j, and σ_j. For illustrative purposes we considered a large number of independent synapses, in this case 2000 contacts per system. Again, the average intrinsic probability, $\langle p\rangle$, was ~0.5 (ranging between 0.1 and 0.9), the average unitary amplitude, $\langle\mu\rangle$, was ~35 pA (ranging between 20 and 50), and the average CV (i.e., $\langle\sigma/\mu\rangle$) was 0.55 (range 0.2–0.9) in each system.

Fig. 2 *B* shows the relationship between the intrinsic and equivalent values. The plane of open dots at the left of this three-dimensional parameter space shows the values for the intrinsic probability and amplitude were evenly distributed within the plane defined by the fixed value of n. The large filled circle on the right-most plane shows the values for the fully equivalent parameters. As expected from the results presented in Fig. 2 *A*, the equivalent number of synapses, $\tilde{n} = 1552$, is smaller than the intrinsic value of 2000. The equivalent probability of release, $\tilde{p}$, is higher than the average intrinsic probability of release, $\langle p\rangle$, and the equivalent mean, $\tilde{\mu}$, is nearly identical to the average uePSC mean, $\langle\mu\rangle$. The estimates of the equivalent parameters and of averages of the intrinsic parameters in six such simulations were (±SD): $\tilde{n} = 1553 \pm 58$; $\tilde{p} = 0.64 \pm 0.01$; $\langle p\rangle = 0.50 \pm 0.01$; $\tilde{\mu} = 35.07 \pm 0.02$ pA; $\langle\mu\rangle = 35.08 \pm 0.02$ pA. Thus, differences between actual and estimated parameters are fairly constant despite the wide range of possible combinations.

We also performed six similar simulations with a system that had only 50 nonuniform synapses and average intrinsic probability $\langle p\rangle = 0.48 \pm 0.01$, and amplitude $\langle\mu\rangle = 35.14 \pm 0.87$ pA (not shown). The resulting equivalent parameters were: $\tilde{n} = 38.6 \pm 1.4$; $\tilde{p} = 0.62 \pm 0.01$; $\tilde{\mu} = 34.6 \pm 1.2$ pA. The proportional differences between intrinsic and equivalent parameters were virtually identical to those observed when a system of 2000 synapses was considered.

n, p, μ versus ñ, p̃, μ̃: dependence on the extent of variability

In the examples given above, each intrinsic parameter varied over a wide range. We now investigate the accuracy of the equivalent approximation in subsets of their possible ranges selected to approximate actual synaptic parameters. For example, although uePSCs are generally variable in amplitude, mean uePSC amplitudes often appear to be similar within an individual cell (Liu and Tsien, 1995a,b; Oleskevich et al., 1999). We thus examine the equivalent parameter approximation as a function of the degree of variability of μ_j.

The results of these simulations are shown in Fig. 3. The influence of three different extents of variability for μ between the different synapses is illustrated. In Fig. 3, *A–C*, μ ranged between 25 and 30 pA, increasing to the range 25–45 pA and then 25–60 pA in the next two columns. In each case, n ranged from 1 to 15, p ranged from 0.7 to 1.0 (as recently shown in synaptic experiments done at physiological temperatures, i.e., 35–37°C; Markram et al., 1997; Hardingham and Larkman, 1998), and σ^2 was between 100 and 250 pA^2. Intrinsic parameters were drawn from these ranges at random for 500 simulations in each column, and the equivalent parameters calculated. The relation between intrinsic and equivalent values for each parameter is shown in each panel. Note that for low variability of μ between synapses (despite high variability of the quantal response within a synapse), the estimates given by the equivalent parameters are very accurate, with a progressive decline in that accuracy as the variability of μ increases. When deviations occur due to variability in μ, they do so in the direction of higher probability and lower number, as expected for conditions that increase $CV_{p\mu}$.

Similar results were obtained when we kept μ within a narrow range of values (25–30 pA), p was allowed to vary (all other ranges were as given above). The results of 500 such simulations are shown in Fig. 4. The equivalent parameters degraded slightly at low values for $\langle p\rangle$, where the $CV_{p\mu}$ will be larger. Nevertheless, the approximation was quite good in each case.

