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Spike detection from noisy neural data in linear-probe recordings

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Abstract

Simultaneous recordings of multiple neuron activities with multi-channel extracellular electrodes are widely used for studying information processing by the brain's neural circuits. In this method, the recorded signals containing the spike events of a number of adjacent or distant neurons must be correctly sorted into spike trains of individual neurons, and a variety of methods have been proposed for this spike sorting. However, spike sorting is computationally difficult because the recorded signals are often contaminated by biological noise. Here, we propose a novel method for spike detection, which is the first stage of spike sorting and hence crucially determines overall sorting performance. Our method utilizes a model of extracellular recording data that takes into account variations in spike waveforms, such as the widths and amplitudes of spikes, by detecting the peaks of band-pass-filtered data. We show that the new method significantly improves the cost–performance of multi-channel electrode recordings by increasing the number of cleanly sorted neurons.

Introduction

Functions of the brain emerge essentially from the orchestrated activation of many neurons in neuronal networks. Optical recordings, typically calcium imaging, are advantageous over electrophysiological recordings in the simultaneous recording of very many neurons and in the identification of the cell types of recorded neurons. However, the validity of such methods is restricted by the low temporal resolution (~ several hundreds of milliseconds) of recorded signals, and the reliable detection of single spikes is technically challenging. In contrast, extracellular recordings with multi-channel electrodes allow us to observe neural population activity with a high temporal resolution of millisecond range. Although multi-unit recordings cannot reliably identify the cell types, the fine temporal resolution makes the method particularly useful for studying the spiking dynamics of a neural population.

A technical challenge in multi-neuron recordings is a reliable separation of spikes belonging to the individual neurons from the recorded signals, which contain spikes of many neurons around the electrode. Several methods, including our own (Takekawa *et al.*, 2010, 2012), have been proposed for spike sorting (Hulata *et al.*, 2002; Pouzat *et al.*, 2002; Shoham *et al.*, 2003; Quijano Quiroga *et al.*, 2004; Zhang *et al.*, 2004). Spike sorting in general consists of three steps, i.e. the detection of spikes from the raw data, the

extraction of characteristic features of the detected spikes, and the clustering of spikes belonging to different neurons based on the extracted features (Schmidt, 1984; Lewicki, 1998). For instance, in our previous methods, feature extraction was performed based on principal component analysis of the multimodality of distributions, and spike clustering was formulated based on the variational Bayes method. These algorithms are implemented using open software, EToS1 and EToS3 (<http://etos.sourceforge.net/>), and EToS3 has been widely appreciated as a high-performance spike sorting system.

However, the previous methods have difficulty in parameter setting and the detection of spikes when spike waveforms are non-stationary and/or of low amplitude. Because the spatial distance between the electrode and a recorded neuron varies, the amplitude of the recorded spike waveforms varies significantly. In addition, the widths of spikes vary from neuron to neuron because the recorded neuron group generally contains multiple cell types. The frequency of spike firing also varies from neuron to neuron, implying that the number of spike samples differs for different neurons. In fact, the previous methods could not isolate many neurons if neuronal firing is sparse and the number of spike samples is limited. Here we propose a novel method for spike detection that considers all the above difficulties. This method can efficiently detect spikes with small amplitudes to increase the number of spike samples. We show the virtues of the method using artificial data and extracellular recording data recorded with a linear probe. The proposed method can be combined with a broad class of methods for feature extraction and spike clustering (Quijano Quiroga *et al.*, 2004; Zhang *et al.*, 2004; Ghanbari *et al.*, 2011).

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Materials and methods

Model of extracellular recording data

We assume that an observed data point x at time t arises from sparse positive signal s (spikes) with additive noise ε : $x(t) = s(t) + \varepsilon(t)$, where $s(t) = 0$ if no spikes exist at that time. The distribution of data points across time bins is then described as the following sum: $p(x) = p(x, s > 0) + p(x, s = 0)$. Throughout this paper, the amplitudes of simulated data are shown in arbitrary units although in reality they are in the range of several tens of microvolts. We estimate the probability $P(s > 0|x) = p(x, s > 0)/p(x)$ that an observed datum x belongs to a spike, where the equality follows from Bayes theorem. To this end, we estimate the number of true positive (TP) and false positive (FP) signals in the spikes detected with threshold X as follows: $N_{\text{TP}} = N \int_X^\infty p(x, s > 0)dx$ and $N_{\text{FP}} = N - N_{\text{TP}}$, where N is the total number of detected signals. Then, the number of TP signals in observed data $\{x_i\}$ can be evaluated as $N_{\text{TP}} = \sum_{x_i \geq X} P(s > 0|x_i)$, using $N_{\text{TP}} = N \int_X^\infty p(x)P(s > 0|x)dx$.

Note that the value of X remains arbitrary and we want to determine it according to a reasonable criterion. Although other choices may be also useful, we determine the value of the threshold by maximizing $N_{\text{TP}} - N_{\text{FP}}$ with respect to X . From the above expression of N_{TP} , we can show that this occurs when $N'_{\text{TP}} = N'_{\text{FP}}$, or $P(s > 0|x) = 1/2$, where the prime means derivative with respect to X . Therefore, this criterion implies that the probability that a data point x represents a spike is $1/2$ at the threshold.

