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

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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.

Keywords

quantal exocytosis; spike detection; signal processing; carbon-fiber amperometry

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2. 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). 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 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.

3. Material and methods

3.1.1. 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 CaCl₂, 1.2 MgCl₂, 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 CaCl₂, 2 MgCl₂, 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 –3dB bandwidth of ~220 Hz. The decimated sampling rate of 1kHz 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.

3.1.2. 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:

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

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

The standard error of the least-squares fit is given by:

$$SE = \sqrt{\frac{\sum (y(t) - \hat{y}(t))^2}{N - 1}}$$

A criterion score for the fit is calculated as the ratio (Clements and Bekkers, 1997)

$$\text{criterion score} = a/SE$$

and thus, is a measure of the “signal-to-noise” ratio of the fit, or how strongly the segment of the data is represented by the template. This is repeated by moving the template point-by-point along the data trace in order to produce a criterion score for every possible data segment. A spike is detected each time the criterion score exceeds a threshold, and the time when the criterion score reaches a peak value is the most likely starting time for the spike. It should be noted that even when the least-squares fit appears poor by visual inspection, it can produce a criterion score sufficiently large to detect the spike with high confidence.

3.1.3. Template waveform

Amperometric spikes can often be adequately fit by a function consisting of an exponential rise with time constant τ_r followed by an exponential decline with a much slower time constant (τ_d) of the form:

$$f(t) = \left(1 - \exp\left(-\frac{t}{\tau_r}\right)\right) * \exp\left(-\frac{t}{\tau_d}\right); t > 0$$

The template contains a brief pedestal before the exponential rise (Clements and Bekkers, 1997), which helps produce a more accurate determination of the baseline b , and, more importantly, helps to ensure that a high criterion score (good fit) is only obtained when the template is precisely aligned in time with the spike found in the data. A pedestal of duration t_p is defined as:

$$f(t) = 0; -t_p < t < 0$$

3.1.4. Selection of template parameters

Four templates are sufficient to detect essentially all amperometric spikes in both bovine and mouse chromaffin cells (see Results). The rising and falling time constants were selected by looking for clustering of time constants found by fitting spikes from representative recordings. Template 1 has a slow rise ($\tau_r = 50$ ms for bovine, 70 ms for mice) and slow decay ($\tau_d = 150$ ms for bovine, 350 ms for mice). Template 2 has a slow rise ($\tau_r = 30$ ms for bovine, 70 ms for mice) and an intermediate decay time ($\tau_d = 80$ ms for bovine, 180 ms for mice). Template 3 has a fast rise ($\tau_r = 3$ ms for bovine, 10 ms for mice) and fast decay ($\tau_d = 8$ ms for bovine, 20 ms for mice) and template 4 has a fast rise ($\tau_r = 3$ ms for bovine, 10 ms for mice) and a slow decay ($\tau_d = 80$ ms for both bovine and mice). All templates are normalized to unit peak amplitude and have 20 ms pedestals. For bovine chromaffin cells,

templates are 200 ms in duration, whereas mice spike templates are either 250 ms or 380 ms in duration. Reducing the template duration to 200 ms did not adversely affect performance (data not shown).

3.1.5. Comparison with other detection software

Software developed by Eugene Mosharov (Mosharov and Sulzer, 2005) was used as the default derivative-threshold (DT) approach to detecting spikes. This software requires the user to specify a smoothing frequency, background region and peak threshold. A spike-free region in the amperometric trace was input to be the background region. The spike detection program was run at thresholds ranging from 0–80 pA/ms. ClampFit (Molecular Devices, Sunnyvale, CA, USA), a commercial spike detection application, uses templates selected by the user to detect spikes. The input templates applied were the same as those used for MF approach. The detection threshold was varied from 0 to 20 and the detection performance was measured at each threshold. MiniAnalysis (Synaptosoft, Fort Lee, NJ, USA) is another commercial spike detection application that uses a derivative threshold approach. This spike detection routine was run at thresholds ranging from 0 to 20 pA/ms.