Synaptic systems with a mixture of high- and low-output synapses

Although hippocampal synapses appear to be fairly stationary, there is some evidence that hippocampal synapses can be classified into two groups, high- and low-output synapses exhibiting high and low values of p, respectively. For example, Rosenmund et al. (1993) have presented evidence that with autaptic synapses on hippocampal neurons in culture, the majority (>75%) of synapses are low-output and exhibited a value for p of around 0.09, and the remaining exhibited a value for p of around 0.5. At physiological temperatures $\tilde{p}$ is generally >0.7, and can approach 1 (Har-

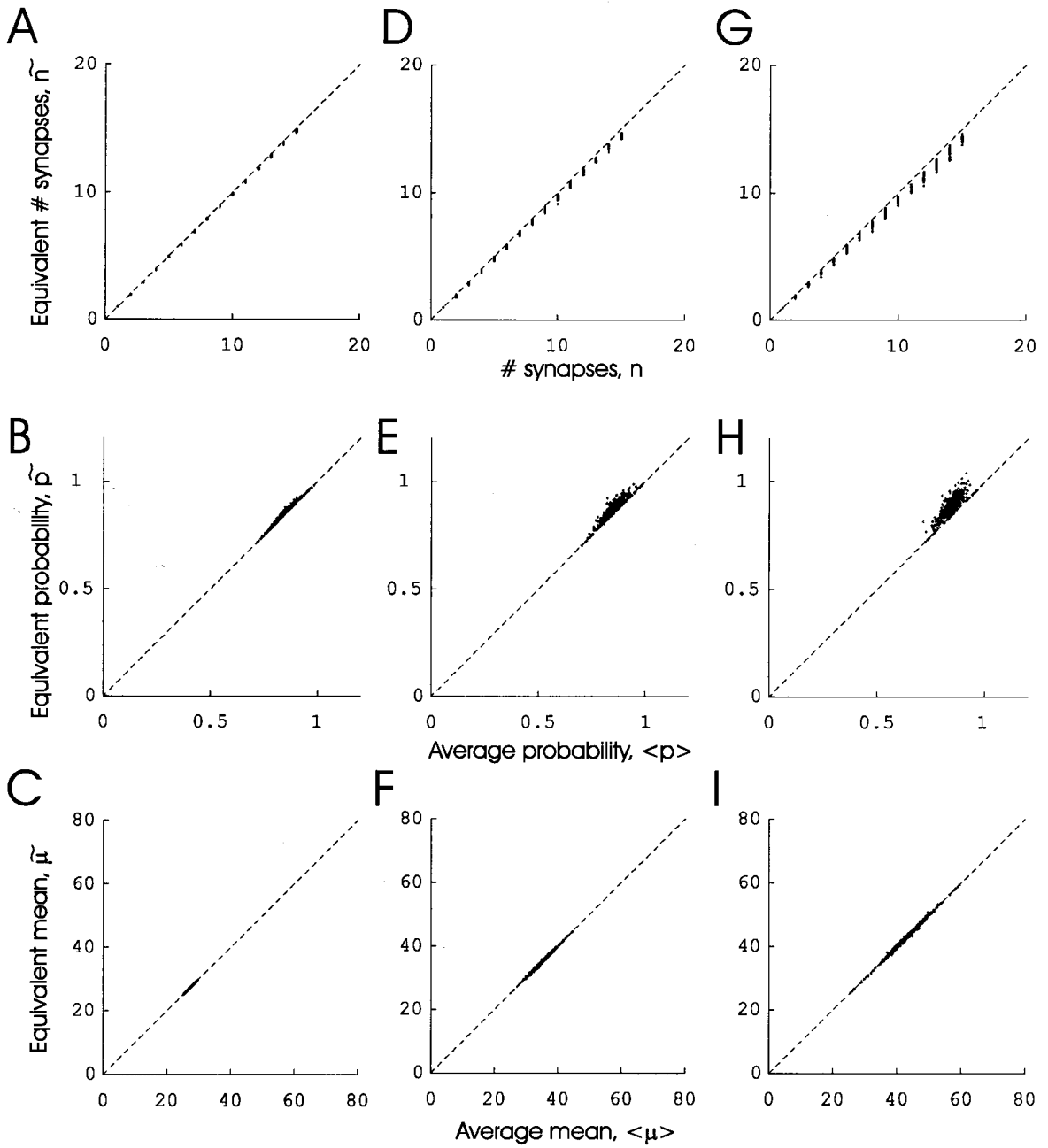

FIGURE 3 Relation between equivalent and mean intrinsic quantal parameters for different ranges of variability of μ. Three columns of figures demonstrate relationships between n and $\tilde{n}$ (*A, D, G*), $\langle p \rangle$ and $\tilde{p}$ (*B, E, H*), $\langle \mu \rangle$ and $\tilde{\mu}$ (*C, F, I*). The following ranges of variability of μ have been used to generate these data: *A–C*, μ_{min} = 25 pA, μ_{max} = 30 pA; *D–F*, μ_{min} = 25 pA, μ_{max} = 45 pA; *G–I*, μ_{min} = 25 pA, μ_{max} = 60 pA. In all simulations the probability of releases varied randomly among synapses between p_{min} = 0.7 and p_{max} = 0.99; uePSC variance varied randomly between σ^2_{min} = 100 pA^2 and σ^2_{max} = 250 pA^2, and the number of synapses used in simulation varied randomly between 1 and 15. Dashed lines form a 45° angle to either of the axes. Figures in each column correspond to the same simulation, and 500 systems have been used for each simulation.

