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Errors in the estimation of the variance: Implications for multiple-probability fluctuation analysis

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Abstract

Synapses play a crucial role in information processing in the brain. Amplitude fluctuations of synaptic responses can be used to extract information about the mechanisms underlying synaptic transmission and its modulation. In particular, multiple-probability fluctuation analysis can be used to estimate the number of functional release sites, the mean probability of release and the amplitude of the mean quantal response from fits of the relationship between the variance and mean amplitude of postsynaptic responses, recorded at different probabilities. To determine these quantal parameters, calculate their uncertainties and the goodness-of-fit of the model, it is important to weight the contribution of each data point in the fitting procedure. We therefore investigated the errors associated with measuring the variance by determining the best estimators of the variance of the variance and have used simulations of synaptic transmission to test their accuracy and reliability under different experimental conditions. For central synapses, which generally have a low number of release sites, the amplitude distribution of synaptic responses is not normal, thus the use of a theoretical variance of the variance based on the normal assumption is not a good approximation. However, appropriate estimators can be derived for the population and for limited sample sizes using a more general expression that involves higher moments and introducing unbiased estimators based on the *h*-statistics. Our results are likely to be relevant for various applications of fluctuation analysis when few channels or release sites are present.

Keywords: Variance–mean analysis; Fluctuation analysis; Synaptic transmission; Quantal analysis; Least squares; Variance of variance; Sample moments; *h*-Statistics

1. Introduction

1.1. Quantal transmission

Neuronal communication in the brain is mediated predominantly by chemical synapses, which operate through the release, from the presynaptic neuron, of a neurotransmitter that diffuses in the synaptic cleft and induces electrical changes in the postsynaptic cell. According to the quantal hypothesis of transmitter release (del Castillo and Katz, 1954; Katz, 1969), neurotransmitter is packaged in discrete quantities (*quanta*) that are released in an all-or-none fashion at specific sites. Assuming that each site acts independently, binomial and Poisson statistics can be used to model the stochastic behavior of synaptic transmission. Thus, synaptic efficacy can be described using three quantal parameters (Katz, 1969; Vere-Jones, 1966): the number of functional release sites (N), the mean probability of release (P) and the amplitude of the response to a single *quantum* (Q). Changes in one or more of these parameters account for modifications in synaptic strength.

1.2. Multiple-probability fluctuation analysis

Several "quantal analysis" methods have been developed to estimate quantal parameters and/or to identify the site of changes in synaptic efficacy, for example by examining the failure rate or the amplitude histograms of synaptic responses (Katz, 1969). However, these methods can be difficult to apply at central synapses when the signal-to-noise ratio is low due to the small quantal size and non-uniform Q compromises the discrimination of peaks in the amplitude histograms (Redman, 1990; Walmsley, 1995). To overcome some of these difficulties, new approaches inspired by stationary and non-stationary noise analysis of ion channels (Heinemann and Conti, 1992; Sigworth, 1980) have been developed as an extension of previous methods used in the 1970s and 1980s (Clamann et al.,

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1989; Miyamoto, 1975). Multiple-probability fluctuation analysis (MPFA: Silver et al., 1998; also known as variance–mean analysis: Reid and Clements, 1999) is a recent method that allows estimation of quantal parameters from the relationship between the variance and mean of synaptic responses recorded under different release probability conditions (Clements and Silver, 2000; Silver, 2003). Quantal parameters are estimated by fitting the variance–mean relationship with a binomial or multinomial model using the least squares optimization method. MPFA has been applied at a number of central synapses (Foster and Regehr, 2004; Lawrence et al., 2004; Oleskevich et al., 2000; Sargent et al., 2005; Silver et al., 1998, 2003; Sims and Hartell, 2005). These studies have shown that the number of functional release sites per connection can vary from one to several hundred and the release probability per site varies widely at central synapses.