3.1.6. Quantification of detection performance using Receiver Operating Characteristic plots

The performance of various spike-detection algorithms was measured using Receiver Operating Characteristic (ROC) plots. The two important indicators of performance are the ability to detect actual spikes (high sensitivity) while minimizing false positives (high specificity). Sensitivity is quantified by the detected fraction, defined as:

$$\text{Detected Fraction} = (\text{Num. of true spikes detected}) / (\text{Total num. of actual spikes in the recording})$$

Lack of specificity is quantified by the false-positive fraction, defined as:

$$\text{False-positive fraction} = (\text{Num. of false positives detected}) / (\text{Num. of true spikes detected} + \text{Num of false positives detected})$$

As the threshold value for detection is increased, the false-positive fraction decreases (higher specificity), however, the detected fraction also decreases (lower sensitivity). Therefore, the tradeoff between specificity and sensitivity is quantified by plotting the detected fraction versus the false-positive fraction as the threshold value is increased from a low to a high value (Receiver Operating Characteristic, Fig. 1C). An ideal detection algorithm will resemble the dashed curve in Fig. 1C and detect 100% of events with 0% false positives (upper left corner of ROC) when an appropriate threshold is used. On the other hand, a poorer-performing algorithm will feature a more prominent tradeoff of sensitivity versus selectivity as the threshold is varied (solid black curve in Fig. 1C) such that false positives inevitably accompany detection of a large fraction of events.

In order to quantify the detected fraction and false-positive fraction, one must define the true condition, i.e., identify all the actual spikes in the recording. We used manual analysis of

recordings as the “gold standard” for identifying spikes. Cases where it is ambiguous whether a signal is a pre-spike foot or an independent spike were excluded from analysis. A spike detected by an algorithm is considered “true” if the rising edge is within a defined time tolerance of the rising edge determined manually. The tolerance was set to be the duration of the template. Algorithms were implemented using Igor Pro software (Wavemetrics, Lake Oswego, OR, USA).

4. Results

4.1.1. Multiple templates are required to detect exocytotic spikes with varying time courses

As illustrated in Fig. 2A, B, exocytotic spikes in chromaffin cells vary considerably in time course, therefore a single template is insufficient to detect both slow and fast spikes. We therefore chose four templates that represent a range of spike dynamics found in chromaffin cells (Fig 2C, left). Template 1 has a slow rise and slow decay, template 2 has a slow rise and intermediate decay time, template 3 has a fast rise and fast decay and template 4 has a fast rise and a slow decay. Finally, a sum of templates was used to fit spikes with double exponential decays. Fig 2C (blue traces) presents criterion scores for the four templates calculated from a sample trace. Note that, in contrast to detection of spontaneous post-synaptic events (Clements and Bekkers, 1997), no individual template produces criterion score peaks that enable resolution of all of the spikes. The red star indicates the template with the highest criterion score for each spike. Thus use of multiple templates is not only essential for exocytotic spike detection, but also leads to classification of spikes into broad categories defined by the best-match template (van Kempen et al., 2011).

4.1.2. Use of two thresholds reduces false-positives

A spike is detected when the criterion score exceeds a threshold, and the time when the score reaches a peak identifies the start time of the detected spike. The criterion score then declines as the template is moved past the start-time of the spike, subsequently rising again as it approaches the next spike. Therefore, the algorithm needs to reset the search for a new spike upon a decline in the criterion score.

The simplest approach is to reset the search when the criterion score drops below the same threshold used to detect the spike (Clements and Bekkers, 1997). However, this can lead to false positives.

Fig 3A shows an example trace containing a single spike, yet two additional spurious spikes are detected (red traces) as the criterion score fluctuates around the threshold value as the template is moved across the declining phase of the exocytotic spike.