dingham and Larkman, 1998). Modulation involving changes in $\langle p \rangle$ could then arise from an interconversion between low- and high-output synapses. We therefore simulated the influence of the proportion of low-output synapse on correspondence of the equivalent quantal parameters with the actual average quantal parameters of the high-output synapses (Fig. 5).

In the simulation presented in Fig. 5, the number of synapses was kept constant at 100 and distributed randomly between two groups. In one set of simulations the low-

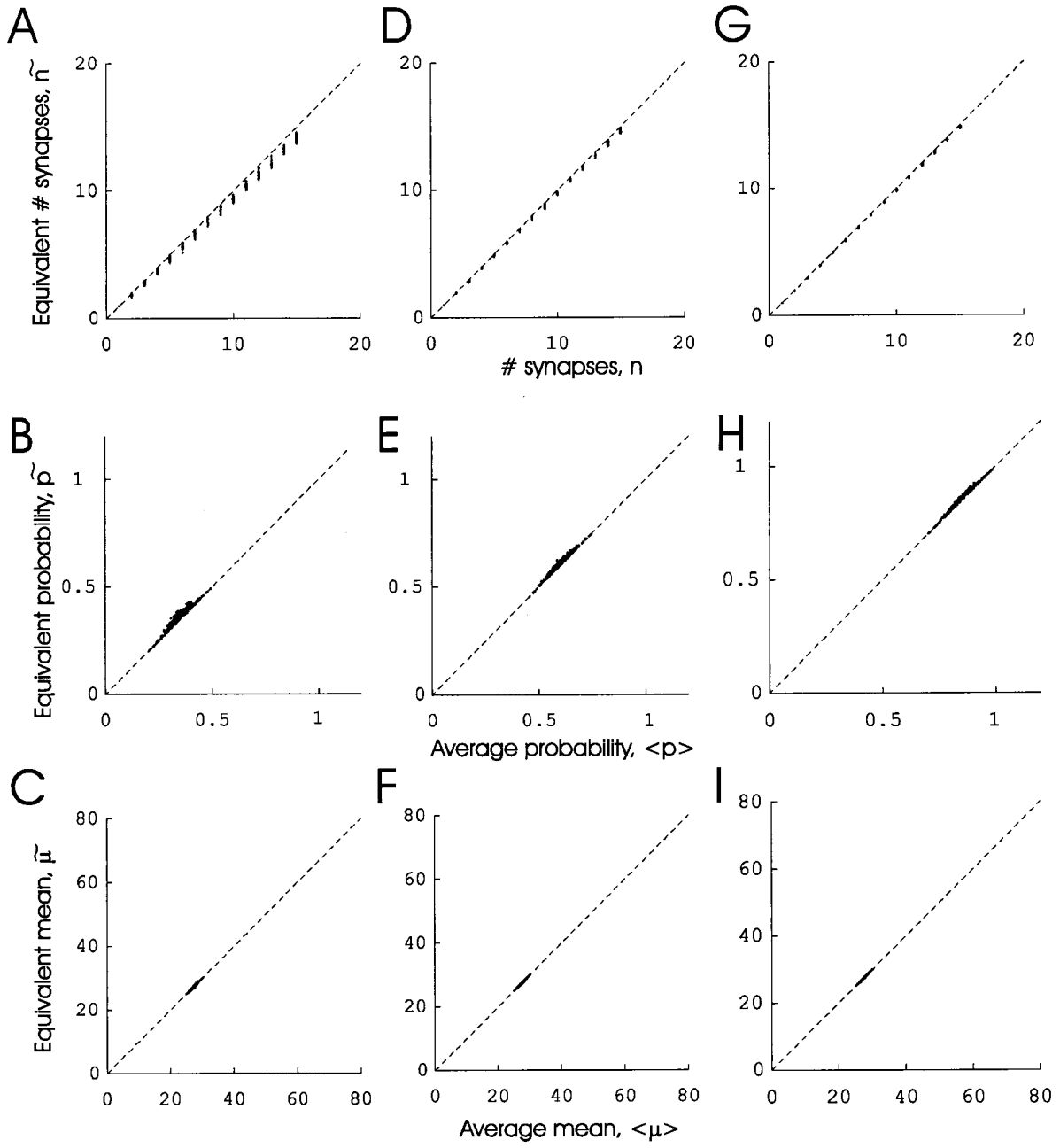

FIGURE 4 Dependence of equivalent parameters on the ranges of variability of p. Three columns of figures demonstrate relationships between n and $\tilde{n}$ (A, D, G), $\langle p \rangle$ and $\tilde{p}$ (B, E, H), $\langle \mu \rangle$ and $\tilde{\mu}$ (C, F, I). The following ranges of variability of p have been used to generate these data: A–C, $p_{min} = 0.2$, $p_{max} = 0.5$; D–F, $p_{min} = 0.45$, $p_{max} = 0.75$; G–I, $p_{min} = 0.7$, $p_{max} = 0.99$. In all simulations uePSC mean amplitudes varied randomly among synapses between $\mu_{min} = 25$ pA and $\mu_{max} = 30$ pA; uePSC variance varied randomly between $\sigma^2_{min} = 100$ pA^2 and $\sigma^2_{max} = 250$ pA^2, and the number of synapses used in simulations varied randomly between 1 and 15. Dashed lines form a 45° angle to either of the axes. Figures in each column correspond to the same simulation, and 500 systems have been used in each simulation.

output group was assigned a value of p between 0.03 and 0.06, and in another set of simulations between 0.07 and 0.099. The high-output group was assigned a value of p 10 times greater, either between 0.3 and 0.6 or 0.7 and 0.99, respectively. For all groups, μ was assigned a value between 25 and 45 pA and σ^2 between 100 and 250 pA^2. Despite this variability, the expected equivalent quantal parameters are close to those of the high-output group, even when this group represents only 25% of the total. Thus, $\tilde{n}$ falls linearly as the number of high-output synapses falls from 100% to 25% (Fig. 5 *A*), while $\tilde{p}$ remains relatively constant (Fig. 5 *B*). This is true regardless of whether the