Delta-function spike model

Previously, Nenadic & Burdic (2005) considered a model in which signals are represented by delta-function-like spikes occurring with a fixed amplitude λ according to the following probability distribution (Nenadic & Burdic, 2005):

$$p_\delta(s) = r\delta(s - \lambda) + (1 - r)\delta(s), \quad (0 \leq r \leq 1) \quad (1)$$

where $\delta(x)$ is Dirac's delta function (see Fig. 1a and b). Then, because $x = s + \varepsilon$, if ε is Gaussian white noise with mean μ and variance σ^2 (see Fig. 1c and d), we can represent the probability distribution function of observed data points x as

$$p_\delta(x) = r\mathcal{N}(x|\mu + \lambda, \sigma^2) + (1 - r)\mathcal{N}(x|\mu, \sigma^2). \quad (2)$$

Then, by estimating the values of parameters μ , σ , λ and r from the observed data, we may derive the optimum value of threshold X_δ as

$$X_\delta = \arg \max_x [N'_{\delta, \text{TP}}(X) - N'_{\delta, \text{FP}}(X)] = \mu + \frac{\lambda}{2} + \frac{\sigma^2}{\lambda} \log \frac{1 - r}{r}, \quad (3)$$

from the condition $N'_{\text{TP}, \delta} - N'_{\text{FP}, \delta} = p(x, s > 0) - p(x, s = 0) = 0$, or

$$r\mathcal{N}(x|\mu + \lambda, \sigma^2) - (1 - r)\mathcal{N}(x|\mu, \sigma^2) = 0. \quad (4)$$

Finite-width spike model

The above approach does not take into account either the width of spikes or possible variations in signal amplitudes. In fact, extracellular recording data generally contain signals with various amplitudes, including very weak ones (see Fig. 1f and g). It is therefore unlikely that a mixture of two Gaussians accurately describes the probability distribution function of x .

Therefore, we propose a novel model of extracellular recording data to estimate the optimum threshold when spikes have finite

widths (or duration) and variable amplitudes. The proposed method has four steps: (1) band-pass filtering of the observed data; (2) detection of the temporal location of positive peaks in the filtered data; (3) estimation of parameters for the distribution of the peaks; and (4) derivation of an appropriate threshold from the model. The first step is necessary to remove high-frequency noise from the data. The second step is crucial for avoiding the difficult estimation of the distribution of the filtered data over time when spike waveforms may vary in an unknown manner. Thus, the amplitudes of peak z are distributed in the filtered data according to

$$p_w(z) = \frac{r}{\sigma} X\left(\frac{z - \mu - \lambda}{\sigma}\right) + \frac{1 - r}{\sigma} Y\left(\frac{z - \mu}{\sigma}\right), \quad (5)$$

where $X(\eta)$ is the distribution of normalized Gaussian white noise $\eta(t)$ after filtering, and $Y(\eta)$ is the distribution of the peak amplitudes of this filtered normalized noise. In short, the first term represents contributions from signals, in which z is distributed around $\mu + \lambda$, and the second term represents contributions from the peaks of noise. In this study, we use the numerical tables of X and Y calculated for $\eta(t)$. Then, as in Eqn (2), we have to estimate the values of four parameters, μ , σ , λ and r , in Eqn (5), implying that the finite-width model is computationally as economical as the delta-function model.

Finite-width variable-amplitude spike model

Finally, we present a model in which the amplitude of finite-width spikes varies from time to time. Unless otherwise stated, we use this model in this study. Note that, due to the presence of noise, a true spike may not give a peak of the observed data when the amplitude is very small. Roughly speaking, this occurs when the amplitude of a spike is overwhelmed by that of noise. Therefore, we estimate the probability of detecting a spike with normalized amplitude $a = \lambda/\sigma$ as $\text{erf}(\beta a)$ in terms of the error function and a positive constant β . Then, Eqn (5) can be rewritten as

$$p_{\text{aw}}(z) = \frac{r}{\sigma} A\left(\frac{z - \mu}{\sigma}\right) + \frac{1 - r}{\sigma} Y\left(\frac{z - \mu}{\sigma}\right), \quad (6)$$

where the signal contributions should be replaced with

$$A(\eta) = \int_0^\infty p_{\text{amp}}(a) \text{erf}(\beta a) X(\eta - a) da. \quad (7)$$

Compared with similar models previously proposed (Bolstad *et al.*, 2003; Xie *et al.*, 2009), our model takes into account the contribution of noise peaks by the second term in Eqn (6) and the influence of noise on the detection of small signal peaks by the error function in Eqn (7).

Determination of signal threshold using our model

In the present study, we assume that the amplitude of signals obeys an exponential distribution, $p_{\text{amp}}(a) = \alpha e^{-\alpha a}$ ($a > 0$) with a positive constant α . We may also set $\text{erf}(\beta a) \simeq 1 - e^{-\beta a}$ to analytically calculate the integration in Eqn (7). Then, noting that $X(\eta - a)$ can be regarded as a Gaussian distribution with mean $\mu + a$ and variance σ , we obtain

$$A(\eta) \propto e^{\frac{1}{2}\lambda^2 - \alpha\eta} \text{erfc}\left(\frac{\alpha - \eta}{\sqrt{2}}\right) - e^{\frac{1}{2}(\alpha + \beta)^2 - (\alpha + \beta)\eta} \text{erfc}\left(\frac{\alpha + \beta - \eta}{\sqrt{2}}\right), \quad (8)$$

in terms of the complementary error function. The above approximation works excellently in most cases except for very broad spikes.