1.3. The binomial model

The binomial model is more appropriate than the Poisson for describing neurotransmitter release at central synapses (Kuno, 1964), since some of these connections are characterized by few release sites and a release probability that can be quite high under physiological conditions (Silver et al., 2003). According to this model, the mean peak amplitude of the synaptic current I and the associated variance σ^2 are given by

$$I = NPQ, \tag{1}$$

$$\sigma^2 = NQ^2P(1-P), \tag{2}$$

so that a parabolic relationship holds between them:

$$\sigma^2 = IQ - \frac{I^2}{N}. \tag{3}$$

Fig. 1A shows Monte Carlo simulations of postsynaptic currents at three different probabilities for a connection with five release sites (a value typical for central synapses). The mean synaptic current increases linearly with P, while trial-to-trial fluctuations arise presynaptically from the variability in the number of *quanta* released. The theoretical relationship between variance and mean of synaptic current is clearly parabolic (Eq. (3)) and the black dots show the tested probabilities (Fig. 1A). The empty squares show the relationship between variance and mean measured from samples of 200 simulated currents for each P.

1.4. The multinomial model

1.4.1. Uniform release probability

Several studies have shown that the assumptions required for applying a simple binomial model often do not hold for central synapses. A multinomial model has therefore been developed in order to take into account both asynchronous release and non-uniform Q (Frerking and Wilson, 1996; Silver, 2003). Quantal variability can be present at the single site level (intrasite variability) and across sites (intersite variability). Intrasite variability arises from fluctuations in the size of quantal events from trial-to-trial and from asynchrony in their latency, and can be quantified in terms of the associated coefficients of variation (CV_{QS} and CV_{QL}, respectively). Intersite variability arises from differences in the mean quantal size from site to

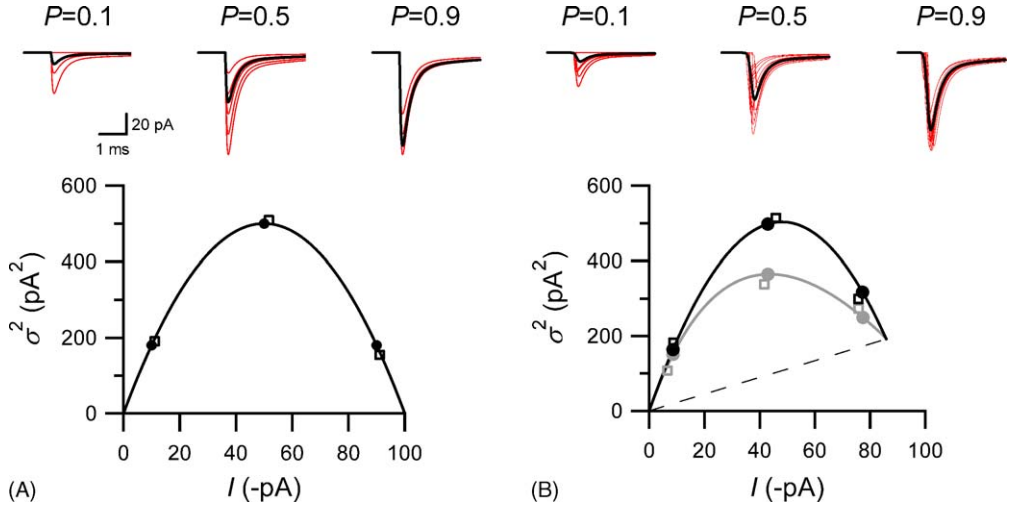

Fig. 1. Binomial and multinomial models of synaptic transmission. (A) Simulated synaptic currents (red traces, 15 traces superimposed) and mean current (black trace, average from 200 traces) for a synapse with $N=5$, $Q=-20$ pA at three different release probabilities. The theoretical variance–mean curve obtained from Eq. (3) is plotted in the graph at the bottom. The black dots represent the theoretical data points associated with the tested release probabilities (Eqs. (1) and (2)), whereas the empty squares are values from sets of 200 simulated currents. (B) Same as in (A) for synapses with $N=5$, intrasite and intersite quantal variability ($CV_{QS}=CV_{QII}=0.3$ and $CV_{QL}=0.2$). The current traces and the black line and markers in the variance–mean plot refer to a synapse with uniform P, while the grey line and markers in the graph refer to a non-uniform release probability case ($\alpha=1$). The variance–mean plots and the theoretical data points are obtained from Eqs. (4)–(7) or Eqs. (8)–(12) for the uniform or non-uniform case, respectively. The dashed line shows the variance contributed by intrasite variability. The mean quantal amplitude across sites ($\bar{Q}$) was set to -20 pA, that gave $\bar{Q}_p=-17.2$ pA in the presence of asynchronous release. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)