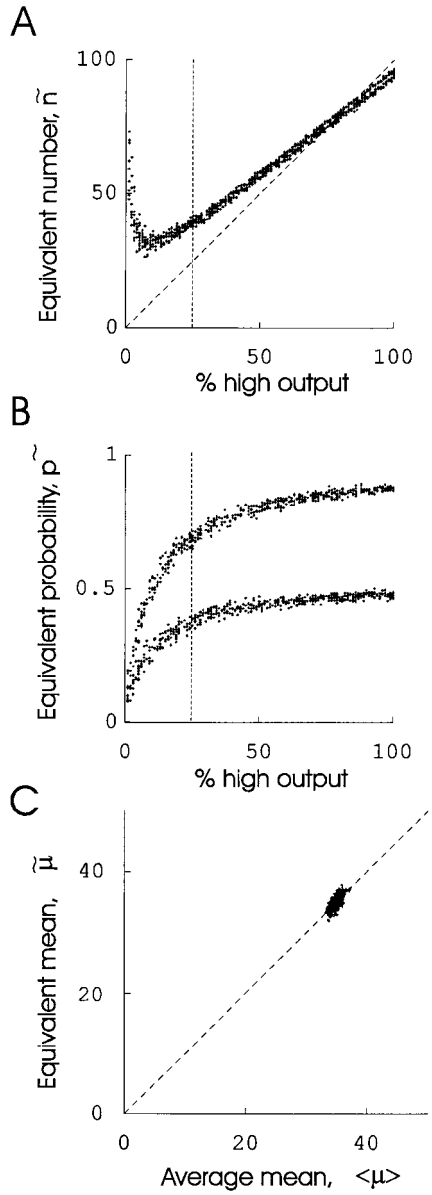

FIGURE 5 Influence of low-output synapses on the relation between equivalent parameters and mean intrinsic parameters for high-output synapses. In each panel we consider two sets of systems of 100 synapses, both made up of two populations of synapses; high-output and low-output synapses differ in release probability by a factor of 10. In the first set the range of values of p for the high-output synapses is 0.7–0.99, in the other the range is 0.3–0.6. Hence, the range of values of p for the low-output synapses within a given randomly generated set will be between 0.03–0.06 and 0.07–0.099, respectively. For all sets, μ was assigned a value between 25 and 45 pA and σ^2 between 100 and 250 pA^2. For each set the percentage of high- to low-output synapses varies between 0 and 100. (*A*) Effect on equivalent number, $\tilde{n}$. The influence of low-output synapses on the number of uniform synapses needed to generate an equivalent uniform distribution is small until they represent >50% of synapses. Even when low-output synapses represent 75% of the synapses, the equivalent parameters primarily reflect the number of high-output synapses. (*B*) Effect on equivalent probability, $\tilde{p}$. The same lack of effect is seen with the predicted equivalent probability. Even though $\langle p \rangle$ is changing considerably, $\tilde{p}$ remains fairly constant and close to the value of $\langle p \rangle$ for the high-output synapses. (*C*) Effect on equivalent mean, $\tilde{\mu}$. Despite considerable variation in $\tilde{p}$ and $\tilde{n}$, the value of $\tilde{\mu}$ remains constant and a good estimate of the actual mean value for the intrinsic system.

range of p for high-output synapses is between 0.3 and 0.6 or between 0.7 and 0.99. The estimate of $\langle \mu \rangle$ is not affected by this variability (Fig. 5 *C*). Thus, fully formed reserve synapses (i.e., that can be recruited if needed) need not be completely silent. Low-output synapses only distort the system when they exceed 75–80% of the total. It is perhaps not a coincidence that a 4:1 ratio appears to represent the ratio of low- to high-output synapses observed in the hippocampus (Rosenmund et al., 1993).

Consider now a mixed population of synapses with mean release probabilities clustered around two values, $\langle p_H \rangle$ and $\langle p_L \rangle$, which are high and low, respectively. If these synapses are indistinguishable postsynaptically (i.e., $\widetilde{CV}_H = \widetilde{CV}_L$; $\tilde{\mu}_H = \tilde{\mu}_L$) and exist in a proportion $k = (n_L/n_H)$, then from Eq. 28a, since variances add, and setting

$$K = \left(1 + \frac{k\langle p_L \rangle}{\langle p_H \rangle}\right)\left(1 + \frac{1}{k}\frac{(1 + CV^2_{p_L})}{(1 + CV^2_{p_H})}\right),$$

$$\sigma^2_{(E)} = \mu_{(E)}\tilde{\mu}(1 + C\tilde{V}^2)\{1 - \langle p_H \rangle(1 + CV^2_{p_H})K\} \quad (29)$$

Thus, until the parameter K becomes significant, the low-output synapses will be inaudible but still available for rapid recruitment.

An illustrative example of deconvolution of equivalent quantal parameters from a nonuniform system

Various numerical and analytical techniques have recently been developed for deconvolution of ePSC amplitude distributions (Dempster et al., 1977; Ling and Tolhurst, 1983; Smith et al., 1991; Kullmann, 1989, 1992; Dityatev and Clamann, 1993; Stricker et al., 1994; Stricker and Redman, 1994). Here we illustrate the feasibility of the approach using simulated data.

Consider a system of four nonuniform synapses (Fig. 6), whose uePSC amplitude distributions are described by single Gaussian functions. The incomplete uePSC mean and variance and the probability of release for each synapse were chosen randomly from a pool of values using a random value generator. An overall uePSC amplitude distribution was then built (Fig. 6 *A*) as a result of an algebraic sum of those four randomly designated uePSC amplitude distributions (not shown). A convolution of the same four nonuniform uePSC amplitude distributions gave rise to the ePSC amplitude distributions (Fig. 6, *B–D, dotted lines*). Unique deconvolution of unimodal ePSC distributions requires independent estimates of at least one pair of parameters (see Theory). It appears that the underlying unitary events during