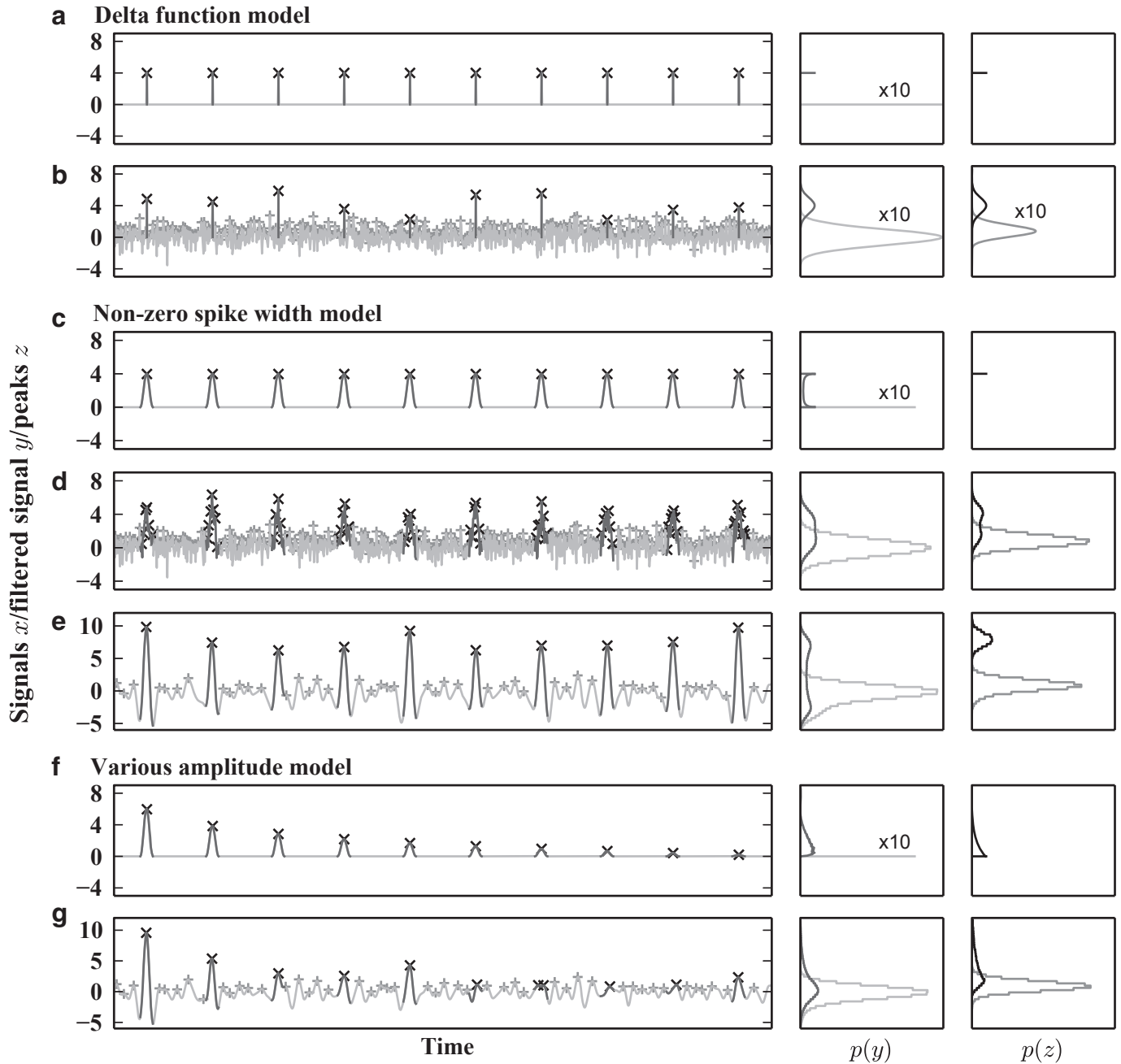


FIG. 1. Schematic illustration of our model of electrode data. Each column consists of the time course of signals with or without noise (left), the distributions of signals and noise (centre), and those of signal peaks and noise peaks (right). (a) Spike signal $s(t)$ and (b) the corresponding noisy observed data $x(t)$ in the delta-function (width-less) model. (c) Spike signal with a finite width and (d) the corresponding observed data in the finite-width model. Note that the rightmost panels look similar in (a) and (c), implying that finite spike widths do not raise technical difficulties. (e) We obtain the filtered data $y(t)$ from the observed data shown in (d). (f) Spike signal and (g) the corresponding filtered signal for the variable amplitude model. Note that the rightmost panel looks similar to that in (e), implying that our method can handle varying spike amplitudes without difficulties.

We determine the values of parameters μ , σ , α , β and r by maximizing the log-likelihood $\sum_z \log p_{aw}(z)$ of detected peaks using Eqns (6) and (8). We use a quadratic interpolation to obtain the accurate times and amplitudes of peaks (Takekawa, et al., 2012). Then, we count peak z as a spike when the condition $P(s > 0|z) > 0.5$ is satisfied. The numerical table of $Y(\eta)$ was calculated using the kernel density estimation of simulated noise (Bashtannyk & Hyndman, 2001; Oliphant, 2007). The optimization problem of maximum-likelihood estimation was solved numerically using the L-BFGS-B algorithm (Zhu et al., 1997; Oliphant, 2007). The trapezoidal formula is used to

calculate the normalizing constant of Eqn (8) from detected peaks. The model parameters can then be easily evaluated by sampling a small number of peaks (several hundred). This scheme works well for the present purpose, yet the computational cost is sufficiently low.

Peak-time detection with multiple filters

As described in the previous section, band-pass filtering is essential for our method to detect signals effectively, because band-pass filtering reduces high-frequency noise components and makes it easy to

detect clear peaks. Band-pass filtering also removes a low-frequency local field potential (LFP) from the raw broadband extracellular recording data. Therefore, we apply band-pass filtering to the raw data as an initial fundamental procedure. Moreover, a lower error rate can be achieved by selecting the best trial of different filters.

Here, we introduce a Mexican-hat wavelet as a band-pass filter, which emphasizes signals with a specific width. The Mexican-hat wavelet is defined with the target signal width w :

$$f_{\text{mxh}}(t|w) \propto \left(1 - 8\left(\frac{t}{w}\right)^2\right) \exp\left(-4\left(\frac{t}{w}\right)^2\right). \quad (9)$$

We also introduce a conventional filter with two frequencies for comparison:

$$f_{\text{band}}(t|w_H, w_L) \propto \frac{1}{w_H} \text{sinc}\left(\frac{t}{w_H}\right) - \frac{1}{w_L} \text{sinc}\left(\frac{t}{w_L}\right), \quad (10)$$

where $\text{sinc}(x)$ is the sampling function and $[w_H, w_L]$ is the scale of signal width, i.e. $\frac{2\pi}{w_L}$ and $\frac{2\pi}{w_H}$ are the low and high cut-off frequencies of the filter, respectively. The filtered data y of raw extracellular recording data x filtered with $f_{\text{mxh}}(t|w)$ are defined as:

$$y(t) = \int f_{\text{mxh}}(\tau|w)x(t + \tau)d\tau. \quad (11)$$

Because spike width can vary from neuron to neuron, an optimum filter can be different for different data. We therefore evaluated the performance of multiple filters to select the best one for any given data. We may combine multiple filters to optimally improve the performance. However, such integration of multiple filters seems difficult and was not attempted here.