In order to reduce false positives, we used two thresholds. A higher threshold is applied to detect a spike, but then the criterion score must decline below a lower threshold before the search for a new spike is initiated. Fig 3B shows the same spike from Fig 3A where use of the second lower threshold prevents the detection of spurious spikes because the criterion score does not drop below the lower threshold and then rise above the higher threshold until the next spike is encountered. We found that setting the lower threshold to 50% of the value of the higher threshold was sufficient to avoid false positives (Figs 4–6).

4.1.3. The matched-filter approach is not very sensitive to filtering and can detect spikes with a sloping baseline

The matched filter approach uses hundreds of recorded data values to calculate each criterion score and is therefore not highly sensitive to random noise fluctuations resulting from insufficient filtering of the data. Fig. 4A presents criterion scores for template 3 applied to a raw data trace compared to the same data trace after the 10:1 decimation filtering that we normally use. Note that each of the five amperometric spikes have clearly defined criterion-score peaks even for a ~10-fold increase in bandwidth above the minimum necessary to resolve fast spikes. Nevertheless, it is recommended that traces be filtered at a cutoff frequency no higher than necessary to preserve spike dynamics in order to minimize noise and potential false positives. In addition, using a lower sampling rate speeds the detection algorithm because fewer points are used in the calculation. The matched filter approach is also not very sensitive to a sloping baseline because, unlike amplitude-threshold algorithms, it does not rely on precise identification of baseline values. Fig. 4B presents criterion scores for a data trace before and after adding a sloping offset to simulate an extreme case of a sloping baseline. Note this trace also shows an example where both spikes in an overlapping pair are clearly detected.

4.1.4. The matched-filter approach detects amperometric spikes with pre-spike features

Spike events resulting from exocytosis are often preceded by small-amplitude features that result from efflux of transmitter through a narrow fusion pore (Alvarez de Toledo et al., 1993; Chow et al., 1992). Fig. 5 presents examples demonstrating successful identification of spikes preceded by either plateauing (Fig. 5A) or ramping (Fig. 5B) pre-spike features. Note that the pre-spike features themselves are not incorrectly identified as “false positive” spikes because they do not have the characteristic shapes represented in the spike template library. However, if a pre-spike event displays both a rising and falling phase preceding a second larger spike event, the first event may be identified as a separate spike. In these cases, it is often unclear whether the first event is a foot signal or a small, independent spike.

4.1.5. Extending the matched-filtering approach to detect spikes with a sum of two templates

Some exocytotic spikes decay in a double exponential fashion, having a first fast decay followed by a second slower decay (Segura et al., 2000). We extended the matched-filter algorithm to produce least-square fits with a sum of two templates: $f_1(t)$ and $f_2(t)$. The fit is therefore of the form:

$$\hat{y}(t) = a_1 f_1(t) + a_2 f_2(t) + b = X * \beta$$

where X is the template matrix given by:

$$X = [f_1(t) \mid f_2(t) \mid \mathbf{1}]$$

and β is the parameter vector given by:

$$\beta = \begin{bmatrix} a_1 \\ a_2 \\ b \end{bmatrix}$$

The Least Squares solution for the parameter vector is therefore given by:

$$\hat{\beta} = [X^T X]^{-1} X^T y$$

Thus, the LS fits to a sum of two templates are calculated for each possible starting point of a segment of data in the same manner that fits are calculated for each individual template.

The criterion score for a double-template fit is calculated as:

$$\text{criterion score} = \frac{(a_1 + a_2)}{SE}$$

Note that fitting data with a sum of two templates is preferable to creating a new template with a double-exponential decay, because it allows the relative amplitudes of the two exponential components to be freely adjusted to obtain a better fit to the spike. As noted by the red stars in Fig 2C, the double-template fit can often produce higher criterion scores (better fits) than use of a single template. The double-template fit was accepted as the best fit, and thus classifies the spike in the double-exponential category, only if 1) neither amplitude is negative, and 2) the smaller amplitude is at least 10% as large as the larger amplitude.

