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A matched-filter algorithm to detect amperometric spikes resulting from quantal secretion

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HIGHLIGHTS

- A "matched filtering" approach for detecting amperometric spikes is proposed.
- The approach relies on the use of a library of prototypical spike templates.
- Data segments are matched against each template to yield a criterion score.
- Spikes are detected when the criterion score exceeds a threshold.
- The algorithm outperforms the derivative-threshold detection approach.

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ABSTRACT

Background: Electrochemical microelectrodes located immediately adjacent to the cell surface can detect spikes of amperometric current during exocytosis as the transmitter released from a single vesicle is oxidized on the electrode surface. Automated techniques to detect spikes are needed in order to quantify the spike rate as a measure of the rate of exocytosis.
New method: We have developed a Matched Filter (MF) detection algorithm that scans the data set with a library of prototype spike templates while performing a least-squares fit to determine the amplitude and standard error. The ratio of the fit amplitude to the standard error constitutes a criterion score that is assigned for each time point and for each template. A spike is detected when the criterion score exceeds a threshold and the highest-scoring template and the time of peak score is identified. The search for the next spike commences only after the score falls below a second, lower threshold to reduce false positives. The approach was extended to detect spikes with double-exponential decays with the sum of two templates.
Results: Receiver Operating Characteristic plots (ROCs) demonstrate that the algorithm detects >95% of manually identified spikes with a false-positive rate of ~2%.
Comparison with existing methods: ROCs demonstrate that the MF algorithm performs better than algorithms that detect spikes based on a derivative-threshold approach.
Conclusions: The MF approach performs well and leads into approaches to identify spike parameters.

1. Introduction

Quantal exocytosis, or the release of transmitter from a single vesicle, can be recorded as spikes of amperometric current using electrochemical microelectrodes if the released transmitter is readily oxidizable, as in the case of catecholamines (Chow et al., 1992; Wightman et al., 1991; Travis and Wightman, 1998). The analysis of these spikes provides information about the time course of release from individual vesicles, and the spike area, or charge, reports the charge or quantum of transmitter released from the vesicle. Quantal size and release kinetics provide important information about how exocytosis is regulated and illuminate mechanisms whereby proteins and lipids drive vesicle fusion (see (Borges et al., 2008) for a review) and may be altered in disease states and thus be targets

Abbreviations: MF, matched filter; EMF, extended matched filter; DT, derivative threshold; ROC, receiver operating characteristics.

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for therapeutic interventions, e.g., (de Diego et al., 2012). Detecting when each spike occurs is the first step of analyzing spike parameters and also quantifies the rate of release events as the spike frequency. Manual spike detection in amperometric recordings is labor intensive and therefore automated spike detection software is often used (Friedrich and Ashery, 2010; Mosharov and Sulzer, 2005; Segura et al., 2000).

The most widely used automated spike detection algorithms for this application detect an event when the derivative of the trace exceeds a set threshold (Gómez et al., 2002; Mosharov and Sulzer, 2005; Segura et al., 2000). There are two main disadvantages in such an approach. Firstly, data smoothing/filtering is required both before and after computing the derivative in order to prevent spurious detection. Excessive smoothing may lead to spikes being undetected whereas insufficient smoothing can result in detection of noisy transients as spikes (false positives). The onus is on the user to determine optimal filtering parameters. Secondly, this method is biased against slowly rising spikes, which can go undetected in this approach. Moreover, this method only uses information from a brief interval in the spike, the rapidly rising phase, and does not exploit information from the bulk of the spike time course when the amplitude is declining.

Event detection is a common problem in signal processing with a rich literature (Poor, 2013). A powerful approach to detect events in a noisy background is to exploit *a priori* knowledge about the event time course through use of an idealized event template. Matched filtering refers to the process of correlating the measured signal with the idealized event template in order to detect events when the correlation exceeds a threshold. Whereas matched filtering has been used in detection applications such as radar for many years, its use for detecting quantal exocytosis via amperometry has not been systematically studied. Examples of biological signals that have been detected using template-based approaches are extracellular action potentials (Kim and McNames, 2007) and spontaneous post-synaptic electrophysiological events ("minis"). In the approach described by Clement and Bekkers, a template is used that represents an ideal postsynaptic mini event. The template is correlated point-by-point throughout the recording and a score is calculated for each time point. Events are detected when the score exceeds a threshold value. However, detection of amperometric spikes is a more challenging problem than minis because amperometric spikes vary widely in amplitude and time course.