Simulated extracellular recording data

We consider the case where the data points may contain a sparse signal (spikes) and additive noise. To test our method, we con-

structed artificial data that consist of various spike signals with an LFP component and white Gaussian noise.

We described each spike signal as a single cycle of cosine function, $\frac{\lambda}{2} \left(1 + \cos \frac{2\pi(t-t_{\text{spike}})}{w}\right)$, with the temporal range $t_{\text{spike}} - \frac{w}{2} < t < t_{\text{spike}} + \frac{w}{2}$. Here, the spike amplitude λ obeys the distribution $(\lambda_M - \lambda)^\gamma$ ($\lambda \geq 0$) with maximum amplitude λ_M ($\geq \lambda$), and the spike width w distributes uniformly in $[w_0, w_1]$. The spike frequency is chosen in a biologically realistic range.

Acquisition of experimental data

All experiments were carried out in accordance with a protocol approved by the RIKEN Institutional Animal Care and Use Committee. Male wild-type (C57 BL/6, Japan SLC, Shizuoka, Japan) mice, 5–6 weeks old, were used. The recording sites, the primary somatosensory cortex (S1) and the secondary motor cortex (M2), were determined by transcranial flavoprotein fluorescence imaging. Extracellular activity was recorded using silicon probes with a single shank (A1x32-Poly2-5mm-50s-177, NeuroNexus, MI, USA). The silicon probes were connected to a head stage (RA16AC or NN32AC, Tucker-Davis Technologies, FL, USA). The recording signals were amplified and digitized with 16-bit resolution using a preamplifier (PZ2-32, Tucker-Davis Technologies), and they were filtered (0.1–5000 Hz; RZ5D, Tucker-Davis Technologies). The dura was left intact. The tip of the probe was positioned at depth (1025 or 1050 μm in S1 and 1100 μm in M2) by using a micromanipulator (SMX-2-FB, Sensapex, Oulu, Finland). These depths were inferred from the manipulator depth. The recording sites were kept moist with Ringer's solution containing (in mM): 135 NaCl, 5.4 KCl, 1.0 MgCl_2 , 1.8 CaCl₂ and 5.0 HEPES (pH 7.2 with NaOH) after the craniotomy. Recordings began after a recovery period of at least 1 h. First, the recordings were performed in awake states for more than 1 h. Then mice were anaesthetized with isoflurane (1.0–1.3% gas mixture with the room air). Their body temperature maintained at 36–37 °C with a heating pad (BWT-100, Bio Research Center, Aichi, Japan) during anaesthesia.

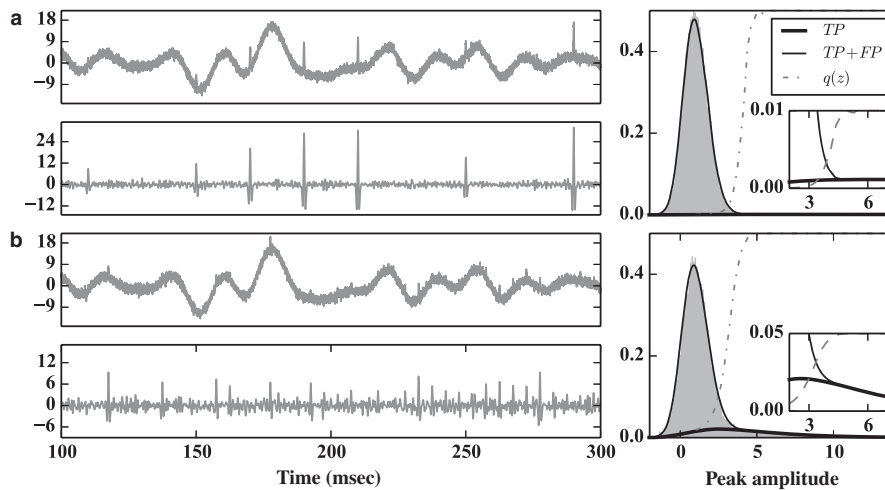


FIG. 2. Evaluation of our model on two types of artificial data. The length of each datum is 60 s, the sampling rate is 30 kHz and the Mexican-hat filter has $w = 1$ ms. (a) The data are the WLfg type characterized by large spike amplitudes and low spike frequencies: spike width 0.6–0.9 ms, maximum amplitude $\lambda = 20$, spike frequency 50 Hz and power of the amplitude distribution $\gamma = 1$. The observed data (left top), filtered signal (left bottom) and the distributions of peaks in the filtered signal (right) are shown. In the right panel, the estimated distributions are shown for TPs (thick line) and TPs + FPs (thin line), and $q(z)$ (dashed line) represents the probability that a data point with given peak amplitude is a true spike. The grey shaded area shows the distribution of actual peaks. Inset: higher magnification of the distributions. (b) The data are the wLFG type containing low-amplitude and high-frequency spikes: spike width 0.3–0.6 ms, maximum amplitude $\lambda = 5$, spike frequency 200 Hz and power of the amplitude distribution $\gamma = 2$.