4.1.6. The matched-filter approach outperforms the derivative-threshold approach

Amperometric recordings from bovine and mouse chromaffin cells were analyzed to compare the Matched Filter (MF) and Extended Matched Filter (EMF, double-template) approaches with the Derivative Threshold (DT) approach. The performance of these approaches for detecting spikes was measured using Receiver Operating Characteristic plots described in the Methods section. In brief, reducing the threshold for detection (criterion score for MF and EMF, derivative in *pA/ms* for DT) results in detection of more spikes (thus higher sensitivity), but will eventually result in a greater number of false positives (thus lower specificity). An optimal algorithm using an ideal threshold will result in 100% detection with 0% false positives, i.e., the upper left corner of an ROC plot. Fig 6A presents a representative ROC plot for a recording from a bovine chromaffin cell containing 80 spikes. The performance of the MF (red) and EMF (black) approaches are similar, and are contrasted to that of the DT approach with several different settings of low-pass filtering. The MF approach detects 100% of the spikes for 2% false positive fraction (criterion score = 2.6). The performance of the DT approach depends on the filtering cutoff frequency (Fig. 6A). Nevertheless, even at the best cutoff frequency (20 Hz), DT detects only 60% of the spikes for a 2% false positive fraction (criterion score = 30 pA/ms). Amperometric events are missed with DT even when the threshold was lowered to the point where the false-positive fraction exceeds 10%. Missed events tend to be smaller and slower, as shown in

examples in Fig. 6C. On the other hand, rapid fluctuations in the signal are sometimes misclassified as spikes using DT. Fig. 6B presents ROC plots for five bovine chromaffin cell recordings that demonstrate similar characteristics. In mouse chromaffin cells (Fig. 7) MF and EMF could detect 100% of the spikes for 0% false positive detection (criterion score = 1.7) whereas DT could detect 60% of the spikes for the same false positive fraction (filter setting, DT= 21 pA/s).

4.1.7. The matched-filter approach outperforms commercial event-detection software

A similar ROC analysis was employed to compare the performance of MF algorithm to commercial applications primarily intended for analysis of spontaneous post-synaptic events: MiniAnalysis (Synaptosoft, Fort Lee, NJ, USA) and ClampFit (Molecular Devices, Sunnyvale, CA, USA).

When the threshold for these methods is set to a value that results in ~2% false positives, the MiniAnalysis and ClampFit applications only detect 45% (at threshold = 3) and 70% (at threshold = 4), respectively, of the spikes in bovine chromaffin cells (Fig. 8A). In contrast, the MF or EMF approaches are able to detect 100% of the spikes for 0% false positives (threshold = 2.6). In mouse cells, MiniAnalysis and ClampFit detect 70% (at threshold = 3) and 75% (at threshold = 3) of the spikes for zero false positives (Fig. 8B). Thus, the MF approach emerged as the superior method among all available detection applications. Examples of missed spikes and false positives for MiniAnalysis and ClampFit are depicted in Fig. 8C, D.

The conditions for detection such as filtering, and detection threshold for each software program were chosen to ensure that the detection performance was optimal. For example, the Derivative Threshold approach was tested at the optimal smoothing frequency; The same template library was used to apply ClampFit software as the MF algorithm. However, the experimenter did not have access to all controls in commercial software and this affects the scope of the ROC plot performance comparison.

5. Discussion

A MF approach was applied for the detection of exocytotic events in amperometric recordings. The performance of this method is superior to existing commercial and user-community applications, as demonstrated by ROC plots. The MF technique provides two main advantages. First, it eliminates the need for low pass filtering the data for noise reduction. DT approaches rely on data smoothing both before and after computing the derivative of the original recording and the best choice of filter setting depends on the signal-to-noise ratio of the recording (Gómez et al., 2002; Mosharov and Sulzer, 2005). Excessive low-pass filtering of data can cause the algorithm to overlook actual spikes whereas inadequate smoothing can increase false positive detection due to noisy transients (Fig. 6). In addition, the setting of the derivative threshold that maximizes sensitivity and selectivity depends on the low-pass filter setting. Hence it is not straightforward to choose low pass filter settings.

