Novel numerical method for isolation of different components within a complex current traces.

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In many neurophysiological studies researchers must separate different parts of complex currents. To reveal different components of currents they usually use the application of pharmacological substances. But in some cases, method of pharmacological separation may be not ideal due to insufficient selectivity of blockers. Also if one needs to investigate modulation of more than one component, many additional blocking schedules must be utilised depending on the number of components that need to be investigated.

We suggest a method that allows to separate any number of steady current components by a combination of least squares and gradient algorithms.

Keywords: synaptic transmission, quantal analysis, patch-clamp.

The investigated currents were analysed off-line. The excitatory postsynaptic current (EPSC) peak amplitude was determined using a computer routine based on the fitting of each current trace by imposition of model curves (pulses) with monoexponential rise and decay phases (Fig. 1). The least squares procedure was used to determine the weight of the model curve, while the time constants and offset were fitted by the gradient method [1] to minimize the mean square error. The time constants of average current were taken as the initial point for fitting each individual EPSC. Each model curve parameters were restricted reasonably by the range of 0.5-2-fold initial value. Model curve amplitude calculated at the point where the first derivative equals to zero was regarded as the maximal amplitude of the component. The peak amplitude of the imposition of all fitted curves was taken as the EPSC amplitude.

In fact, this method is an outgrowth of the widely used routine, where the amplitude of peak is determined as an average value in the predefined time window. The amplitude of EPSC calculated accordingly to both methods provides the minimum of mean square error but the routine used in the present work takes into account all the points of the current trace. Hence this method should be less affected by noise.

The reliability of method was testified with the aid of Monte-Carlo technique. The artificial noise current (bandwidth 0.05-1 kHz) was generated using the random number generator and added to the parent EPSC. In this way, the set of 100 test EPSCs was obtained for each noise amplitude value. Then amplitudes of each current

trace were determined using the method described above, the initial parameters of fitting curve in each case were chosen randomly and differed from the parameters of the parent trace for not less than 30 %. The error of the method was evaluated as mean square deviation of the amplitude of fitted curve from the amplitude of parent trace:

$$SD_{error} = \sqrt{\sum_{n} \left(I_{fit}^{n} - I_0 \right)^2 / n} ,$$

SD_{error} is the error of method, I_0 is the amplitude of current trace, I_{fit}^{n} is the amplitude of fitting curve for each testing EPSC and n is the total number of testing EPSCs.

The error in the EPSC amplitude determination was evaluated for three types of parent traces: pharmacologically isolated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) currents and the composite EPSC from rat hippocampal pyramidal neurones with approximately equal fractions of AMPA and NMDA currents. As one could see from the dependencies of error on the relative noise amplitude (SD_{noise}/I_0) presented in Fig. 2, the method used provides a sufficient accuracy even at two-fold noise to signal ratio. Thus, the proposed method is highly tolerant of noise and enables to obtain the noise free amplitude distribution for followed quantal analysis of properties of the "pure" component.

