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Making quantal analysis more convenient, fast, and accurate: User-friendly software QUANTAN

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Abstract

Quantal analysis of synaptic transmission is an important tool for understanding the mechanisms of synaptic plasticity and synaptic regulation. Although several custom-made and commercial algorithms have been created for the analysis of spontaneous synaptic activity, software for the analysis of action potential evoked release remains very limited. The present paper describes a user-friendly software package QUANTAN which has been created to analyze electrical recordings of postsynaptic responses. The program package is written using Borland C++ under Windows platform. QUANTAN employs and compares several algorithms to extract the average quantal content of synaptic responses, including direct quantal counts, the analysis of synaptic amplitudes, and the analysis of integrated current traces. The integration of several methods in one user-friendly program package makes quantal analysis of action potential evoked release more reliable and accurate. To evaluate the variability in quantal content, QUANTAN performs deconvolution of the distributions of amplitudes or areas of synaptic responses employing a ridge regression method. Other capabilities of QUANTAN include the analysis of the time-course and stationarity of quantal release. In summary, QUANTAN uses digital records of synaptic responses as an input and computes the distribution of quantal content and synaptic parameters. QUANTAN is freely available to other scholars over the internet.

Keywords: Synaptic transmission; Quantal content; EPSC; mEPSC; Quantal size; Deconvolution; Synaptic latency; Binomial model

1. Introduction

Neurotransmitters are packed in presynaptic vesicles and released from axonal terminals as multimolecular packages termed neurosecretory quanta. Neuronal secretion is stochastic in origin (for review: Redman, 1990; Bennett and Kearns, 2000; Atwood and Karunanithi, 2002; Sakaba et al., 2002; Stevens, 2003), and the number of quanta released in response to an action potential, as well the amount of transmitter released by a single vesicle, varies from trial to trial. The probabilistic nature of neurosecretion considerably complicates investigation of synaptic regulation and plasticity. At the same time, statistical analysis of quantal release proved to be a powerful tool in understanding the regulation of the release probability, the number of releasable quanta, and quantal size (Van der Kloot and Molgo, 1994; Oleskevich et al., 2000; Uteshev et al., 2000; Meyer et al., 2001; Bykhovskaia et al., 2001; Searl and Silinsky, 2002; Tsvetkov et al., 2002; Neher and Sakaba, 2003; Sargent et al., 2005; Biro et al., 2005).

Spontaneous release events can be detected as miniature excitatory postsynaptic potentials (mEPSP) or currents (mEPSC) using amplitude thresholds for the recorded or differentiated signal. Several custom-made (Cochran, 1993; Ankri et al., 1994) and commercial (SynaptoSoft) program packages have been created for the automatic detection and analysis of mEPSPs. A more challenging problem is to determine how many quanta are released in response to a presynaptic action potential, since these quantal events usually overlap and cannot be reliably discerned. At the same time, extracting the number of quanta released in response to an action potential from the recordings of excitatory postsynaptic currents (EPSCs) or potentials (EPSPs) is an ultimate condition for the statistical analysis of quantal release. The problem of the detection of evoked quantal events can be solved differently for different synapses at different experimental conditions. Under the conditions when multiquantal responses are infrequent and the instrumental noise is sufficiently low, quanta can be detected visually (Katz and Miledi, 1965; Wernig, 1972; Zucker, 1973) as inflections or multiple peaks of EPSP traces.

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A software package that performs this procedure automatically by analyzing differentiated signals has been created (Bykhovskaia et al., 1996).

However, quantal events often occur synchronously or nearly synchronously, and at these conditions quantal events cannot be reliably discerned. In addition, measured synaptic responses may include variable contributions of noise or may be intrinsically variable. Therefore, deconvolution algorithms have been developed to extract the distribution of quantal content from the noisy distribution of EPSP sizes (Van der Kloot, 1988, 1997; Korn et al., 1993; Stricker et al., 1994; Vorobieva et al., 1999; Bekkers, 2003; Stricker and Redman, 2003). Deconvolution methods usually rely on the information about quantal events obtained from the analysis of mEPSPs or unitary evoked EPSP. Thus, there is a need for a software package that could be universally suitable for the analysis of spontaneous synaptic activity, counting quantal events at low-output synapses, and evaluation of quantal content at high-output synapses by a deconvolution method.