A second advantage of the MF technique is that detection bias is explicit in the choice of the templates to be included in the library. The MF method uses information from both the rising and falling phases of the spike to enable detection that is rationally biased by the choices made in constructing the template library. In contrast, the DT approach relies on a rapidly rising phase of the spike to enable detection, and therefore introduces a bias against detecting slowly rising spikes.

An alternative approach to detect exocytotic spikes is to subtract the time-varying baseline from the signal and then detect spikes that exceed amplitude thresholds (Friedrich and Ashery, 2010). However, tracking the time-varying baseline with pA precision requires that the baseline be stable over the averaging time window and relies on user input of the noise level. Also, in an amplitude-based algorithm small-amplitude flickers may be mistaken as spikes (Friedrich and Ashery, 2010) whereas our approach rejects such flickers because their time courses do not match the templates. Avoiding false positives inevitably makes amplitude-based algorithms more biased against small-amplitude events than template-based algorithms.

All reasonable efforts were made to reduce bias in comparing algorithms. Events were classified as “hits” or “misses” (false positives) using automated criteria described in Methods. User-adjustable parameters were selected for each algorithm that maximized performance. Nevertheless, there is the possibility that bias can be introduced by the user in the application of the various software packages.

Selection of an appropriate template duration is important for the algorithm to function appropriately. Use of a long-duration template makes it difficult to resolve spikes closely spaced in time whereas a brief template poses the risk of increasing the false positive fraction. We found that a template size of ~200 ms is optimal for bovine and mouse chromaffin cell recordings. The short pedestal that precedes the template is also critical for high-performance detection, and we found a 20 ms pedestal works well.

Template libraries need to be chosen for the specific biological preparation and the type of event one wants to detect. We selected the time constants for the rising and falling phases for bovine versus mouse chromaffin cells after looking for clusters of time constants in exponential fits to sample data sets. Development of semi-automated methods for creating template libraries would be a useful extension of the approach, but is beyond the scope of this study.

The identification of the template that produces the highest criterion score (Fig. 2C) can be used to classify events into categories that describe the nature of the fusion event (e.g., fast versus slow opening of the fusion pore). Addition of new templates may be useful to identify and classify other types of fusion events such as kiss and run (stand-alone feet).

It should be noted that the Least Squares fitting that is central to this approach is computationally efficient because it is a closed-form solution, unlike iterative curve fitting which is necessary to solve for fitting parameters that are not linearly related to the signal, such as the exponential time constants.

This work concentrates on computationally efficient detection of spikes; however, this is usually followed by estimation of relevant spike parameters such as charge (area), duration (FWHM) and the duration and charge of the “foot signal” that often precedes the spike. One approach to quantify a spike is to fit it with an exponential time course similar to the templates we used, but allow the time constants to vary during an iterative fitting process. The MF approach is well suited to provide “seed” values from the LS fit template with the highest criterion score for iterative curve fitting, including the start time, amplitude and time constants.

The EMF approach to fit data to a sum of two templates did not perform substantially better to detect spikes than the simpler approach based on a single template. Nevertheless, this approach performed equal to MF approach for detection and also provides excellent seed values enable efficient fitting of spikes with double-exponential decays during the parameter estimation phase (data not shown).

6. Conclusions

Our implementation of the MF algorithm is a highly specific and sensitive amperometric spike detection approach. MF outperforms the classic approach of Derivative Threshold (DT) as well as other commercial and user-community applications such as ClampFit and MiniAnalysis.

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Abbreviations

MF	Matched Filter
EMF	Extended Matched Filter
DT	Derivative Threshold
ROC	Receiver operating characteristics

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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

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
(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.

