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Estimating amperometric spike parameters resulting from quantal exocytosis using curve fitting seeded by a matched-filter algorithm

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Abstract

Background: Quantal exocytosis of oxidizable neurotransmitters can be detected as spikes of amperometric current using electrochemical microelectrodes. Measurements of spike parameters indicate the maximal transmitter flux, flux duration, and amount of transmitter released from individual vesicles. Automated analysis algorithms need to reject spikes that overlap in time. In addition, many spikes are preceded by small amplitude “foot” signals, attributed to slow release of transmitter through a fusion pore. Accurate pre-spike baseline determination is essential for estimating fusion-pore duration and the amount of transmitter released through the fusion pore.

New Method: We developed an estimation approach that is based on fitting a multi-exponential function to the data. Our previously described matched-filter algorithm is used to identify the sections of data to fit and provides seed values to facilitate convergence of the iterative fit. The new estimation algorithm includes overlap rejection, a two-step fitting procedure and a novel baseline estimation procedure.

Results: Histograms of spike parameters demonstrate excellent agreement of the new approach with manually computed parameters.

Comparison with Existing Methods: Parameter estimates generated using the new approach are closer to blind manual estimates than commonly used existing methods. The improved performance is due to better detection of valid spikes and rejection of overlapping spikes. Moreover, since the complete time course of the spike is fit to a function, more complete information about the spike time course is captured.

Conclusions: The matched-filter seeded algorithm reliably rejects overlaps and estimates spike and foot signal parameters in a fully automated manner.

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Keywords

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

1. Introduction

Single-cell amperometry is a powerful technique for studying exocytosis. In this method, electrochemical microelectrodes located directly adjacent to the cell membrane measure neurotransmitter released from individual vesicles as “spikes” or bursts of amperometric current as the transmitter is oxidized on the surface of the electrode (Wightman et al., 1991). Close examination of these events reveals that slow release of transmitter through a nanometer-scale fusion pore produces a pre-spike foot signal (Chow et al., 1992; de Toledo et al., 1993) that is followed by a rapid rising phase as the fusion pore expands. The spike decays in a single or double-exponential manner as the contents of the vesicle are emptied or as release is terminated upon reclosure of the release pathway. Therefore, the temporal dynamics of amperometric spikes can be used to understand the release process from individual vesicles in great detail and to determine how the release process is modified by drugs, second messengers, and perturbation of proteins that participate in the fusion and release process (see (Borges et al., 2006) for a review).

The steps in amperometric spike analysis consist of spike detection followed by estimation of parameters. We previously described a matched filter (MF) template-based spike-detection algorithm that detects ~97% of manually identified spikes for a mere ~2% false positive rate (Balaji Ramachandran and Gillis, 2018). In brief, this algorithm uses a least-squares approach to fit prototypical spike templates consisting of a fast exponential rise and a slower exponential decay to each segment of data in a recording. The amplitude of the fit of each template is divided by the standard error of the fit to determine a criterion score at each possible data segment. A spike is detected when the criterion score exceeds a threshold, and the template with the highest criterion score for the spike is selected as a first-pass representation of the spike. We also described an extension of the matched-filter algorithm that finds the least-squares fit of data segments to the sum of two templates as a better representation of spikes that contain double-exponential decays. The superior performance of the detection algorithms compared to existing approaches was demonstrated using receiver operating characteristic plots.

The next step is estimation of spike parameters such as peak amplitude $I_{\max}$, and charge Q (Jankowski et al., 1993; Schroeder et al., 1992). $I_{\max}$ indicates the maximal transmitter efflux rate and Q indicates the total amount of transmitter released. The kinetics of transmitter release are typically described using two parameters. First, the slope of a line fitted to the rising phase of the spike is used as a measure of release speed and the full-width-half-maximal time (t_{50}) of the spike is used as a measure of release duration. Whereas these two parameters provide basic information about release kinetics, they do not fully describe the spike time course, which is often multi-exponential (Wang et al., 2001). Fitting a function consisting of an exponential rise and a sum of one or two exponential decays can, in principle, describe the full time course of typical spikes, but fitting exponential time

constants is complicated in that the time course is a non-linear function of the time constants, therefore an iterative fitting routine must be applied. Iterative curve fitting is highly sensitive to seed values (initial parameter estimates), therefore *a priori* estimates of parameters are required to produce reliable fits to spikes. Here we describe an approach to use our previously described template-based match-filtering algorithm to provide seed values for the start time, amplitude and time constants to enable reliable curve fitting to spikes that vary considerably in time course.