This manuscript describes user-friendly software QUANTAN that has been created to perform quantal analysis of synaptic currents or potentials at various experimental conditions. The software performs multiple tasks required for statistical analysis of transmitter release, including filtering of electrical recordings, event detection, analysis of the release time-course, separation of synchronous and asynchronous release components, calculation of quantal content and its variability, and fitting the quantal release by binomial and Poissonian statistical models. QUANTAN combines our previously developed algorithms for direct quantal counts at low-output synapses (Bykhovskaia et al., 1996) and deconvolution of the distribution of EPSC sizes at high-output synapses (Vorobieva et al., 1999); further, it incorporates an algorithm for evaluation of the accuracy of direct quantal counts, which facilitates the choice for the appropriate detection method. Furthermore, it employs several different methods to calculate quantal content, thus possible errors can be assessed. The software has been extensively tested for the analysis of EPSCs recorded focally from the lobster (Bykhovskaia et al., 2001, 2004; Kapitsky et al., 2005), mouse (Samigullin et al., 2004; Coleman et al., 2007), and drosophila (Akbergenova and Bykhovskaia, 2007) neuromuscular junctions (nmj). QUANTAN is freely available to other scholars over the internet.

2. Methods and results

2.1. Electrophysiology

EPSCs were recorded focally from the mouse and the lobster nmjs (Fig. 1). Dissection procedures, solutions compositions, and visualization of synaptic terminals are described elsewhere (Bykhovskaia et al., 2004; Samigullin et al., 2004; Kapitsky et al., 2005). Briefly, the axon was stimulated electrically via suction electrode, and synaptic activity was recorded focally from visualized (2-Di-4-Asp or FM1-43, molecular probes) synaptic terminals (Fig. 1A) using fire-polished macropatch electrodes of 5–15 μm diameter. Recordings (Fig. 1B) were digitized using

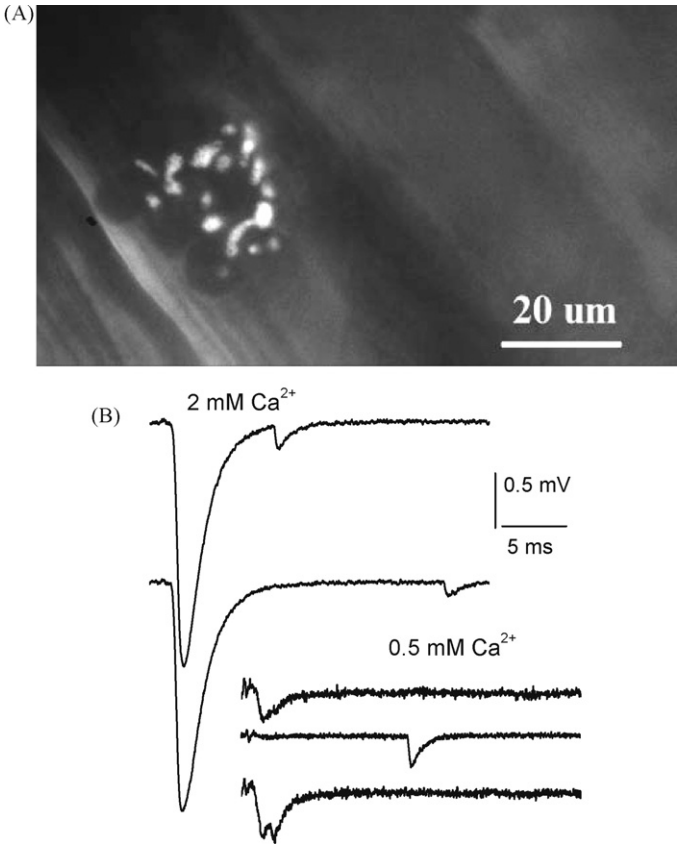

Fig. 1. Focal recordings from the mouse diaphragm nmj. (A) Recording site: an endplate stained with 2-Di-4-Asp. (B) Examples of EPSCs recorded at physiological conditions (2 mM Ca^{2+}) and at the reduced Ca^{2+} (0.5 mM). Recordings at 2 mM Ca^{2+} demonstrate multiquantal EPSCs followed by asynchronous quanta. Recordings at 0.5 mM Ca^{2+} demonstrate a unitary EPSC (top), a transmission failure followed by an asynchronous mEPSC (middle), and a double quantal EPSC (bottom).

PClamp 6.2 or PClamp 8.0 (Axon Instruments) with 10–50 μs resolution.

2.2. General organization of the program package QUANTAN

Software QUANTAN is written using C++ Builder (Borland) and operates under Windows platform. It includes the modules for quantal detection, the analysis of average EPSC traces and evaluation of the detection accuracy, evaluation of the quantal size (q), calculation of the distribution of quantal content (m) employing direct counts or deconvolution, and fitting the obtained distribution of m by binomial or Poissonian statistical model (Fig. 2). In addition, QUANTAN includes a module for transformation of data files (Fig. 2B, "File transformation"), which allows cutting or rearranging episodes in the original data files. Input data files contain recordings of synaptic responses digitized by PClamp (Axon Instruments, version 6 or higher).

2.3. Event detection

To accurately detect EPSCs, mEPSCs, or multiple quantal peaks in a single EPSC, we improved and further developed an