Fig. 2. The heterogeneity of spike time courses necessitates the use of multiple templates for detection

(A) A sample amperometric recording (B) Samples of amperometric spikes illustrating the heterogeneity of time courses and amplitudes. (C) *Solid black trace*: sample data segment with spikes. *Blue traces*: criterion scores for each of the templates depicted on the left. The *red stars* indicate the template with the highest criterion score for each spike. The *black stars* represent the highest-scoring template when using only single exponentially decaying templates, but where the sum of two templates (T3 + T4) approach resulted in a higher score (*red stars*).

Fig. 3. A two-threshold approach reduces false positives

(A) A single threshold algorithm can produce false positives as the criterion score fluctuates above and below the threshold on the falling phase of a spike. (B) False positives are avoided if the criterion score must fall below a second, lower threshold before initiating a search for the next spike. *Black*: data trace. *Red*: least-squares fits determined at the peaks of the criterion scores *Blue*: Criterion scores

Fig. 4.

(A) **Top:** Amperometric trace sampled at 10 kHz (gray) and decimated to 1 kHz (blue). **Black arrows** point to true spikes. **Bottom:** Criterion scores corresponding to the unfiltered (dashed gray) and the decimated (dashed blue) data. **Inset** expanded single spike shows the original traces (top) and the criterion scores (bottom). **Red dotted line** is the criterion threshold of 2.6.

(B) **Top:** Original amperometric trace (red) and trace with a baseline slope of -10 pA/s (black). All the arrows point to true spikes. **Green arrows** point to overlapping spikes.

Bottom: Criterion scores for original trace (*dashed red*) and trace with -10pA/s baseline slope (*dashed black*). *Blue dotted line* is the criterion threshold of 2.6.

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Fig. 5.
(A) Top: Amperometric trace (*black*) preceded by a ramp + plateau type foot signal with the MF fit (*red dotted*). **Bottom:** Criterion scores of four templates (*varying shades of blue*). **(B) Top:** Amperometric trace (*black*) preceded by a ramp type foot signal with the MF fit (*red dotted*). **Bottom:** Criterion scores of four templates (*varying shades of blue*).

Fig. 6. The MF algorithm outperforms the DT approach in bovine chromaffin cell recordings (A) ROC plot for a recording comparing the DT algorithm with several filter settings with the MF and EMF approaches. Several criterion scores (CS) are indicated by arrows on the plot. (B) ROC plot for five recordings comparing the performance of spike detection algorithms. Blue: DT, red: MF, black: EMF. Each data set is represented by a unique symbol. Inset: expanded view near the ideal corner. (C) Examples of missed events and false positives using the DT approach. Black traces: section of amperometric data showing missed spike (left) and false positive (right), Red trace: MF fit to spike detected by MF algorithm, green trace: current derivative (dI/dt)

Fig. 7. The MF algorithm outperforms the DT approach in recordings made from mouse chromaffin cells

(A) Amperometric trace (B) Receiver Operating Characteristic plots comparing the performance of spike detection algorithms for five recordings. *Blue: DT, red: MF, black: EMF*. Each data set is represented by a unique symbol. Inset: expanded view near the ideal corner. (C) Examples of missed events and false positives using the DT approach. *Black traces: section of amperometric data showing a missed spike (left) and a false positive (right), Red trace: MF fit to spike detected by MF algorithm, green trace: current derivative (dI/dt).*

Fig. 8. MF outperforms ClampFit and MiniAnalysis

for bovine (A) and mouse (B) chromaffin cells. Receiver Operating Characteristic plots comparing the performance of spike detection algorithms for five amperometric recordings. MF (red), EMF (black), ClampFit (yellow) and MiniAnalysis (green) are shown. Each data set is represented by a unique symbol Inset: Expanded view of ideal corner. Spikes missed and false positives detected by ClampFit and MiniAnalysis are shown for bovine (C) and mouse (D) chromaffin cell data. Red trace: MF fit to spike detected by MF algorithm