Estimating parameters from spikes that overlap in time is problematic because they do not decay back to baseline and it is ambiguous to locate their start and end points. Previous algorithms sometimes fail to identify overlapping spikes, either incorrectly identifying two closely occurring spikes as a single spike and consequent skew estimates of spike parameters, or the mis-identifying the first of two overlapping spikes as a foot signal. Here we describe an automated overlap-identification algorithm that uses information about the time constant of decay from our matched-filter detection algorithm to reject spikes that are spaced too close in time to allow reliable parameter estimation.

Pre-spike foot signals provide information about the amount of neurotransmitter released through a narrow fusion pore prior to expansion and also the lifetime of the fusion pore. Determining the pre-spike baseline is critical for accurately identifying the starting time of the pre-spike foot. Here we present a novel extension of our template-based detection algorithm that looks backwards from identified spikes to identify the flat baseline immediately preceding the pre-spike feature.

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 CaCl₂, 1.2 MgCl₂, 10 HEPES, and 11 glucose, pH 7.2. A high potassium concentration solution 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 (Gillis et al., 2017). Recordings were filtered at 3 kHz and sampled at 10 ksamples/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. Decimation results in faster computational speed and a 1 kHz decimated sampling rate is sufficient to resolve the fastest rise time of events in chromaffin cells. Recordings made from mouse chromaffin cells were provided by the laboratory of Kevin Currie (Vanderbilt Univ.) and acquired as previously described (Jewell et al., 2011). Mouse chromaffin cell recordings were filtered at 2 kHz and sampled at 10 ksamples/s.

2.2. Comparison with other estimation software

Software developed by Mosharov and Sulzer (Mosharov and Sulzer, 2005) was used for detecting and estimating spikes to compare with the algorithm described here. The Mosharov and Sulzer software requires the user to specify a smoothing frequency, background region and peak threshold. The smoothing frequency determined to give optimal performance was 20 Hz, so this was used for all comparisons. A spike-free region in the amperometric trace was selected to be the background region. The peak threshold was set to be 3.

2.3. Estimation algorithm

Fig. 1(A) presents a typical amperometric spike recorded from a bovine adrenal chromaffin cell and common parameters to be estimated including the full width half maximum duration (t_{50}), peak amplitude ($I_{\max}$) and area (Q). Pre-spike foot area (Q_{foot}) and duration (t_{foot}) are also indicated.

Fig. 1(B) depicts the overall process flow of the parameter-estimation approach. The first step in spike estimation is the identification of a spike. Our previously described matched-filtering algorithm uses four prototype spike templates that vary in time course. Each template is least-squares fit to all possible segments of data in a recording and the amplitude of the fit of each template is divided by the standard error of the fit to determine a criterion score at each time point. A spike is detected when the criterion score exceeds a threshold, and the template and start time (t_0) with the highest criterion scores for the spike are identified. If the sum of two templates produces a higher criterion score and the smaller template amplitude is at least 10% the size of the larger template amplitude, the event is classified as “double exponential” for parameter identification. Note that fits of templates to the data is by least-squares linear regression, and is therefore a closed-form solution without iteration.

The matched-filter detection process yields a set of initial parameter values for the spike including start time (t_0), rise time constant (τ_r), decay time constant(s) ($\tau_{d1,2}$), amplitudes of the exponential decay(s) (a_1, a_2) and baseline (b). Next, these template parameters are passed onto our overlap rejection algorithm described in detail in section 3.2.1. If adjacent spikes are found to be overlapping, they are rejected for estimation. If adjacent spikes are not overlapping, spike parameters are passed to a two-step curve fitting algorithm. In the first step two iterative curve fits are performed, (i) the rising phase, defined as the interval between the start time and the peak, is fitted by an exponential seeded by parameters t_0 , τ_r , amplitude (a_1+a_2) and baseline (b) (ii) the decaying phase of the spike, starting at its peak, is fit by a single or double-exponential decay seeded by matched-filter parameters t_0 , $\tau_{d1,2}$, a_1, a_2 and baseline (b). In the second step, the parameters obtained from the rising and falling-phase fits are seeded into the combined function to fit the entire spike. The output parameters of this curve-fitting step are then used to compute spike parameters including Q , t_{50} and $I_{\max}$. In the final segment of our algorithm, the pre-spike criterion scores are used to determine the baseline and pre-spike foot signals are identified and analyzed to compute t_{foot} and Q_{foot} .