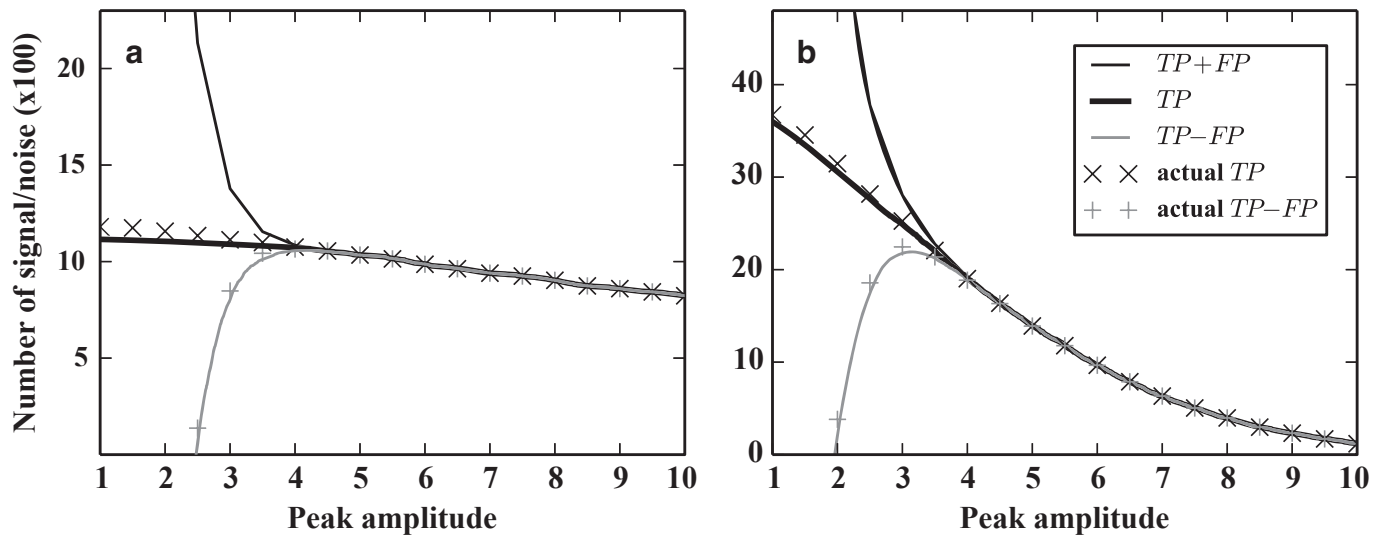


FIG. 3. Number ratio of signals and noise for artificial data. (a, b) The numbers of TPs and FPs estimated by our model are compared with the actual numbers of signal spikes and error spikes by using the same artificial data sets used in Fig. 2(a) and (b), respectively. Vertical lines represent the values of detection threshold determined by the model to maximize TP - FP.

Results

Performance of error rate estimation

The performance of the method was assessed by using 16 different types of artificial data sets generated by all possible combinations of the following four properties: spike width $w = 0.3$ – 0.6 or 0.6 – 0.9 ms; maximum amplitude $\lambda = 5$ or 20 ; spike frequency = 50 or 200 Hz; shape parameter $\gamma = 1$ or 2 (Materials and methods). Below, we denote these data types by four labels – [w or W], [l or L], [f or F] and [g or G]. For example, the wlfG-type data have width of 0.3 – 0.6 ms, maximum amplitude of 5 , spike frequency of 50 Hz and shape parameter 1 . The artificial spike data shown in Fig. 2 show two difficult cases with opposite characteristics. Data 1 contain low-frequency signals with large amplitudes (Fig. 2a, left) while data 2 contain high-frequency signals with small amplitudes (Fig. 2b, left). In both cases, the proposed model fits the distribution of the observed peaks (TP + FP) surprisingly well except for tiny deviations near the top (right panels) with the following estimated parameters: (a) $P = 0.039$, $\mu = -0.084$, $\sigma = 1.045$, $1/\alpha = 26.172$, $1/\beta = 8.155$; (b) $P = 0.123$, $\mu = -0.202$, $\sigma = 1.119$, $1/\alpha = 3.255$, $1/\beta = 4.271$. Here, the parameter p refers to the spike-vs.-noise ratio determined from the firing rate, μ and σ^2 to the estimated mean and variance of the noise, α determines the characteristic spike amplitude and β describes the influence of undetectable low-amplitude spikes (Materials and methods). We can evaluate the performance of the model by estimating the number of TP peaks in all detected peaks and comparing this with that of actual TP peaks. As shown in Fig. 3, the number of estimated TP peaks coincides well with that of actual TP peaks for both data sets.

We compared performance in spike detection between the present model and other models on the 16 types of artificial data sets in Table 1, in which ' X_δ ' refers to the delta-function model with the corresponding optimal threshold and ' $\mu + 2\sigma$ ', for example, to the same model in which the detection threshold is set to 2σ of the estimated noise distribution with median absolute deviation (Hoaglin *et al.*, 1983; Quian Quiroga *et al.*, 2004; Takekawa *et al.*, 2012). For each data set, the top rows show the hit score, i.e. the estimated value of TP minus FP, and bottom rows show the total number of

detected spikes. The results demonstrate that the proposed model shows better performance than the other models in all the cases. Thus, the proposed model gives an excellent estimation of the

TABLE 1. Comparison between our method with optimal threshold and others

| Data | Our method | X_δ | $\mu + 2\sigma$ | $\mu + 3\sigma$ | $\mu + 4\sigma$ | $\mu + 5\sigma$ |
|-------|------------|------------|-----------------|-----------------|-----------------|-----------------|
| wlfG | 1518 | 1304 | −4360 | 1361 | 1439 | 1155 |
| wlfG | 1647 | 1308 | 8629 | 2220 | 1467 | 1155 |
| wlfG | 1125 | 920 | −4918 | 980 | 1044 | 735 |
| wlfG | 1230 | 924 | 8592 | 1871 | 1066 | 735 |
| wlfG | 6896 | 4984 | 5316 | 6604 | 5158 | 3837 |
| wlfG | 7506 | 4988 | 11 254 | 6810 | 5166 | 3839 |
| wlfG | 5349 | 3508 | 3395 | 5103 | 3682 | 2402 |
| wlfG | 6317 | 3514 | 10 841 | 5455 | 3690 | 2404 |
| wlfG | 2522 | 2068 | −2845 | 2342 | 2501 | 2400 |
| wlfG | 2540 | 2068 | 8355 | 2951 | 2513 | 2400 |
| wlfG | 2342 | 1861 | −3134 | 2156 | 2312 | 2177 |
| wlfG | 2370 | 1861 | 8415 | 2805 | 2326 | 2177 |
| wlfG | 10 280 | 8070 | 9870 | 10 166 | 9559 | 8960 |
| wlfG | 10 336 | 8070 | 11 710 | 10 182 | 9559 | 8960 |
| wlfG | 9660 | 7256 | 8989 | 9492 | 8715 | 8007 |
| wlfG | 9747 | 7256 | 11 620 | 9528 | 8715 | 8007 |
| wlfG | 1534 | 1338 | −4253 | 1372 | 1445 | 1152 |
| wlfG | 1657 | 1342 | 8496 | 2213 | 1469 | 1152 |
| wlfG | 1122 | 931 | −4890 | 963 | 1033 | 741 |
| wlfG | 1259 | 939 | 8471 | 1874 | 1073 | 745 |
| wlfG | 6879 | 4967 | 5404 | 6550 | 5121 | 3784 |
| wlfG | 7643 | 4983 | 10 961 | 6794 | 5144 | 3788 |
| wlfG | 5232 | 3504 | 3404 | 5016 | 3625 | 2381 |
| wlfG | 6313 | 3541 | 10 571 | 5446 | 3668 | 2383 |
| wlfG | 2531 | 2075 | −2627 | 2367 | 2513 | 2401 |
| wlfG | 2549 | 2075 | 8133 | 2928 | 2525 | 2401 |
| wlfG | 2342 | 1877 | −2986 | 2164 | 2317 | 2183 |
| wlfG | 2438 | 1877 | 8235 | 2795 | 2337 | 2183 |
| wlfG | 10 294 | 8043 | 10 064 | 10 116 | 9494 | 8864 |
| wlfG | 10 358 | 8043 | 11 402 | 10 128 | 9496 | 8864 |
| wlfG | 9677 | 7217 | 9262 | 9438 | 8634 | 7922 |
| wlfG | 9792 | 7217 | 11 252 | 9476 | 8636 | 7922 |
| Total | 79 303 | 59 923 | 25 691 | 76 190 | 68 592 | 59 101 |
| | 83 702 | 60 006 | 156 937 | 83 476 | 68 850 | 59 115 |

