Automatic processing of amperometric data.

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Amperometry is a widely used technique for monitoring the secretion of catecholamines by exocytosis. Amperometric signals are generated by the oxidation of quantally released catecholamines close to tip of a carbon fibre electrode. Each event resulting from an exocytosis is called ‘secretory spike’. Several kinetic parameters can be extracted from spikes to get important information about the catecholamine storage and the time course of exocytosis. The large amount of data obtained from these experiments requires the use of computer programs. Here, we describe a software, written for Igor Pro (Wavemetrics, Lake Oswego, OR, USA) that allows the off-line analysis of amperometric signals, which includes: i) the automatic analysis of a large collection of experiments user independent, ii) the visual check and correction of the located spikes, iii) data pooling from several experiments to create galleries with hundreds of thousands of spikes[1].

Due to the high amplification of amperometric signals, they are usually contaminated with electromagnetic noise and its removal implies the use of digital filtering in order to improve the signal/noise ratio. Previous authors have used general filtering methods (FIR, smooth, band reject filter, etc.)[2] applied through the whole record affecting differently every spike that contains uneven frequency components. Here, we propose new filtering algorithms that apply low-pass digital filters with variable cut-off frequencies depending on the spectra of discrete segments of the record.

RESULTS AND DISCUSSION

We present the new version of our software for the automatic analysis of amperometrical records[1], which results much more flexible and user friendly. It includes a quick new way to choose folders and files. Now, the spike review becomes easier and faster due to its new method for applying corrections. In addition, the parameters can be plotted onto every event (figure 1A). The current version allows performing the statistical analysis either by spike- or cell-based way. The program and its user manual (Mac and PC compatible) are periodically updated and available as freeware at the web address:

http://webpages.ull.es/users/rborges/

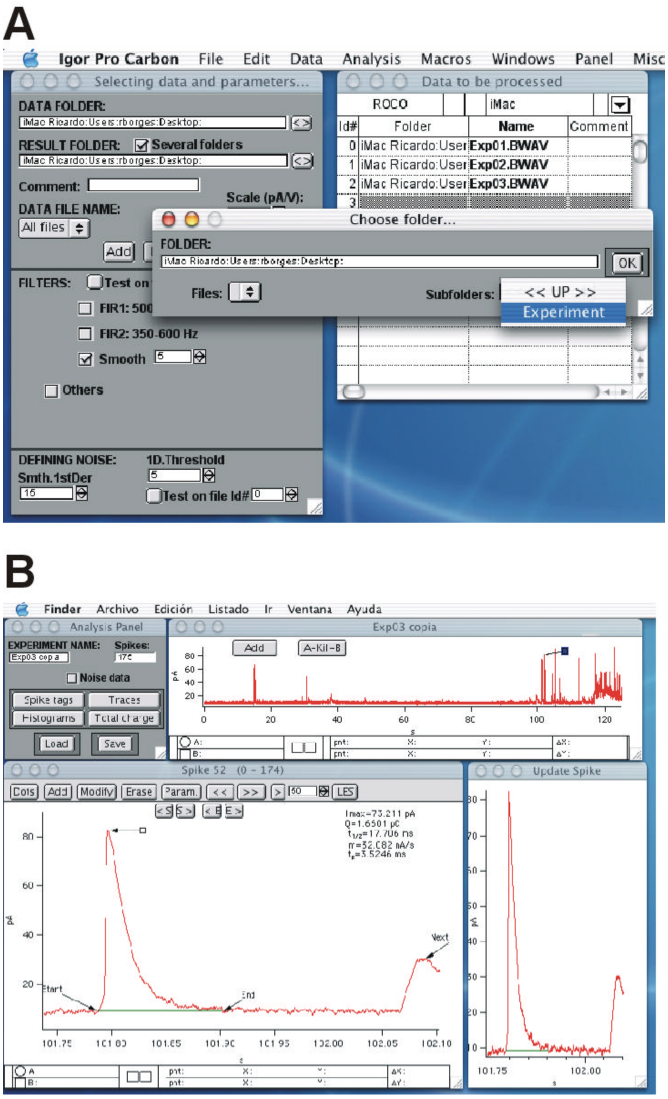

Figure 1. Analysis programs. The screens for the 'Spike Analysis' **A)** and for the 'Spike View' **B)** of the automatic analysis are presented. The 'Spike Analysis' panel is divided into three main parts: selection of data files, choosing of filters and specification of spike identification criteria. Once all previously mentioned fields have been filled or selected, user can start the analysis of the whole list of data files by pressing the 'Run' button. The researcher can check the spikes found by the previous automatic 'Spike analysis' and manual corrections can be introduced in the 'Spike View' screen.

The program is divided into three main parts (figure 1B) that appear in the 'Macros' menu of Igor: i) digital filtering, automatic localization and characterization of spikes from a number of experiments; ii) visual review of results for making manual corrections; iii) creation of galleries of spike parameters pooling results from a large number of experiments. User only needs to introduce the list of files to be processed, the type and characteristics of digital filter, and the spike identification criteria. The program iterates the analysis for every experimental file without any further user activity.