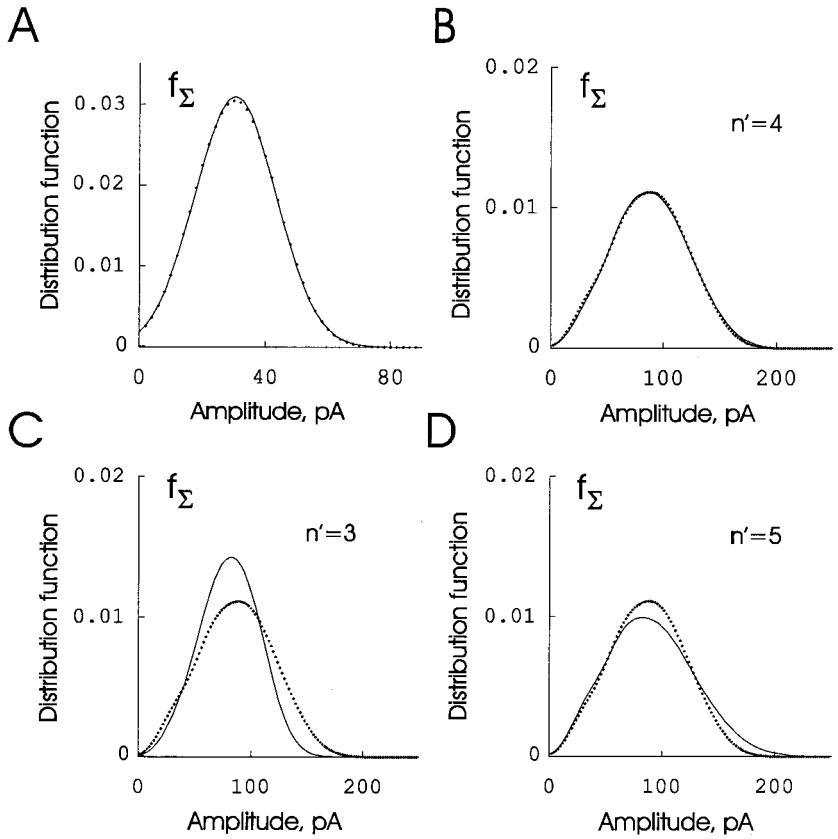

FIGURE 6 Equivalent approximation in the case of unimodal ePSC amplitude distributions. (*A*) Four arbitrary sets of intrinsic synaptic parameters corresponding to four independent synapses have been obtained using a random value generator (not shown), and an overall unimodal uPSC amplitude distribution has been built as a sum of four uPSC amplitude distributions and digitized with the step size of 2 pA (*dotted lines*) and fitted by a single Gaussian function (*solid line*). The following random values for the intrinsic synaptic parameters have been generated: μ = {39.2395, 28.4381, 29.8069, 24.8404} (pA), ($\langle\mu\rangle$ = 30.58 pA); p = {0.690621, 0.775514, 0.674932, 0.702032}, ($\langle p\rangle$ = 0.710775). σ = {10.5209, 13.6437, 11.9397, 10.4485} (pA), ($\langle\sigma\rangle$ = 11.6382 pA). The equivalent values of the parameters were the following: $\tilde{n}$ = 3.89647, $\tilde{\mu}$ = 30.4985 pA, $\tilde{p}$ = 0.72966. The mean and variance estimated from the fit of the uePSC amplitude distribution shown in (*A*) were: μ_{uPSC} = 30.4848 pA and σ_{uPSC} = 13.0285 pA. (*B–D*) ePSC amplitude distribution function has been generated by convoluting four random nonuniform uePSC amplitude distribution functions used to generate the overall uePSC distribution shown in (*A*) (a theoretical convolution is possible for the Gaussian functions). Equation 13b, derived for the uniform systems, was then used to fit the ePSC amplitude distribution of the nonuniform system. As a result of the fit the apparent values of the probability of release were obtained with various initial guesses for the number of synapses: (*B*) $n' = 4$; (*C*) $n' = 3$; (*D*) $n' = 5$; the correct number of synapses has been revealed, i.e., $n_{\mathrm{app}} = n = 4 \approx \tilde{n}$.

spontaneous or asynchronously released uni-quantal events and evoked synchronously released events are identical and are released from the same pool (Raastad et al., 1992; Oliet et al., 1996; Liu and Tsien, 1995a,b; Callister and Walmsley, 1996; Taschenberger et al., 1995; Rosenmund and Stevens, 1996). Thus quantal parameters should simultaneously describe the underlying behavior of both types of observed activity. This will be especially true when asynchronous release is measured during an action potential-induced after-discharge of uPSCs such as occurs in the presence of strontium (Abdul-Ghani et al., 1996; Rumpel and Behrends, 1999; Bekkers and Clements, 1999). In this case, the equivalent parameters $\tilde{\mu}$ and $\tilde{\sigma}^2$ can be obtained from fitting the overall uePSC amplitude distribution obtained from a strontium induced after-discharge, similar to that shown in Fig. 6 *A*. These values, in turn, can be used to uniquely deconvolute the ePSC distribution obtained by recording from pairs of hippocampal neurons, as shown in Fig. 6 *B*. If n' is forced to be 3 or 5, the fit is not acceptable.