2.3.1 Rejection of overlapping spikes using matched-filtering template parameters

—When a high spike frequency (rate of release) is measured, it is common for spikes to overlap in time (Wightman et al., 1991). Overlapping spikes are poor candidates for parametric analysis since their time courses and areas cannot readily be resolved.

Therefore, the first step in spike estimation is the identification and rejection of overlapping spikes. Traditional approaches to rejecting overlaps rely on user intervention to define the acceptable extent of overlap, and, in our experience, often fail to identify two spikes located close together in time. Here we describe an algorithm that detects overlapping spikes using two criteria.

Criterion 1: For every pair of successive spikes, the second spike (S_2) is rejected for analysis if it occurs prior to the time when the decay of the first spike (S_1) falls below a minimum current ($I_{\min}$, typically 1 pA). The rationale for this criterion is that the first spike has not decayed sufficiently to avoid disrupting the baseline for the second spike. The least-squares fit of the template(s) is used to predict the decay of S_1 . In the case of a single-exponential decay, the falling phase of a spike is approximately given by,

$$f(t) = I_{\max} \exp\left(-\frac{t}{\tau_d}\right)$$

where, $I_{\max}$ is the peak amplitude of the amperometric trace. By setting this function to $I_{\min}$, the minimum acceptable interval from the peak until a second spike (S_2) occurs is given by:

$$t_{\text{window}} = \tau_d \ln\left(\frac{I_{\max}}{I_{\min}}\right)$$

Criterion 2: S_1 is rejected for analysis if S_2 occurs less than three times the slower decay time constant ($3\tau_{d2}$) from the time of the peak of the first spike. The rationale for this criterion is that a duration of three- time constants is needed to ensure that the spike time course can be fit with high confidence.

Fig 2 presents examples of overlapping spikes. In Fig 2 (A) the first spike is accepted because the $3\tau_{d2}$ time point (blue asterisk) occurs before the second spike started. However, in Fig 2 (B) the first spike is rejected because another spike occurs before the first spike decays to $3\tau_{d2}$.

2.3.2. Iterative curve fitting of spikes—The next step in spike estimation is curve fitting to a spike model. The templates used to detect spikes are a function consisting of a fast-exponential rise followed by a slower exponential decay:

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

Where, τ_r is the rise time constant and τ_d , is the decay time constants that are selected as representative of “typical” spike time courses. This least-squares fit of the template to the data ($y(t)$) is of the form,

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

Where the amplitude a and baseline / offset b are found using the (non-iterative) least-squares algorithm (Balaji Ramachandran and Gillis, 2018). Our extended matched filtering approach fits spikes to a sum of templates with different decaying time constants in the form:

$$\hat{y}(t) = a_1 f_1(t) + a_2 f_2(t) + b; \quad t > 0$$

Whereas least-square fits of templates to spikes enables excellent spike identification, the fits are imprecise because the time constants of the templates are fixed. Adjustments of the time constants are necessary to enable estimation of spike parameters based on precise fits of the exponential function (Equation 1) to the data. Since the function has a non-linear dependence on the time constants, an iterative curve-fitting approach is needed (e.g., (Levenberg, 1944; Marquardt, 1963)), and appropriate convergence of the fit is highly sensitive to initial parameter estimates (seed values). Parameters obtained from the least-square template fits were used as seed values in an iterative curve fitting routine. The first step is to fit the rising phase of spikes to a function of the form,

$$\hat{y}(t)_{rise} = a \left(1 - \exp \left(-\frac{(t-t_0)}{\tau_r} \right) \right) + b$$

$$; \quad t_0 < t < t_{peak}$$

Subsequently, the decaying phase of the spike is fit to a single or double exponential, depending on the classification. For a double-exponential fit the decaying phase is of the form

