Topic Introduction

Measuring the Basic Physiological Properties of Synapses

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Studying synaptic physiology remains an important endeavor for understanding the mechanisms that underlie neurotransmitter release as well as synaptic development and refinement. It is also critical to understanding brain function. The basic conceptual framework for synaptic physiology was worked out by Katz and colleagues and has coalesced around three factors: the number of releasable vesicles (N), the probability of release (P), and quantal size (Q). Here, I discuss a few key experiments related to these quantities and how they are assessed.

BASIC PHYSIOLOGICAL PROPERTIES OF SYNAPSES

The basic mechanisms of synaptic transmission are well understood from many decades of physiological and molecular experiments. However, there are many synaptic proteins whose roles in modulating this process are not clear. In addition, much remains to be learned about synaptic function. The reasons any given synapse has the characteristics it does are largely unknown, particularly considering the sheer number of different types of synapses between all the different cell types in the brain. It is difficult to imagine understanding brain function without a reckoning of this diversity, daunting although it is. For those interested in function or mechanism, there are still many reasons to characterize synapses using electrophysiology. The accompanying protocol describes how to prepare brain slices for electrophysiology, with an emphasis on synaptic physiology in voltage clamp (see Protocol: **Preparing Brain Slices to Study Basic Synaptic Properties** [Xu-Friedman 2017]). This approach remains the gold standard for understanding the properties of individual neurons, as well as the connections between neurons.

Synapses are anatomically and molecularly specialized cellular compartments. The presynaptic neuron possesses clusters of vesicles that express an array of proteins that guide them through their cycle of activity—by mobilizing and docking them to the plasma membrane, filling them with the appropriate neurotransmitter, and triggering their fusion with the presynaptic membrane when presynaptic electrical activity drives a rise in intracellular calcium (Sudhof and Rothman 2009). On the other side of the synaptic cleft are postsynaptic specializations. In electron micrographs, these cluster into the postsynaptic density, which contains neurotransmitter receptors as well as signaling and structural elements (Sheng and Kim 2011).

THE EXCITATORY POSTSYNAPTIC CURRENT (EPSC)

A helpful framework for understanding the physiology of synapses was derived many decades ago, and reached a clear statement with the work of Bernard Katz. This framework considers the synaptic

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potential as dependent on three factors: the number of releasable vesicles (N), the probability with which each vesicle releases its contents (P), and the quantal size of the current resulting from the release of neurotransmitter (Q). For simplicity, this introduction will focus on excitatory synapses. (Inhibitory are thought to be largely similar.) The excitatory postsynaptic current (EPSC) is then a product of these factors: $EPSC = NPQ$.

Quantal Size (Q)

The term “quanta” originated with Katz following the discoveries of Fatt and Katz (1952), in which they observed miniature versions of the normal, synaptic potential recorded at neuromuscular synapses (called endplates) evoked by motor neuron stimulation. These miniature endplate potentials (mEPPs) were only observed near the endplate and had similar reversal potential and sensitivity to receptor antagonists as normal-sized EPPs. It is noteworthy that the electron microscope had not yet provided images of the packages themselves, vesicles at presynaptic terminals.

Fatt and Katz (1952) showed that mEPPs, which occur spontaneously, were the individual building blocks of EPPs evoked by motor neuron stimulation. The individual blocks are presumably not distinguishable because so many of them are released simultaneously during a normal EPP. However, by lowering the concentration of calcium around the preparation, only a few quanta were released following each stimulation and the amplitudes of the evoked EPPs clustered at multiples of the mEPP size.

Measurement of Q can be done simply by patching a postsynaptic cell and recording long bouts of spontaneous electrical events. Many researchers distinguish between spontaneous synaptic currents, which may include action-potential-evoked synaptic currents, and true “minis” recorded in the presence of tetrodotoxin to prevent spontaneous activity.