DISCUSSION

We have presented here a rational approach toward the analysis of nonuniform synaptic systems. With a system that has an arbitrary number of identical synapses it is generally possible to determine, using a combination of ePSC and uePSC amplitude distributions, a unique quantitative description of the underlying synaptic properties. However, this task becomes impossible when those syn-

apses are nonuniform and only the ePSC distribution is available. The alternate form of quantification described and justified in the present study is that of equivalent systems, in which the actual experimental observations are modeled by a uniform system that can be described completely by four parameters. In our approach equivalent parameters are approximated by apparent parameters, providing a useful analytical link between the real quantal properties of nonuniform synaptic systems and the experimental results. Our calculations demonstrate that an equivalent system provides a reasonable summary estimate of the heterogeneous properties of the underlying nonuniform synapses. We discuss the practical accuracy of our approach, its limitations, and its implications for interpreting synaptic function, as well as for a wide range of different complex systems.

The theoretical treatment presented here does not require that the distributions be unimodal or even have regularly spaced peaks. It provides a theoretical justification to the intuitive supposition that the equivalent estimate of distributed parameters such as release probability or mean amplitude would be a weighted average. Our simulations show that this result is applicable over a wide range of possible values for the number of synaptic connections. In particular, for systems with large numbers of synapses (with concomitant unimodal distributions) the equivalent presentation provides an excellent estimation of the average unitary amplitude and its variance, and close estimates of synaptic number and release probability. These latter two parameters deviate from the average intrinsic values in a systematic way, with a tendency toward lower numbers of equivalent synapses, each with a higher probability of release (see Brown et al., 1976; Quastel, 1997). The systematic deviation becomes larger as the spread of unitary amplitudes becomes greater, yet it always remains within a reasonable range. Indeed, as will be discussed below, this deviation points toward a fundamental property of nonuniform systems, and is therefore instructive.

We have also justified that whereas the determination of unique parameters is impossible from a single unimodal ePSC distribution, the independent specification of defined pairs of parameters will permit a unique solution. We have derived which pairs are required. Conveniently, one of these pairs consists of the mean unitary amplitude and its variance, which are directly obtainable for the uePSC amplitude distribution, thus permitting a unique solution when both the ePSC and uePSC distributions can be measured simultaneously in the same synaptic system.

The simulations we have presented here provide a critical test of the theory. Indeed, the parameters chosen in our simulations were often more extreme than one would ever expect in a functioning system, with very wide ranges of variation for each possible parameter. Another difference between our nonuniform parameters and those expected in an actual synaptic system is the manner in which they are evenly distributed. We chose our parameters from their ranges with uniformly distributed random numbers, whereas actual nonuniform synaptic systems would be expected to have values clustered with more normal distributions around their average values. Because our simulations have a greater fraction of parameters at the extremes of their ranges, we expect that the deviation between intrinsic and equivalent values for the real systems would be less than demonstrated here.

The approach we present thus provides a framework for experimental determination of descriptive parameters in a given synaptic system and the meaning of the obtained experimental values. Amplitude distributions for ePSC and uePSC events are constructed and examined for their form. If both the ePSC and uePSC distributions are unimodal, then the experimenter would have no direct means to determine whether the underlying parameters were uniform. Nevertheless, deconvolution under the assumption of uniformity yields a unique set of descriptive parameters. If the synaptic systems were in fact uniform, then these apparent parameters approximate the actual intrinsic parameters. However, if those systems were actually nonuniform, then the apparent parameters approximate the equivalent system, which in turn provides all a good description of the average intrinsic properties of the actual synapses. Moreover, this approach does not demand independence of the intrinsic parameters from each other. Even a subsequent demonstration of nonuniformity and existence of a feedback between pre and postsynaptic terminals, via a retrograde transmission, would not invalidate the estimates if average descriptors are sought.

On the weighted average, Q_{av}

An approach of weighted averages, Q_{av} and P_{av}, has been recently introduced to monitor pre and postsynaptic contributions to changes in synaptic activity that occur during LTP (Reid and Clements, 1999; Clements and Silver, 2000). In particular, assuming that pre and postsynaptic events and contributions to LTP are independent, the authors introduced a specific parameter, Q_{av}, which was presented as independent of the presynaptic events and therefore could be used as a selective indicator of the postsynaptic changes during LTP. This approach is somewhat similar to ours in that the analysis of a complex nonuniform synaptic system is reduced to consideration of a simpler system, with fewer descriptive parameters. However, our analysis shows that the parameter Q_{av} is in fact dependent on the presynaptic probability of release and is, therefore, a characteristic of a mixture of pre and postsynaptic properties. Also, it cannot be precisely determined by graphical analysis of parabolic variance versus mean plots, as claimed by the authors.