$$\hat{y}(t)_{decay} = a_1 \exp \left(-\frac{(t-t_0)}{\tau_{d1}} \right) + a_2 \exp \left(-\frac{(t-t_0)}{\tau_{d2}} \right) + b$$

$$; \quad t > t_{peak}$$

Examples of fits to the rising and decaying phases are shown in the top of Fig 3. In the final fitting step, entire spikes are fit by seeding output parameters from the separate rising and decaying fits to Equation 1 (for the double-exponential case):

$$\hat{y}(t) = \left(1 - \exp \left(-\frac{(t-t_0)}{\tau_r} \right) \right) \left(a_1 \exp \left(-\frac{(t-t_0)}{\tau_{d1}} \right) + a_2 \exp \left(-\frac{(t-t_0)}{\tau_{d2}} \right) \right) + b \quad (\text{Equation 1})$$

$$; \quad t_0 < t < t_{peak} + 3 * \tau_{d2}$$

Seed values for τ_r , and t_0 are from the rising phase fit whereas $a_{1,2}$, b , and $\tau_{d1,2}$ are from the decaying phase fit. Fig 3 *Bottom* depicts typical examples where excellent fits to the entire spike are obtained.

After the fit, the entire time course, amplitude and area of each spike is described by either three parameters (single exponential decay: τ_r , a , τ_d) or five parameters (double exponential decay: τ_r , a_1 , a_2 , τ_{d1} , τ_{d2}). These parameters are then used to calculate the spike parameters of interest to the investigator including the area under the curve or charge (Q), full width half maximum (t_{50}) and peak amplitude ($I_{\max}$). Specifically, the roots of the spike function for a y value of $I_{\max}/2$ are located on either side of the peak and the time difference between these roots is calculated as t_{50} . The function maximum is computed as $I_{\max}$. The spike function is integrated to compute Q. In addition, measures of the spike rising phase such as the 25% to 75% rise time can be calculated from equation 1.

2.3.3. Baseline identification and pre-spike foot analysis—The next step of the algorithm is to estimate parameters describing the pre-spike foot. Accurately identifying and analyzing pre-spike foot signals requires very precise determination of pre-spike baseline current. Thus, the value of parameter b obtained by fitting Equation 1 is not accurate enough for this purpose. We devised an algorithm to compute pre-spike baseline current as an extension of our template- based matched filtering approach used for spike detection. As templates are least-squares fit to each segment of data, a criterion score is created at each time point that is the ratio of the amplitude of the template fit divided by the standard error of the fit to the data segment:

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

In the case of fits to a sum of two templates (double-exponential decay), the criterion score is given by:

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

where a_1 and a_2 are the least-squares amplitudes found for the two decaying exponentials. Peaks in the criterion score that exceed a threshold identify spikes during the detection phase. On the other hand, a criterion score of zero occurs when the data segment is flat, i.e., the amplitude of a spike fit is zero, indicating a potential baseline segment. Use of the criterion score to determine a stable baseline region is, in some cases, preferable to measurement of standard deviation of the raw current because it is less sensitive to a sloping baseline (Fig. 4B in (Balaji Ramachandran and Gillis, 2018) and Supplemental Fig S1) or spurious interference, e.g., residual line noise.

Fig. 4 depicts a typical amperometric spike with corresponding criterion scores. Starting from the peak of the criterion score (beginning of spike), the algorithm searches backwards in time until the first zero crossing is detected (green circle in Fig. 4). This zero crossing occurs as the template and the data first begin to align. This is preceded in time by a negative

criterion score where the template decaying phase aligns with the spike rising phase, leading to a negative correlation. The algorithm continues to search backward in time until the second zero crossing of the criterion score occurs (red circle in Fig. 4), which indicates the region where the baseline is flat. In order to test for a stable baseline, the algorithm determines the standard deviation of the criterion score for a segment of data preceding the second zero crossing with a duration equal to that of the template (line segment in Fig. 4). If the standard deviation of the criterion score for this segment is less than or equal to 0.05, this segment of the amperometric data is averaged to give the baseline value. If the standard deviation is greater to 0.05 the segment is moved backward in time point by point until a stable baseline is identified. This procedure for determining the pre-spike baseline is repeated for each spike.