Release Probability (P)

In the studies by Fatt and Katz (1952), the EPP amplitudes evoked in low calcium concentrations varied highly from trial to trial, such that release appeared random. Del Castillo and Katz (1954) analyzed the distribution of EPP amplitudes to compare them against random distributions. They began with the binomial model of release, which depends on two parameters, N and P ; however, measuring N and P was experimentally intractable. Thus, they simplified their analysis to the Poisson distribution, which only requires a single parameter m , the average number of vesicles released per trial. This simplification is appropriate when N is large and P is small, as appears to be the case at the neuromuscular junction in low calcium. The parameter m can be estimated by dividing the average EPP by the average mEPP, allowing calculation of the expected frequency of single release events, double release events, etc. The match between theory and data were very good using this approach, called “quantal analysis,” at the neuromuscular junction (Boyd and Martin 1956). These experiments indicate that neurotransmitter release is fundamentally a random process, likely obeying binomial statistics.

In contrast to the neuromuscular junction, quantal analysis in central synapses has usually failed to show such clear results. However, it is generally accepted that central synapses are similar in essence to the neuromuscular junction. It is often sufficient to get a relative measure of P by delivering pairs of stimuli at short intervals, and calculating the amplitude of the second EPSC relative to the first (the paired pulse ratio, or PPR). A low PPR reflects high P , and a high PPR reflects low P . The PPR does not yield a direct value of P , but is a sensitive measure of small changes in P .

There are many factors that could cause EPSC amplitudes to depart from binomial statistics in quantal analysis. One is that centrally, release sites are quite variable in size, leading to highly variable miniature EPSC amplitudes, so the distribution of EPSC amplitudes is too broad to recognize individual quanta. A second issue is that individual quanta likely do not summate linearly to produce the EPSC. The most likely reason for this is postsynaptic receptor saturation. Saturation occurs when the neurotransmitter from a single vesicle occupies a large fraction of receptors, such that additional neurotransmitter release has a diminished effect. Saturation can be shown by comparing the effects of low-affinity receptor antagonists on EPSCs under different conditions. Such antagonists block better

when the local glutamate concentration is low than when it is high. In an important experiment by Wadiche and Jahr (2001) at the climbing fiber synapse onto Purkinje cells in the cerebellum, the second in a pair of EPSCs was more strongly blocked relative to the first by a low-affinity antagonist. This is consistent with the glutamate concentration being lower for the second EPSC at the average release site. The most likely reason for this is that multiple vesicles could be released per release site. Furthermore, the amount of glutamate release is not normally proportional to EPSC amplitude in the absence of low-affinity antagonist, probably because of receptor saturation. Similar effects have been found at other synapses (Neher and Sakaba 2001; Foster et al. 2002; Chanda and Xu-Friedman 2010), suggesting receptor saturation is likely widespread.

Releasable Pool

Receptor saturation makes it difficult to assess presynaptic neurotransmitter release using postsynaptic receptors. One solution to this problem is the use of low-affinity antagonists. An alternative approach is to assay release using capacitance measurements of the presynaptic terminal as a direct measure of vesicle fusion. This technique is based on the fact that each vesicle, when it fuses with the plasma membrane, increases the total capacitance of the cell (Lindau and Neher 1988). Measuring capacitance requires patching the presynaptic terminal, so is only possible for large terminals. For example, capacitance was measured at the synaptic terminals of retinal bipolar cells in response to depolarization. For brief depolarization, the capacitance appeared to show an initial plateau at a capacitance equivalent to 1100 vesicles (Mennerick and Matthews 1996), and a second plateau equivalent to 5500 vesicles after much longer depolarization (von Gersdorff and Matthews 1994). These values appear to roughly match the numbers of vesicles counted in serial electron microscope reconstructions of retinal bipolar cell terminals, the smaller population closest to the presynaptic plasma membrane, and the larger population extending further into the synaptic specializations of these cells (von Gersdorff et al. 1996). This indicates that different physiological pools of vesicles have anatomical equivalents, and that they can be depleted.

There are additional approaches for characterizing N and P . One, mean-variance analysis, is based on the binomial model of release and the relationship between the EPSC mean and variance as measured at multiple release probabilities (i.e., calcium concentrations). This method is well described by Silver (2003). The second approach, the integration method, assesses the releasable pool by evoking enough EPSCs to deplete it and integrating their amplitudes, after correcting for synaptic recovery (Schneggenburger et al. 1999). This method has the virtue of simplicity, although it assumes a constant rate of recovery, which is almost certainly not the case (Dittman and Regehr 1998; Wang and Kaczmarek 1998; Sakaba and Neher 2001).

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