values of parameters to discriminate between true spikes and false spikes.

Choice of good filters

Table 2 summarizes the effects of the conventional band-pass filter and the integration of the results with multiple filters on the 16 types of artificial data. We cannot, in principle, define the actual numbers of TPs and FPs for artificial data with variable spike amplitudes. However, the virtue of our model is that it gives an estimate of the probability that a detected peak with given amplitude is a true spike. Therefore, we can derive the expected ratio between the two quantities by using the estimated distributions of signals and noise in our model. Table 2 shows the estimated numbers of TPs and FPs by using this ratio and the total number of detected spikes, and compares the results with the actual numbers of TPs and FPs embedded in the simulated data. The top rows display the hit score and bottom rows the total number of detected spikes as in Table 1. The best scores are underlined, and the filters estimated as optimal are shown in bold type. In many cases, these filters showed the best scores. Generally, the best performance is obtained for the filter width that matches the width of spikes. If an adequate filter width is chosen, our filter generally exhibits better performance of spike detection than the conventional band-pass filter. We also attempted to combine multiple filters to further improve the performance. However, the improvement was not significant and the results are not shown here.

Spike detection in extracellular recordings with a single electrode

It is important to see whether and how our method works on experimental data. We therefore tested our method on a data set recorded extracellularly from the secondary motor cortex of awake mice by using a 16-channel linear probe. We analysed the data of 10-min length obtained at a single channel. Data analyses for all 16 channels are given in the next section. We used a filter with $w = 0.9$ as it yielded an excellent score for artificial data. To see the effect of detection threshold, we show results for the optimal value and its neighbouring values at $\pm 0.2\sigma$. We sorted the detected spikes by using feature extraction and spike clustering of the EToS3 spike-sorting algorithm (Takekawa *et al.*, 2012).

The clusters obtained with the optimal (2.8σ), smaller (2.6σ) and larger (3.0σ) detection thresholds are displayed in Fig. 4. This data set is an especially difficult case containing only low-amplitude spikes. Note also that noise affects spike sorting more severely in single-electrode recordings (the present case) than in conventional electrode recordings. Nevertheless, the top three clusters are cleanly isolated for all three threshold values, whereas bottom four clusters may be excluded from the analysis. Both optimal and larger threshold values yielded the same number of clusters, but the 4th cluster (from the top) is presumably contaminated for the larger threshold. In addition, using the large threshold could have decreased the total number of spikes. Note also that reducing the detection threshold below the optimal value generally does not increase the number of clean clusters because the number of FPs