The start time for a pre-spike foot is defined as when the signal rises two standard deviations above the baseline value (Mosharov and Sulzer, 2005; Zhao et al., 2013) with the standard deviation determined from the baseline data segment. The end of the foot signal is defined as t_0 determined from the iterative curve fit of the corresponding spike (equation 1). This is the point where the exponential rise of the spike begins (i.e., the maximum slope), and hence signifies the end of the foot signal.

3. Results

3.1. A two-criterion approach based on template fits successfully determines if successive spikes are overlapping

Detecting and rejecting overlapping spikes is a necessary first step to make accurate estimates of spike parameters. Our recently described template-based detection algorithm generates estimates of the decay time constant(s) based on the templates that produce the highest criterion score. In order to detect overlapping spikes, the decay time constant (or slower time constant in the case of spikes that decay with two exponentials), together with the peak amplitude $I_{\max}$, are used to set the time window within which a second spike will be rejected (Criterion 1 defined in section 3.2.1). A second spike is rejected if it occurs within this time window because the first spike has not decayed sufficiently to avoid disrupting the baseline of the second spike (e.g., Fig. 2). Fig 5(A) presents examples to illustrate that application of Criterion 1 allows retention of well-separated spikes for analysis whereas overlapping spikes are rejected. Fig 5(B), (C) show that a commonly used overlap rejection algorithm (Mosharov and Sulzer, 2005) fails to reliably reject overlapping spikes. In the Mosharov algorithm, the user sets the extent of allowable overlap, defined as the percentage ratio of the minima between adjacent spikes and the maximal height of the taller spike. A similar approach to reject overlapping spikes is described by Friedrich and Ashery (Friedrich and Ashery, 2010). Spikes accepted using an overlap setting of 50% (a typical level, (Mosharov and Sulzer, 2005)) are shown in Fig 5(B), whereas Fig 5(C) show that clearly overlapping spikes are accepted even for an overlap setting as low as 0.5%. The inability to reject overlaps primarily results from misidentification of the ending time of a spike (Fig 5(B), (C) gray bars) whereas our algorithm, following iterative fitting, correctly identifies the end time of spikes (Fig 5(A)).

Another parameter that affects rejection of overlapping spikes in the Mosharov algorithm is the “allowable baseline drift”. Supplemental Fig S2 A–C presents sample data whereby reducing this parameter improves the ability of the Mosharov algorithm to identify overlapping spikes and the end time of a spike. Nevertheless, misidentification errors persist at all settings for this sample trace. Fig S2 D demonstrates that our algorithm successfully eliminates overlapping spikes in the sample trace with an accuracy that is not sensitive to the precise value of $I_{\min}$ from criterion 1 (range: 0.5 –10 pA).

3.2. Matched filtering template parameters can reliably seed an iterative curve fit of the entire spike time course by equation 1.

Curve-fitting is a desirable means to extract spike parameters because it smooths random fluctuations found in the raw data. Current approaches typically fit a line to an arbitrary segment of the rising phase (e.g., 25% - 75% $I_{\max}$, (Mosharov and Sulzer, 2005)) as a measure of the rate that a fusion pore dilates to allow rapid release. In contrast, our algorithm fits an exponentially rising function to the rising phase of the spike, which provides a good fit to many spikes with less sensitivity to the selection of start and end points of a linear fit. On the other hand, iterative curve fitting is highly sensitive to the accuracy of initial seed values. Therefore, the template fits that are obtained during matched filtering detection can aid in seeding an iterative fitting process. Our approach first fits the rising and decaying phases separately to improve the quality of seed values provided to the combined spike fit function, which contains up to seven free parameters (equation 1). Fig 6(A), (B) and (C) show the fitting of the rising phase, decaying phase and entire spike, respectively, which produces excellent fits to typical spikes determined by visual inspection.

3.3. Iterative curve fits provide accurate parameters for computing Q, $I_{\max}$ and t_{50}

Iterative curve fitting provides values for each spike (τ_r , $\tau_{d1,2}$, $a_{1,2}$) which are used to compute common measures of spike amplitude and time course such as Q (charge), t_{50} (half-maximal width) and $I_{\max}$ (peak amplitude). Data from bovine and mouse chromaffin cells were analyzed using both the new matched filter algorithm and that of (Mosharov and Sulzer, 2005) to allow comparison of spike parameters. These were then compared with manual parameter estimates performed blindly with regard to the results of the other analyses.