The weighted average, Q_{av}, is introduced by Reid and Clements (1999) as $Q_{av} = \Sigma_{j=1}^{n} p_j\mu_j^2/\Sigma_{j=1}^{n} p_j\mu_j$ (here with our notations). They state that the parameter Q_{av} is independent of pre-synaptic events, and therefore $\partial Q_{av}/\partial p_i = 0$, for all synapses. Taking the derivative explicitly, we obtain that $\mu_i\Sigma_{j=1}^{n} p_j\mu_j = \Sigma_{j=1}^{n} p_j\mu_j^2$, or $\mu_i = Q_{av}$. As the index i was chosen totally arbitrarily, the same equality can be

written for other arbitrarily chosen synapses; therefore, $\mu_i = \mu_k = \cdots = \mu_h = \cdots = Q_{av}$, where $1 \leq i, k, h \leq n$. Therefore, the Q_{av} defined by Reid and Clements (1999) is independent of presynaptic events if and only if all synapses are identical in terms of their mean uePSC amplitudes. Indeed, in their derivation, Reid and Clements (1999) assumed that the coefficient of variation was identical for all synapses, i.e., $CV_j = CV$. Identical means and CV values in turn will imply identical variances, which essentially means that all postsynaptic parameters were implicitly assumed to be identical. Therefore, in fact, Reid and Clements (1999) considered only the case of presynaptic nonuniformity and complete postsynaptic uniformity between synapses.

Tuning nonuniform synapses

We showed in the previous section that Q_{av} is independent of presynaptic influences only if the postsynaptic properties of all synapses are identical. It is interesting that Reid and Clements (1999) have reported that the parameter Q_{av} was not influenced in experiments by modulators of presynaptic release, such as cadmium. This might indicate that the mean amplitudes of uePSCs generated at different synapses on a given neuron are indeed similar, an argument that we have discussed above (see also Liu and Tsien, 1995a,b; Bekkers and Clements, 1999). Alternatively, it might imply that pre and postsynaptic parameters, and thus pre and postsynaptic contributions to LTP, are not completely independent from each other (Schikorski and Stevens, 1997), and that a parameter of a different nature, such as the area of the presynaptic active zone, for example, can influence both pre and postsynaptic parameters and in turn can be influenced directly by LTP.

Our results show that as the variability in average unitary amplitude, μ, between individual synapses increases, the estimated number of equivalent synapses decreases compared to the actual system. This systematic deviation has interesting implications for the interpretation of synaptic function. It says, in effect, that uniform synaptic systems are more "efficient" in that they can communicate the same average signal with fewer individual synapses. If synaptic efficiency were to play an important role in a particular system, this result implies that there would be an advantage to "tuning" all synapses within the system to a narrow range of variability of mean amplitudes. Conversely, variability within synapse uePSC may serve as an adaptive measure that allows the nervous system to ignore differences between synapses on a given neuron and to treat them as if they were a precisely equivalent uniform population. Otmakhov et al. (1993) have demonstrated that in the hippocampus, ePSCs representing the summation of as few as three large uPSCs are sufficient to evoke an action potential. On average 10–15 are required, depending on the level of inhibition. Thus, it is likely that synaptic variability will result in variable firing patterns, which may have an impact on this putative tuning process.

Recent results from Liu and Tsien (1995a,b) and from Oleskevich et al. (1999) seem to indicate just this result, with the similar mean uePSC amplitudes in the same neuron, even when mean amplitudes vary considerably between neurons. Indeed, there was a negative correlation between the number of synapses on a neuron and the mean uePSC size. This observation may reflect limiting amounts of postsynaptic domain (PDZ) proteins or other proteins involved in assembling pre and postsynaptic aspects of the synapse (Sheng, 1997; van Rossum and Hanisch, 1999). Conversely, the observed pronounced variability of uePSC amplitudes at individual synapses (CV typically around 0.5) assures that amplitude distributions for uePSC and ePSCs are unimodal, and therefore better defined by an equivalent system. We have previously discussed the origin of this variability (Uteshev and Pennefather, 1996) and suggested that it has a structural component involving off-center release at the synaptic active zone. Being structural in nature, it will be susceptible to adaptive "tuning." There also is considerable evidence for activity-induced changes in the number of available postsynaptic receptors located within a given glutamatergic synapse (Carroll et al., 1999; Morales and Goda, 1999; Watt et al., 2000). A large intrinsic variability in uePSC responses will mask this localized change while still permitting it to influence the apparent equivalent mean of the system.

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