Here we describe application of matched-filtering approach to detect amperometric spikes and quantify its performance using Receiver Operating Characteristic plots. Other innovations include the use of two thresholds to reduce false positives, development of a template library to reflect spike diversity, and detection of events best fit by a sum of two templates.

2. Material and methods

2.1. Experimental recordings

Chromaffin cells were isolated from bovine adrenal glands and cultured as described previously (Yang et al., 2007). The cell bath solution consisted of (in mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES, and 11 glucose, pH 7.2. A solution with an elevated potassium concentration was used to depolarize cells and induce exocytosis (in mM): 55 NaCl, 100 KCl, 5 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, and 10 glucose, titrated to pH 7.2 with KOH. Amperometric recordings from bovine chromaffin cells were made with either carbon fiber microelectrodes (ALA Scientific, East Farmingdale, NY, USA) or self-made micro-fabricated Au electrode arrays (Chen et al., 2003; Kisler et al., 2012; Liu et al., 2011). Recordings were filtered at 3 kHz and sampled at 10k samples/s. In most recordings, every 10 points were averaged to result in a "decimated" sampling rate of 1k samples/s and a −3 dB bandwidth of ~220 Hz. The decimated sampling rate of 1 kHz is sufficient to resolve fast events in chromaffin cells and results in faster computational speed. Recordings made from mouse chromaffin cells were kindly provided by the laboratory of Kevin Currie (Vanderbilt Univ.) and obtained as previously described (Jewell et al., 2011). Recordings were filtered at 2 kHz and sampled at 10k samples/s.

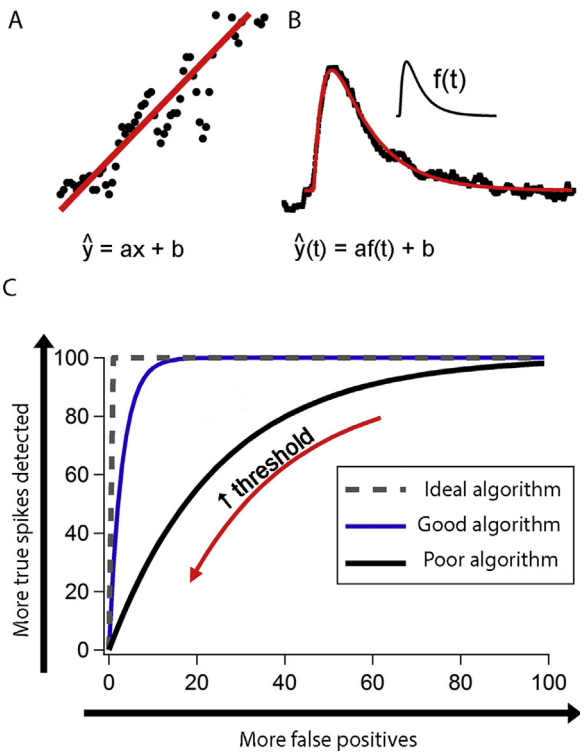

Fig. 1. Least squares can fit an amperometric spike with a template function and a receiving operator characteristic plot can quantify the performance of detection algorithms. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
(A) Least squares fit of a line *(red)* to sample data *(black)* to yield the slope (*a*) and y intercept (*b*). (B) Least-squares fit of a function *f(t)* to an amperometric spike to yield the spike amplitude (*a*) and offset/baseline (*b*). (C) A receiver operating characteristic is a plot of sensitivity vs specificity as the threshold for detection varies from low to high. A higher threshold tends to reduce false positives at the expense of missing events (lower sensitivity). An ideal detection algorithm *(dotted gray)* detects 100% of spikes with 0% false positives when a proper threshold is used; a poor performing test will resemble the *solid black* curve because detecting a large fraction of the events with a lower threshold invariably leads to many false positives; a good test will resemble the *solid blue* curve.

2.2. Matched filter

Linear regression is a familiar technique whereby a set of data points is fitted by a line with slope *a* and intercept *b* to minimize the sum of squared errors between the data and the fit (Fig. 1A). A straightforward extension of this Least Squares approach is to fit a set of data *y(t)* consisting of *N* points with a template function (*f(t)*) to yield an amplitude *a* and offset *b* given by (Fig. 1B):

$$\hat{y}(t) = af(t) + b$$

where *a* and *b* are the least-squares values given by:

$$a = \frac{\Sigma f(t)y(t) - \Sigma f(t)\Sigma y(t)/N}{\Sigma f^2(t) - \Sigma f(t)\Sigma f(t)/N}$$

$$b = \Sigma y(t) - a\Sigma f(t)$$