1. D. M. Himmelblau, Applied nonlinear programming (The University of Texas, Austin: McGraw-Hill, 1972) 321-324.

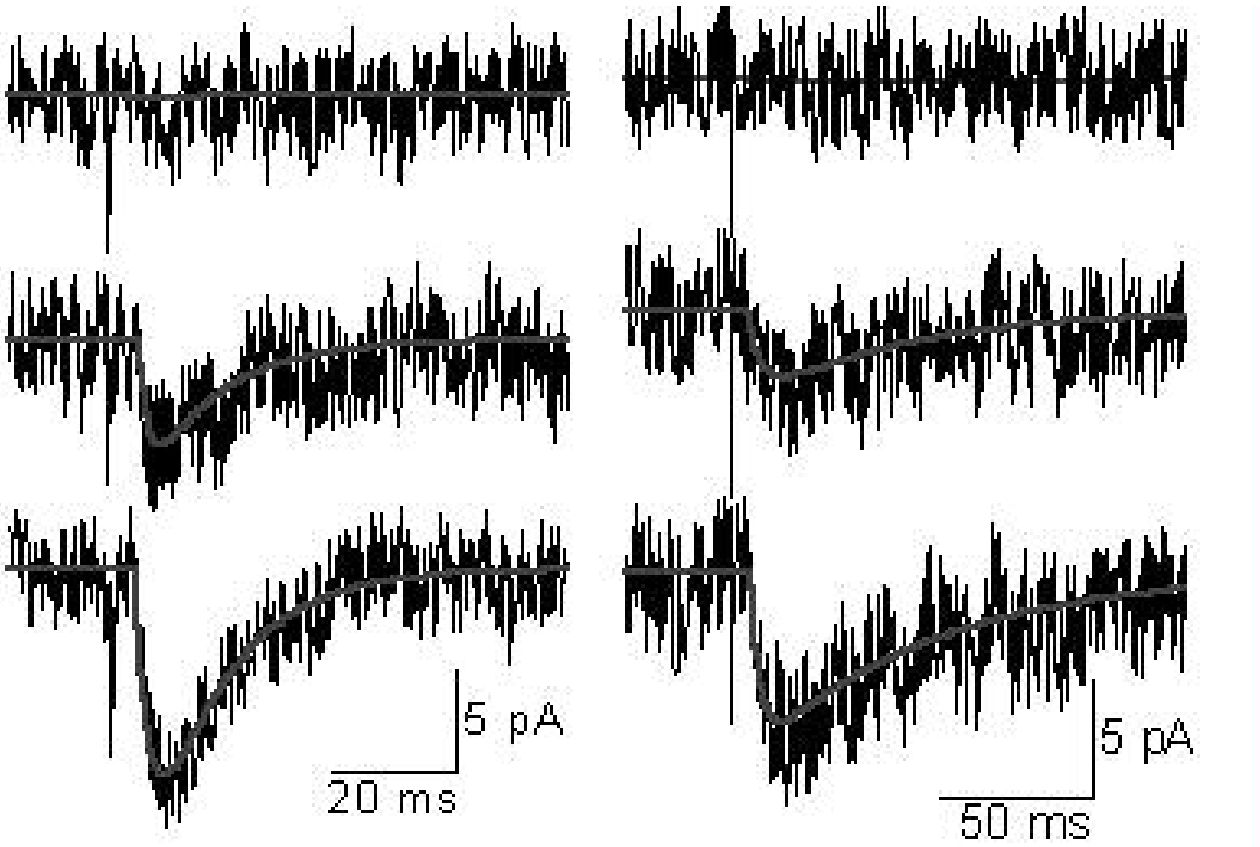

Fig.1. The examples of AMPA and NMDA EPSCs. *Left*, EPSCs recorded in the CA1 pyramidal cell at –75 mV in the presence of 20 μM D-APV. *Right*, EPSCs recorded at –50 mV in the presence of 10 μM CNQX. The traces were fitted as described. The thick line represents the model curve with monoexponential rise and decay. The amplitude of this curve was used the measure of EPSC amplitude. The reliability of this method in the respect of noise is illustrated at Fig. 2.

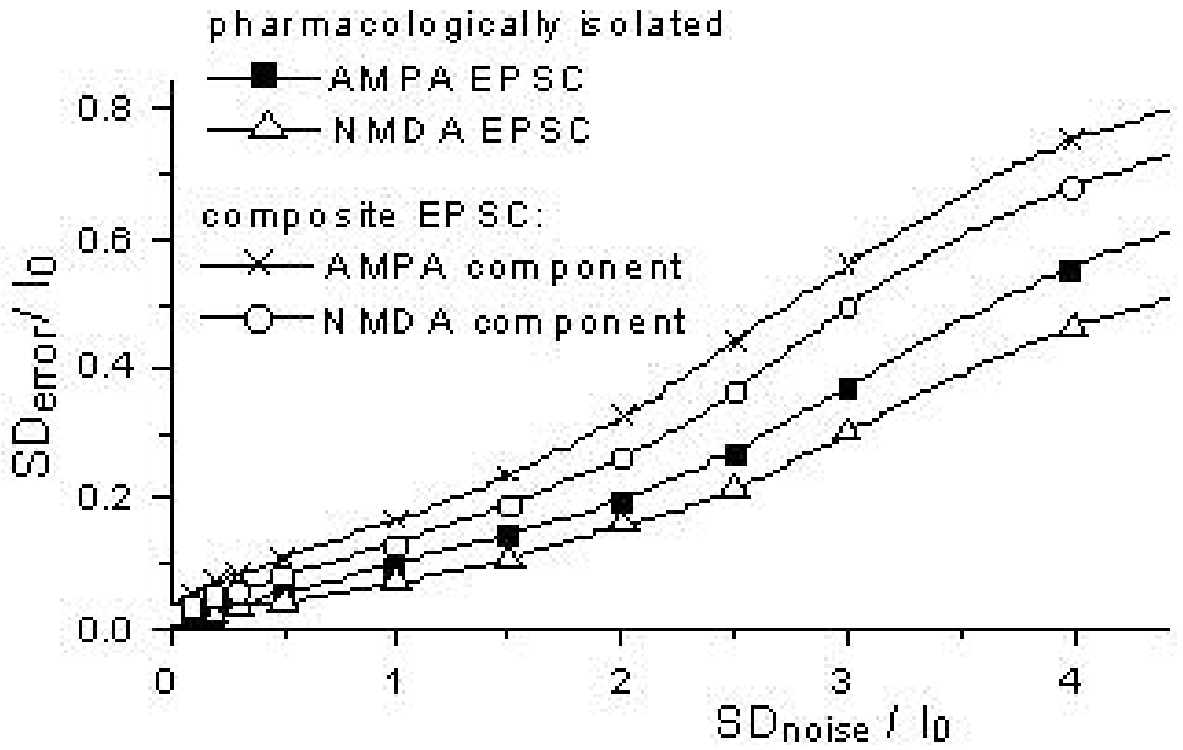

Fig. 2. The dependence of the mean square error of EPSC amplitude determination (SD_{error}) on the relative noise amplitude was evaluated for pharmacologically isolated AMPA and NMDA EPSCs and AMPA and NMDA component of composite EPSCs as described. Note the relative inaccuracy of method is not larger than 20% for noise to signal ratio in the range of 1-2.5.