The program incorporates two of the most popular digital filters: binomial smooth and FIR. However, some noise frequencies are also present as components of the secretory events and these filters severely affect those spikes with rapid ascending/descending slopes. To avoid that, we have implemented two adaptive filters: YAIZA[3], which finds the presence of overfiltration by detecting the Gibbs phenomenon, and DAUTE which combines multi-low-pass filters for selective frequency rejection from discrete recording segments.

IMPROVED DIGITAL FILTERING METHODS

The design of the low-pass filter depends on spike features, because each spike has a different power spectrum, and drugs or experimental conditions could alter its form. The filter must be designed to get a better signal-to-noise ratio but not for adding perturbations or Gibbs phenomena. The Gibbs phenomenon is a distortion in the signal due to an overfiltration that rejects important high frequency components[4]. Consequently, Gibbs phenomenon appears in data regions where sudden signal variation occurs (i.e. fast ascending slope of a spike). We present here new algorithms to enhance the S/N ratio from individual amperometric spikes using different FIR (Finite Impulse Response) filters[5] as well as the spike features.

YAIZA algorithm.

We distinguish among three different regions in the amperometric records of bovine adrenal chromaffin cells: type I, segments where can be applied filters with frequencies under 50 Hz (where the basal trace and very slow spikes are); type II, regions with frequencies between 50 and 150 Hz (spikes with medium fast ascending slopes); and type III**,** regions with frequencies over 150 Hz (spikes with fastest slopes).

YAIZA employs three fixed low-pass digital FIR filters**:** L1, whose cut-off frequency (*fc*) is 500 Hz, L2 (*fc* = 150 Hz) and L3 (*fc* =50 Hz). The correct filter will be chosen to avoid Gibbs phenomena. For instance, if Gibbs phenomenon is detected in a given spike region after use the L3 filter, YAIZA will apply the L2 just in this region. Whether Gibbs phenomenon persists L1 will be used.

DAUTE algorithm.

The DAUTE is a new signal-processing algorithm for the digital filtering of amperometrical records. This system uses a bank of low-pass FIR filters that act sequentially on discrete segments of data to suppress the noise, taking into account not modifying the original time course of secretory events.

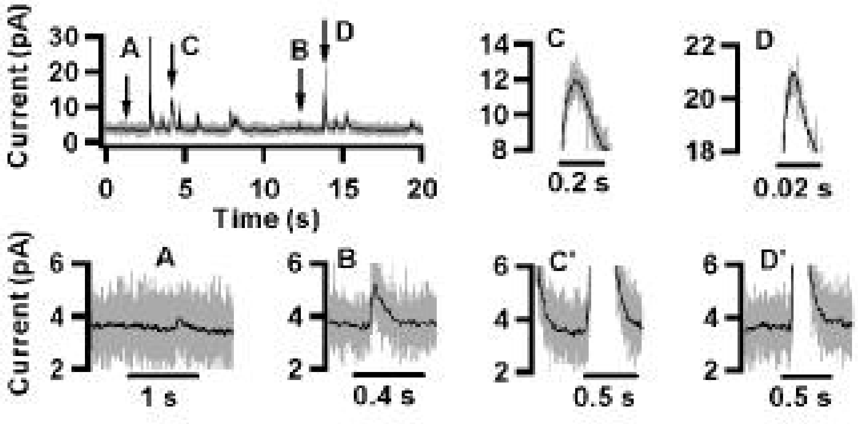

Figure 2. The filtered data with DAUTE algorithm. The gray and black traces are original and filtered data respectively. We show different zoomed segments of the record. The **A** plot shows a noise segment with no spikes, in this case the DAUTE has reduced considerably the noise allowing the detection of a small and slow spike, smaller than 2 pA, in **B**. The **C** and **C'** plots show the top and basal traces of the same spike respectively, In the same way but with other spike is plotted in **D** and **D'**. Therefore, DAUTE has filtered very fast and slow spikes in the same record without introducing Gibbs phenomena. On the other hand, we can now calculate parameters from very small spike (<2 pA) with reliability.

These discrete segments of high frequency are taken from a bank of high-pass FIR filters. The high efficiency of DAUTE facilitates the automatic analysis of spikes and allows the extraction of reliable kinetics data even from very small secretory events (<2 pA; figure 2).

The selection of high-pass (*HPi*) and low-pass (*LPi*) filters is based on sampling frequency being i=1,...,n where n is the number of levels of the filter bank. We usually set n to 6 levels. Following the Nyquist principle, if *fs* is the sample frequency, *fs*/2 is the maximal detectable frequency thus cut-off frequency will be $(fs/2)/2^i$ for the level *i*.

When the high-pass filter bank is applied to data, we obtain *n* filtered signals, *DATAHPi*. DAUTE will use them for detecting the high frequency regions at each level. The next step consists in the application of the low-pass filter bank to data getting *DATALPi*. At the end, the best-filtered data is assessed by combining the *DATALPi*'s with all information picked up on the *DATAHPi's* (Figure 2).

In conclusion, analysis of amperometric records requires the use of software in order to process large amount of data. Therefore, we have described a program for the automatic analysis of individual secretory events. In addition, we have presented new approaches for digital filtering specifically designed for amperometric signals, the fixed level filter (YAIZA) and the self-corrected multi-low-pass digital filter (DAUTE).

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