Fig 7 presents mean values for the three parameters whereas median values are presented in Table 1. Median values obtained from the fully automated MF algorithm are generally within ~10% of values obtained through laborious manual analyses for both bovine and mouse chromaffin cell recordings, whereas the Mosharov and Sulzer algorithm generally gives poorer results. Fig 8 presents histograms of the values for bovine (left column) and mouse (right column) chromaffin cells. It is typical to present histograms of Q raised to the one-third power (Fig 8 (A), (D)) because charge is proportional to vesicle volume and vesicle radius typically has a Gaussian distribution, or consists of two Gaussians representing two populations of vesicles (Jankowski et al., 1993; Schroeder et al., 1996). Log transformed parameters t_{50} and $I_{\max}$ also exhibit double Gaussian distributions (van Kempen et al., 2011). Gaussian fits for the fully automated MF algorithm are in good agreement with those obtained using manual analysis.

The non-parametric Kolmogorov and Smirnov test (Kolmogorov, 1933) was applied to compare the MF as well as the Mosharov and Sulzer computed parameters against manually computed parameters. The KS test computes p values, with values less than the “critical” p value supporting the null hypothesis that the parameter values are significantly different. The MF algorithm produced parameter values that did not significantly differ from manual analysis for bovine parameters Q ($p_{\text{critical}} = 0.105$, $p_{\text{MF}} = 0.143$), I_{max} ($p_{\text{critical}} = 0.104$, $p_{\text{MF}} = 0.486$) and t_{50} ($p_{\text{critical}} = 0.115$, $p_{\text{MF}} = 0.65$). In contrast, the M/S computed bovine parameters were statistically different than those obtained by manual analysis for Q ($p_{\text{critical}} = 0.115$, $p_{\text{MS}} = 3.0 \times 10^{-4}$), I_{max} ($p_{\text{critical}} = 0.114$, $p_{\text{MS}} = 7.38 \times 10^{-5}$) and t_{50} ($p_{\text{critical}} = 0.117$, $p_{\text{MS}} = 2.0 \times 10^{-4}$). In mouse chromaffin cell data, critical p-values were not surpassed by either the MF or M/S methods, indicating statistical differences between both approaches and manually computed parameters. However, the p- values for MF method were several-fold higher than those comparing the M/S and manual methods, indicating a greater similarity between MF and manual methods (data not shown). As indicated in Fig. 7, the percentage deviation of mean parameter values was modest for the MF approach compared to manual analysis for both bovine and mouse events.

3.4. Use of the criterion score from matched filtering can accurately identify pre-spike baseline for foot-signal analysis

In the matched-filtering based foot detection approach, the positive criterion zero crossing was used to determine the baseline. Then the first point that exceeded two standard deviations above the baseline was identified as the start of the foot signal and the end of the foot signal is determined from t_0 of the iterative curve fit of equation 1 to the spike. The resulting foot duration is denoted as t_{foot} whereas the integral of the amperometric signal during the foot is denoted as Q_{foot} . Fig 9 presents box and whisker plots of t_{foot} and Q_{foot} . Note that the fully automated MF-based approach gives better agreement with manual measurements of the foot signal charge and duration than the Mosharov and Sulzer approach. Fig 10 presents several example time courses of foot signals with the green circles denoting the start and end points detected using the automated matched-filtering based approach. Note that the proposed approach can faithfully capture the start and end times of foot signals with plateauing, ramping and sigmoidal- shaped time courses.

4. Discussion

The template-based matched filtering detection algorithm we recently described accurately detects spikes with few false positives (Balaji Ramachandran and Gillis, 2018). Here we show that the approach also generates initial parameter estimates useful to detect overlapping spikes and seed iterative curve fitting to extract complete information about the spike time course.