TABLE 2. Effects of different filters on spike detection in different data types

| Data | [0.4,2] | 0.5 | 0.7 | 0.9 | 1.1 | 1.3 | 1.5 | Opt |
|-------|---------|--------|---------------|---------------|-------------|---------------|---------------|--------|
| wlfg | 1485 | 1355 | 1513 | 1520 | 1500 | 1453 | 1368 | 1520 |
| | 1533 | 1477 | 1583 | <u>1618</u> | 1604 | 1555 | 1446 | 1618 |
| wlfG | 1093 | 910 | 1122 | 1137 | 1102 | 1031 | 955 | 1137 |
| | 1141 | 988 | 1192 | <u>1221</u> | 1200 | 1111 | 1029 | 1221 |
| wlFg | 6620 | 6089 | 6753 | 6952 | 6872 | 6749 | 6567 | 6952 |
| | 6886 | 6963 | 7417 | <u>7528</u> | 7510 | 7531 | 7513 | 7528 |
| wlFG | 5209 | 4377 | 5241 | 5389 | 5302 | 5192 | 5015 | 5389 |
| | 5725 | 5421 | 6203 | <u>6205</u> | 6184 | 6224 | 6251 | 6205 |
| wLfG | 2472 | 2468 | 2539 | 2529 | 2511 | 2491 | 2468 | 2539 |
| | 2502 | 2496 | <u>2567</u> | 2555 | 2527 | 2509 | 2514 | 2567 |
| wLFG | 2296 | 2257 | 2337 | 2351 | 2339 | 2314 | 2257 | 2351 |
| | 2422 | 2297 | 2369 | <u>2423</u> | 2411 | 2380 | 2289 | 2423 |
| wLFg | 9297 | 10 058 | 10 313 | 10 307 | 10 249 | 10 204 | 10 133 | 10 313 |
| | 9297 | 10 164 | <u>10 383</u> | 10 367 | 10 301 | 10 266 | 10 197 | 10 383 |
| wLFG | 9371 | 9356 | 9709 | 9783 | 9668 | 9528 | 9431 | 9783 |
| | 9967 | 9842 | 10 045 | <u>10 075</u> | 9772 | 9618 | 9521 | 10 075 |
| Wlfg | 1223 | 515 | 1233 | 1479 | 1564 | <u>1573</u> | 1544 | 1564 |
| | 1283 | 859 | 1407 | 1605 | 1672 | <u>1675</u> | 1632 | 1672 |
| WlfG | 753 | 268 | 816 | 1063 | 1152 | 1161 | 1123 | 1161 |
| | 799 | 514 | 932 | 1195 | 1256 | <u>1261</u> | 1199 | 1261 |
| WlFg | 5544 | 2249 | 5476 | 6603 | 7034 | <u>7119</u> | 7115 | 7115 |
| | 6302 | 5407 | 6970 | 7405 | 7714 | <u>7793</u> | 7859 | 7859 |
| WlFG | 4041 | 1185 | 3866 | 4997 | 5458 | 5572 | 5566 | 5572 |
| | 5081 | 3927 | 5548 | 6231 | 6372 | <u>6608</u> | 6598 | 6608 |
| WLfg | 2351 | 2023 | 2416 | 2509 | 2543 | 2542 | 2517 | 2543 |
| | 2567 | 2267 | 2478 | 2539 | <u>2561</u> | 2560 | 2533 | 2561 |
| WLfG | 2138 | 1700 | 2201 | 2313 | 2356 | <u>2368</u> | 2352 | 2352 |
| | 2232 | 1980 | 2285 | 2369 | 2382 | <u>2396</u> | 2400 | 2400 |
| WLFg | 9584 | 8320 | 9912 | 10 246 | 10 301 | 10 342 | <u>10 349</u> | 10 342 |
| | 10 056 | 9502 | 10 110 | 10 330 | 10 369 | <u>10 398</u> | <u>10 399</u> | 10 398 |
| WLFG | 8649 | 7061 | 9065 | 9548 | 9724 | 9753 | 9719 | 9753 |
| | 8767 | 8533 | 9449 | 9690 | 9820 | <u>9841</u> | 9815 | 9841 |
| Total | 72 126 | 60 191 | 74 512 | 78 726 | 79 675 | 79 392 | 78 479 | 80 386 |
| | 76 560 | 72 637 | 80 938 | 83 356 | 83 655 | 83 726 | 83 195 | 84 620 |

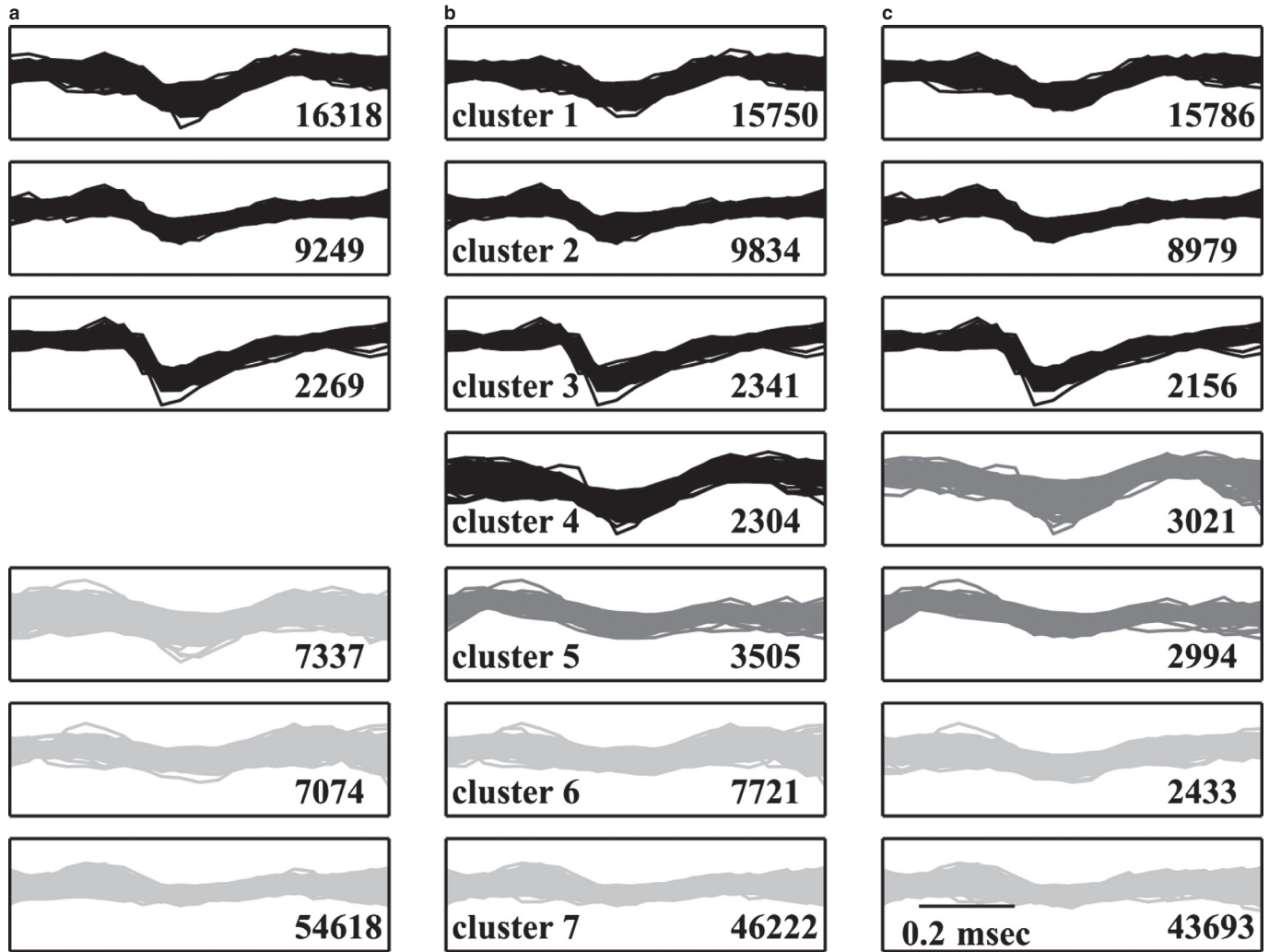


FIG. 4. The performance of our model at a single channel of extracellular recording data. We analysed the data of 10-min length and show the waveforms of all the detected spikes for the initial 30 s. Spikes (negative peaks) detected with (a) threshold smaller than the optimal one, (b) the optimal threshold and (c) threshold greater than the optimal one were clustered. Numbers in the lower right corner indicate the numbers of spikes in the individual clusters.

would increase. Thus, as shown in Fig. 4, the use of an optimal threshold is of crucial importance for the overall performance of spike sorting.

