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Determining synaptic parameters using high-frequency activation

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HIGHLIGHTS

- Experimental methods to estimate RRP size and release probability are compared.
- For high p, low replenishment rates, and high firing frequency 3 methods align well.
- Simulations explain when and why different methods fail for certain conditions.
- A simple model provides better estimates of RRP and p under broader conditions.

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ABSTRACT

Background: The specific properties of a synapse determine how neuronal activity evokes neurotransmitter release. Evaluating changes in synaptic properties during sustained activity is essential to understanding how genetic manipulations and neuromodulators regulate neurotransmitter release. Analyses of postsynaptic responses to high-frequency stimulation have provided estimates of the size of the readily-releasable pool (RRP) of vesicles (N_0) and the probability of vesicular release (p) at multiple synapses.
New method: Here, we introduce a model-based approach at the calyx of Held synapse in which depletion and the rate of replenishment (R) determine the number of available vesicles, and facilitation leads to a use-dependent increase in p when initial p is low.
Results: When p is high and R is low, we find excellent agreement between estimates based on all three methods and the model. However, when p is low or when significant replenishment occurs between stimuli, estimates of different methods diverge, and model estimates are between the extreme estimates provided by the other approaches.
Comparison with other methods: We compare our model-based approach to three other approaches that rely on different simplifying assumptions. Our findings suggest that our model provides a better estimate of N_0 and p than previously-established methods, likely due to inaccurate assumptions about replenishment. More generally, our findings suggest that approaches commonly used to estimate N_0 and p at other synapses are often applied under experimental conditions that yield inaccurate estimates.
Conclusions: Careful application of appropriate methods can greatly improve estimates of synaptic parameters.

1. Introduction

The number of vesicles in the readily-releasable pool (RRP) and the probability of release (p) are fundamental properties of synapses that are used to characterize their basal characteristics and to describe how they are modified by activity, neuromodulators, and genetic manipulations. One way to evaluate RRP size and p is to use a powerful non-physiological means of liberating all vesicles in the RRP, such as prolonged presynaptic voltage steps, photolytic presynaptic calcium release, or applications of hypertonic sucrose, and then using capacitance measurements or postsynaptic voltage-clamp recordings to quantify the RRP (Schneggenburger et al., 2002; Zucker and Regehr, 2002; Schneggenburger and Neher, 2005). These approaches have all provided important insights into synaptic transmission, but for technical reasons, they cannot be readily applied to many types of synapses. Moreover, the pool of vesicles that exocytoses in response to these non-physiological stimuli may not be equivalent to the pool that can be released through physiologically-relevant action

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potential stimuli (Neher, 2015). To overcome these limitations, a second class of approaches has been developed that uses synaptic currents evoked by high-frequency stimulation to estimate p and RRP. Such approaches have the advantage that they are based on synaptic responses evoked under physiological conditions and can be readily applied to many types of synapses.

Here we evaluate different methods that are used to estimate synaptic parameters from responses evoked by high-frequency stimulation. A basic framework has been developed to understand how neurotransmitter is released in response to rapid firing. It is thought that release depends on the size of the RRP (comprised of a number of vesicles, N_0) and the probability of an action potential causing a vesicle to fuse (p). The number of vesicles released by an action potential is equal to $p \times N_0$ (Liley and North, 1953). At synapses with a high initial p, repetitive activation rapidly depletes the RRP and reduces the amount of neurotransmitter release per action potential (Elmqvist and Quastel, 1965). As depletion occurs, a synapse relies on vesicles from a recycling pool of vesicles to replenish the RRP.

Several methods have been developed to determine synaptic properties that are based on this simple framework. The most widely used method to characterize synaptic properties uses high-frequency stimulation to evoke EPSCs (Fig. 1A). According to this approach, rapid stimulation depletes the RRP until the remaining responses rely on the replenishment of the readily-releasable pool. A plot of the cumulative EPSC versus stimulus number has a rapidly changing component during which the RRP is depleted, followed by a linear component once the EPSC reaches a constant amplitude (Fig. 1B, Table 1). N_0 is estimated by extrapolating the linear phase back to the y-axis in order to account for replenishment. This quantity is in units of current, and corresponds to a number of vesicles released when divided by the quantal size, q. Then p is determined as $p = \mathrm{EPSC}_0/N_0$ (Schneggenburger et al., 1999). This method, as well as a variation that uses presynaptic capacitance changes instead of postsynaptic responses, has been applied to characterize synaptic transmission at a variety of synapses, including the calyx of Held, excitatory and inhibitory cultured hippocampal synapses, and the *Drosophila* neuromuscular junction (Schneggenburger et al., 1999; Moulder and Mennerick, 2005; Stevens and Williams, 2007; Fioravante et al., 2011; Liu et al., 2014; Gaviño et al., 2015; Müller et al., 2015). The same data can also be used to estimate synaptic parameters using an approach developed by Elmqvist and Quastel (Elmqvist and Quastel, 1965; Ruiz et al., 2011) that relies on a different set of assumptions (Fig. 1C, Table 1). The Elmqvist and Quastel (EQ) method is less widely used than the train method, but has still been applied at multiple synapses. It relies primarily on EPSCs early in the train and assumes that the decrease in EPSC amplitudes during high-frequency stimulation is a result of depletion of a homogenous pool of synaptic vesicles that comprise the RRP. With this method, a linear fit to the earliest few points of a plot of the EPSC amplitude versus the cumulative EPSC is used to estimate the size of the RRP, and $p = \mathrm{EPSC}_0/N_0$. The data can also be analyzed with what we refer to as the decay method (Ruiz et al., 2011), which has not been used extensively. According to this approach, the amplitude of the nth synaptic response evoked by a train is $\mathrm{EPSC}_n = \mathrm{EPSC}_0(1-p)^n + C$ (C is a constant). An exponential fit to the EPSC amplitude as a function of stimulus number is used to estimate p, and $N_0 = \mathrm{EPSC}_0/p$ (Fig. 1D).