Overlapping spikes were reliably detected and eliminated from analyses using a dual-criteria approach. Note that this approach applies separate criteria for the first and second spike in a pair where the principle concern of accepting the first spike is that it has sufficient time to decay so that accurate decay time constants can be fit to the data. On the other hand, a more stringent criterion is applied to accept the second spike in a pair because the first spike must

nearly completely decay to avoid disrupting the baseline for the second spike and allow foot analysis to proceed. Also note that the detection phase of analysis identifies essentially all spikes to allow accurate estimation of the frequency of exocytosis events near the electrode (Balaji Ramachandran and Gillis, 2018). However, only a subset of spikes can proceed to the analysis phase when events are detected at a high frequency because of overlap. This potentially introduces a bias in spike analysis, regardless of the approach used, if spike parameters are different at high release rates.

Our approach fits equation 1 to spikes to describe the complete amplitude and time course (not including the pre-spike foot) with either three (single exponential) or five (double exponential) parameters. This parameterization is a more complete description of typical spikes than parameters such as $I_{\max}$, Q , and t_{50} . For example, two spikes may have the same t_{50} but substantially different double-exponential decays. Thus, the parametrization by equation 1 can support different post-analyses of spike time course per the wishes of the user.

We find that equation 1 provides excellent fits to typical spikes in bovine and mouse chromaffin cells, but some events, e.g., “stand-alone feet” (Wang et al., 2003) are not adequately described by equation 1. In principle, different fit functions / templates can be created for other event waveforms to allow analysis of these events to proceed in a similar fashion to what is described in this work.

Whereas template fits during the detection phase provide reasonable seed values to allow iterative fitting of equation 1 to the data, we found that first fitting the rising and falling phases separately produces better results by allowing refinement of seed parameters before fitting equation 1 to the data. Visually good fits were obtained and the finely resolved parameter values are used to compute Q , t_{50} and $I_{\max}$. Parameter histograms have centers and spreads within 12% and 4% of manually computed parameter histograms with an overall performance significantly better than the Mosharov and Sulzer algorithms.

Finally, we show that the matched filtering criterion score-based baseline detection algorithm resulted in mean and median values of foot signal charge and duration within 3% and 11% of manually computed values. Developing algorithms to reliably determine baseline values within a fraction of a picoampere is more difficult than it might appear from visual examination of records. Use of the criterion score for this is novel, and has the advantage that scores are unitless, so the method does not require prior knowledge of the noise standard deviation to find a stable region of the signal.

Conclusions

Our matched filtering-based spike and foot estimation algorithm effectively determines spike parameters in a fully automated manner and outperforms previous approaches.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

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

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Highlights

- Algorithms for estimating amperometric spike parameters are described
- A matched-filter detection algorithm provides seed values for curve fitting
- A multi-exponential function is fit to spikes in two steps
- Precise baseline estimation allows accurate measure of pre-spike foot signals
- The fully automated algorithms accurately identify spike parameters

Fig 1.

(A) An example of an amperometric spike preceded by a pre-spike foot. Spike parameters Q , $I_{\max}$, t_{50} and foot parameters t_{foot} , Q_{foot} are indicated. (B) Overall process flow of the estimation algorithm. Matched-filter template parameters were seeded into an overlap rejection algorithm. Well-separated spikes were fit with a (i) rising phase function (ii) decaying phase function. Parameters from the rising and decaying phase fits seeded a combined function that fit both phases of spikes. Q , $I_{\max}$ and t_{50} were computed for spikes. Pre-spike foot signals were estimated to calculate t_{foot} and Q_{foot} .

Fig 2.

Rejection of overlapping spikes using two criteria. (A) The first spike is accepted because it had the opportunity to decay for at least three time constants (*blue star*). However, the second spike is rejected because the first spike has not decayed to < 1 pA when the second spike occurs.

(B) The first spike did not decay to $3\tau_d$ (blue star) and is therefore rejected. The second spike is also rejected because the first spike has not decayed to < 1 pA when second spike occurs.

Fig 3.

Matched-filter template parameters provide seed values for a two-step iterative curve fitting routine. *Top Left:* Rising phases of well separated spikes are fit using a rising exponential function. *Top Right:* Decaying phases of well separated spikes are fit using a single or double exponential function. Parameters from the individual fits are then seeded to fit equation 1 to the entire spike. Fits are indicated in red.

Fig 4.