Spike detection in extracellular recordings with a multi-channel linear probe

Finally, we applied the proposed method to the analysis of experimental data recorded at all 16 channels of a linear probe. Linear probes often provide a convenient and useful method for the simultaneous recording of multi-neuron activities at different cortical depths. Our method detected multiple spikes for each channel of the linear probe, with the probability $P(s > 0|z)$ representing the likelihood that a detected spike is a true spike.

In Fig. 5, we show the groups of multiple spikes that were recorded at adjacent electrodes and were considered to represent spikes propagating from the same neuron. Below, we explain how we performed this grouping. If we detect peaks at adjacent electrodes, we set a certain temporal range around each peak in proportion to the probability $P(s > 0|z)$ calculated for this peak. We determined the proportional constant such that the maximum size of

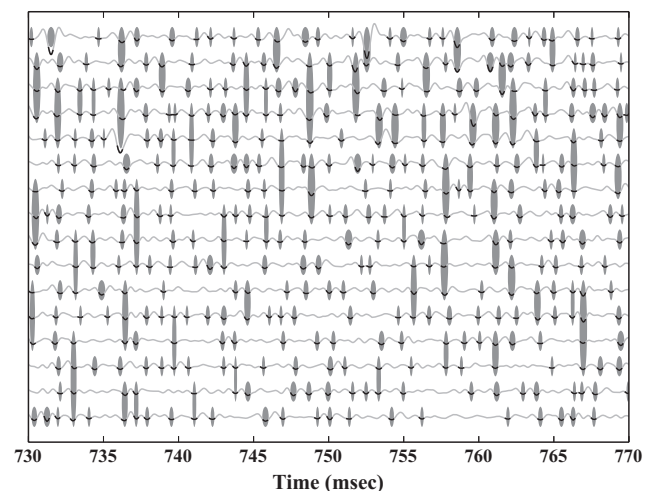


FIG. 5. Spike detection in real data obtained by 16-channel linear probe recordings. Shaded clusters display the simultaneous spikes that detected in neighbouring channels. Results of spike sorting suggest strongly that they belong to the same neuron.

temporal domains was ± 1 ms. We grouped the two peaks if the temporal domains set for the adjacent electrodes overlap with one another. If the domains have no overlap, we regard the two peaks as independent spikes. We repeated this process for all the pairs of adjacent electrodes on the linear probe.

We examined whether the above criterion for grouping is reasonable by sorting the spikes detected at multiple electrodes with EToS3. Preliminary results suggest that about 80% of the spikes grouped by the above rule belong to the same spike cluster (data not shown). This implies that we may sort multi-neuron spikes recorded with a linear probe by the present method without further spike sorting. However, the validity of the above criterion will need to be rigorously examined by future studies.

Conclusion

In this study, we propose a novel method for spike detection by taking the distributed nature of spike amplitudes, widths and frequencies into account. In spike sorting, detected spikes are often excluded from the data set used in the later analyses, if their amplitudes are lower than a certain value. While this manipulation improves the reliability of spike sorting, it may significantly decrease the total number of detected spikes and hence that of simultaneously recorded neurons. The new method increases the total numbers of detected spikes without sensitive parameter tuning even if spike waveforms are non-stationary and show low amplitudes. These features make the present method particularly useful for the analysis of extracellular data recorded with linear probes, which are widely used as a convenient way to study neural activity at multiple cortical depths. Thus, our method greatly improves the cost-performance of multi-neuron recordings.

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Abbreviations

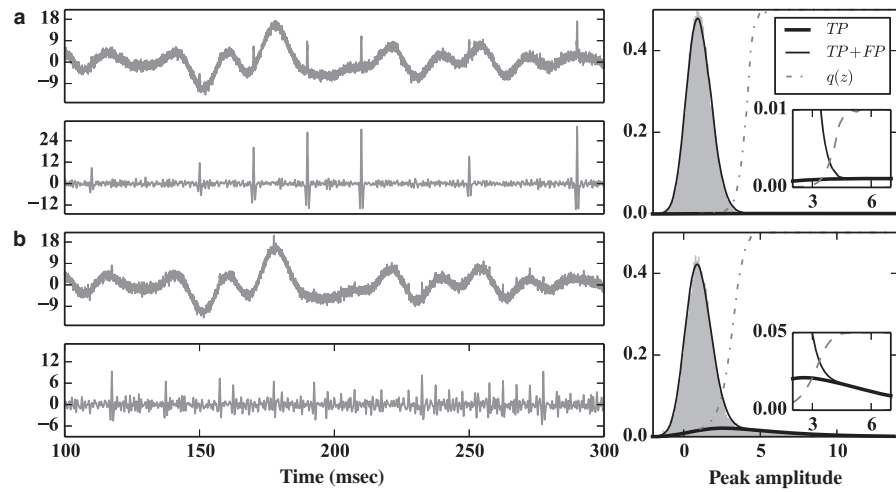
FP, false positive; LFP, local field potential; TP, true positive.

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Graphical Abstract

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We introduce a spike detection method based on a model of extracellular recording data that takes into account variations in spike waveforms, such as the widths and amplitudes of spikes. The proposed method can evaluate appropriate thresholds using the distributions of the peaks of band-pass-filtered data.