All of these methods rely on assumptions that are not valid under all conditions, which can compromise their accuracy in estimating synaptic parameters (Table 1). The EQ and decay methods assume that the effects of replenishment are small and can be ignored, but it is not always clear if this is valid for typical experimental conditions. Although the train method does consider replenishment, it assumes constant replenishment throughout a stimulus train. In fact, replenishment could scale with the availability of empty release sites (Wesseling and Lo, 2002) and accelerate with elevation of presynaptic calcium levels (Kusano and Landau, 1975; Dittman and Regehr, 1998; Wang and Kaczmarek, 1998; Sakaba and Neher, 2001a; Hosoi et al., 2007), which could lead to underestimates of RRP size (but see Neher, 2015). With regard to p, the train method is most flexible, in that it allows for changes in p throughout the stimulus train and heterogeneity of p among individual vesicles in the RRP. In contrast, the EQ and decay methods assume uniform p that remains constant throughout a stimulus train. However, p does not remain constant at facilitating synapses, and p may be non-uniform at many synapses (Dobrunz and Stevens, 1997; Sakaba and Neher, 2001b; Meinrenken et al., 2002; Trommershäuser et al., 2003; Moulder and Mennerick, 2005; Schneggenburger et al., 2012).

In this study, we compare estimates of synaptic parameters at the calyx of Held synapse based on four approaches: the train method (Schneggenburger et al., 1999), the EQ method (Elmqvist and Quastel, 1965), the decay method (Ruiz et al., 2011), and fitting to a depletion-based model. There is good agreement between the estimates obtained with all approaches when p is high, R is low, and stimulus frequency is high. When this is not the case, model estimates of RRP size are greater than those of the train and decay methods but less than those of the EQ method. The values obtained with these four methods can differ significantly under non-ideal conditions and suggest that care is necessary in choosing an approach to analyze such data. Our findings suggest that under non-ideal conditions, a depletion-based model provides better estimates, because the model is based on a more accurate description of replenishment of the RRP than is implicit in the in the train, decay, and EQ methods. We also use the depletion model to explore the effects of changes in p, replenishment rate, and firing frequency on the ability of linear extrapolation estimates to accurately measure N_0 and p. We find that the train method and EQ method perform best in moderate to high p conditions with relatively low replenishment rates and high firing frequencies. These studies provide insight into the optimal approaches to estimate synaptic parameters at the calyx of Held, advocate for simultaneous use of multiple approaches for a given dataset, and have important implications for the study of other synapses.

2. Materials and methods

2.1. Animals and preparation of brain slices

All animals used were wildtype mice (BL6C57/6J, Jackson Laboratories), postnatal day P11–14 of either sex. All animal handling and procedures abided by the guidelines of the Harvard Medical Area Standing Committee on Animals. Mice were deeply anesthetized with isoflurane and killed by decapitation. Transverse 200-μm-thick slices were cut from the brainstem containing the medial nucleus of the trapezoid body (MNTB) with a vibratome slicer. Brains were dissected and sliced at 4 °C in a solution consisting of the following (in mM): 125 NaCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCl, 0.1 $CaCl_2$, 3 $MgCl_2$, 25 glucose, 3 myo-inositol, 2 Na-pyruvate, 0.4 ascorbic acid, continuously bubbled with 95% O_2/5% CO_2 (pH 7.4). Slices were incubated at 32 °C for 20 min in a bicarbonate-buffered solution composed of the following (in mM): 125 NaCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 25 glucose, 3 myo-inositol, 2 Na-pyruvate, 0.4 ascorbic acid, continuously bubbled with 95% O_2/5% CO_2 (pH 7.4). For experiments conducted in an external calcium concentration other than 2 mM, slices were incubated in a solution similar to that above but with varying $CaCl_2$ and $MgCl_2$ concentrations. The concentration of $CaCl_2$ added to that of $MgCl_2$ was always equal to 3 mM when $Ca_e \leq 2$ mM. For experiments with $Ca_e = 3$ mM and 4 mM, $[Mg^{2+}] = 0.1$ mM.