Template-based approach for finding the baseline preceding a spike. Top: amperometric trace (*black*). Bottom: corresponding criterion scores for the template that gave the largest peak score (*blue*). The algorithm searches the criterion scores back in time from the peak (beginning of spike) to find the first (green circle) and second (red circle) zero crossings. The second zero crossing usually denotes a flat segment indicating the baseline. The algorithm searches back in time from the second zero crossing to find a segment where the standard deviation of the criterion scores is 0.05 (line segment). The amperometric trace during this time segment is averaged to give the baseline value.

Fig 5.

A two-criteria approach successfully rejects overlapping spikes for parameter analyses. (A) Amperometric trace with many overlapping spikes. Green arrows indicate the three sufficiently separated spikes according to the dual-criteria algorithm whereas the red Xs indicate detected spikes that failed the test. The gray bars indicate the spike intervals determined by the algorithm. The end time of the spike is taken as three times the slower time constant determined following iterative fits to equation 1. (B) The same segment of amperometric trace with blue arrows indicating spikes accepted using the Mosharov and Sulzer algorithm with an overlap threshold of 50%. Note from the gray bars that the spike end times are misidentified by the algorithm. (C) Same as (B) using an overlap threshold of 0.5%. In parts (B) and (C) the allowable baseline drift was set to 50% (default value).

Fig 6.

A sequential fitting process results in excellent fits of equation 1 to well-separated spikes (A) Red traces are fits to the rising phases of well-separated spikes (indicated by arrows). (B) Next, fits are made to the falling phase of spikes (red traces). (C) Finally, fits of equation 1 to the entire time course are made using seed parameters resulting from the fits to the rising and decaying phases. Insets demonstrate fits on an expanded time scale. Scale bars in light gray insets are 20 pA and 60 ms and scale bars are 10 pA and 5 ms for dark gray insets.

Fig 7.

Mean values of Q , $I_{\max}$ and t_{50} indicate the excellent performance of the new MF parameter-estimation algorithm. Amperometric data from five bovine chromaffin cells (358 spikes, top row) and five mouse chromaffin cells (219 spikes, bottom row) were analyzed. Error bars are $\pm$ SEM.

Fig 8.

Histograms of spike parameters indicate the excellent performance of the new MF parameter-estimation algorithm. Amperometric data from five bovine chromaffin cells (352 spikes, left column) and five mouse chromaffin cells (219 spikes, right column) were analyzed. In each histogram data from manual analysis are blue, the new MF algorithm is in red, and the M/S algorithm is black. Curves represent double Gaussian fits to the data. (A), (D) Histograms of $Q^{1/3}$. (B), (E) Histograms of $\log$ transformed $I_{\max}$ values. (C), (F) Histograms of $\log$ transformed t_{50} values.

Fig. 9.

Box and whisker plots of Q_{foot} , t_{foot} indicate the excellent performance of the new MF parameter- estimation algorithm. Amperometric data from five bovine chromaffin cells (206 foot signals) were analyzed.

Fig.10.

The matched-filter based algorithm can accurately measure pre-spike foot signals with varying time courses. The start and end times determined from the MF algorithm are indicated as green circles and the red circles indicate start and end times for the M/S algorithm. (A) Plateau-type foot signal (B) Ramp type foot signal (C) Sigmoidal foot signal. The blue traces are criterion scores. The *orange circles* denote positive zero crossing of the criterion score. The *brown circles* denote the beginning of the stable region of the criterion score used to determine the baseline.

Table 1.

Median values of Q , $I_{\max}$, and t_{50} computed for bovine and mouse chromaffin cell recordings using the new matched-filter based algorithm (MF), manual analysis, and the algorithm from (Mosharov and Sulzer, 2005) (M/S). The M/S algorithm was tested at an allowable overlap level of 50%

Statistic	Bovine chromaffin cells			Mouse chromaffin cells		
	Median			Median		
Method	MF	Manual	M/S	MF	Manual	M/S
	(n=358)	(n=352)	(n=444)	(n=212)	(n=219)	(n=294)
Q (pC)	1.27	1.21	1.53	0.0891	0.110	0.0075
$I_{\max}$ (pA)	14.8	14.6	9.49	1.59	1.74	0.1674
t_{50} (ms)	108	100	87.5	60.0	64.1	35.10