Optimal sorting of neural spikes with wavelet and filtering techniques

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ABSTRACT

We show that robustness of sorting of neural spikes using the wavelet transform depends strongly on the statistics of experimental noise and the characteristic time scales of spike waveforms. Incorporating adaptive filtering of the extracellular potential into the wavelet sorting algorithm we propose a novel method, the Parametric Wavelet sorting with Advanced Filtering (PWAF), whose classification error approaches the theoretical minimum. Efficiency of the proposed technique is proved with both simulated and real electrophysiological recordings.

Keywords: Neural spikes, wavelet analysis, information processing, spike sorting

1. INTRODUCTION

Most of the neurons in the brain communicate by short lasting electrical pulses – action potentials or spikes that can be recorded extracellularly by a microelectrode.[1] When analyzing the cooperative neural behavior or studying the neural code, spikes thought to be stereotypical events, so not the shape of each spike waveform but the precise timing of firings of the neurons in a population matters. In general, the extracellular electrode picks up signals of several neighboring cells, thus first of all the researcher should classify spikes and associate them with different neurons they originated from (Fig. 1). This problem is called spike sorting or spike separation.[2]

It is typically assumed that each neuron generates spikes of the same shape and amplitude whereas signals from different cells have some individual peculiarities, although their signatures can be quite similar. Thus comparing spike waveforms one can separate them with some degree of reliability. In practice, however, spike sorting represents a complicated task due to a high level of background noise, variability of the spike waveforms, frequently non-well pronounced differences between spikes of different neurons, etc. For example in Fig. 1 at least two different groups of spikes are observable, but a reliable inference needs more careful investigations.

Nowadays techniques of spike sorting proceed through four *independent* steps: (i) filtering of the extracellularly recorded potential, (ii) detection of spiking events, (iii) extraction of discriminative features of the selected spike waveforms, and (iv) delimitation of clusters of spikes in the feature space and association them to different neurons. Among these, steps i) and iii) are the most challenging.

In the simplest but rather frequently used case the extracellularly recorded potential is High Pass Filtered (HPF) with the cutoff frequency around 300 Hz followed by the visual inspection of spike waveforms and a kind of amplitude thresholding (Fig. 1). Such procedure is subjective, complicated, and inefficient (in a typical experiment one can easily have more than 10^4 spikes). Another, significantly more powerful technique is Principal Component Analysis (PCA).[2] Here again spike waveforms are filtered (HPF, 300 Hz) and then scale factors of the first few principal components, so called scores, are used as quantities for representing each spike for sorting. Recently a novel approach for spike feature extraction has been introduced.[3] Within this technique each spike is represented by coefficients of the Wavelet Transform (WT). This method can be more effective than traditional PCA.[3–6] However, it has several disadvantages originated from the arbitrary choice of the mother wavelet and difficulties in an automatic selection of the parameters of the WT.

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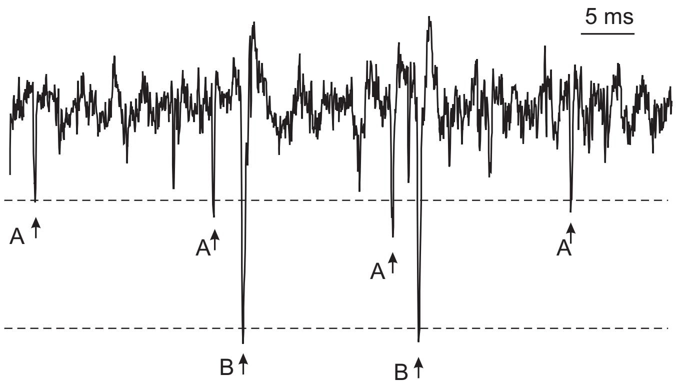

Figure 1. A typical example of the high-pass filtered extracellular potential recorded from the hippocampus of the rat. The simplest way to sort spikes (marked by arrows) is the amplitude thresholding. Two dashed lines delimit amplitudes of spikes belonging to group A and B.

In all abovementioned methods experimental noise is reduced on the first step by a standard filtering that does not account for the noise statistics nor for the spike signatures. Noise in the extracellular recordings has different nature spreading from Johnson noise to the variability of the action potential due to physiological processes, passing through the background activity of distant neurons,[7] and electrode micro-movement.[8] The standard techniques, i.e. amplitude thresholding and PCA, have a long history, and well established recipes for filtering. This is not the case for the WT technique, where a different filtering approach may be superior. As we further show performance of the WT method can be significantly improved incorporating the filtering step into the problem of selection of the optimal feature set.

2. INFLUENCE OF THE NOISE STATISTICS ON THE QUALITY OF NEURONAL SPIKE SEPARATION

To illustrate how the noise statistics affects the spike sorting performance we generated several data sets consisting of (1000+1000) spikes of two different neurons. The original spike waveforms were selected from electrophysiological recordings in the hippocampus. To simulate the effect of the noisy background we mixed a colored noise (band-pass filtered Poisson process) of the frequency band 700 Hz with the spike waveforms. Finally we estimated the performance of each spike sorting technique as the error rate, i.e. the ratio of missclassified spikes to the total number of spikes.

Figure 2a shows the error rate as a function of the base noise frequency. The PCA method gives a high error rate at low frequency noise and then progressively increases performance for high frequency noise. The wavelet technique (as a representative we use the Wavelet-based Spike Classifier, WSC,[3] with qualitative results extended to other method modifications[6]) is a better option for sorting spikes contaminated by a low frequency noise, but it has a strong peak in the range of intermediate noise frequencies. Aiming at seeking for the best filtering strategy we filtered waveforms varying the cutoff frequency of the LPF, and then we performed spike classification on the filtered data. Filtering in general reduces the error rate. However, it affects differently the PCA and WT methods (Fig. 2b). Indeed, the classification error for the WT technique has a minimum at the filter frequency around 2.2 kHz and then rapidly increases. For PCA the error first gradually decreases up to 2 kHz and then practically stays constant. This suggests that low-pass filtering of spikes is worthless for PCA, and is essential for the WT methods, where the cutoff frequency should be appropriately selected.

3. THEORETICAL BASES OF THE PROPOSED SPIKE SORTING APPROACH

Let us now consider the problem of wavelet-based spike sorting in details. We start from a data set of $(N+M)$ spikes of two different neurons contaminated by noise. Denoting the original noise-free spike waveforms as $w_A(t)$ and $w_B(t)$, the recorded spikes are:

$$s_i(t) = \xi_i(t) + \begin{cases} w_A(t), & i = 1, 2, ..., N, \\ w_B(t), & i = N+1, ..., N+M, \end{cases} \qquad (1)$$

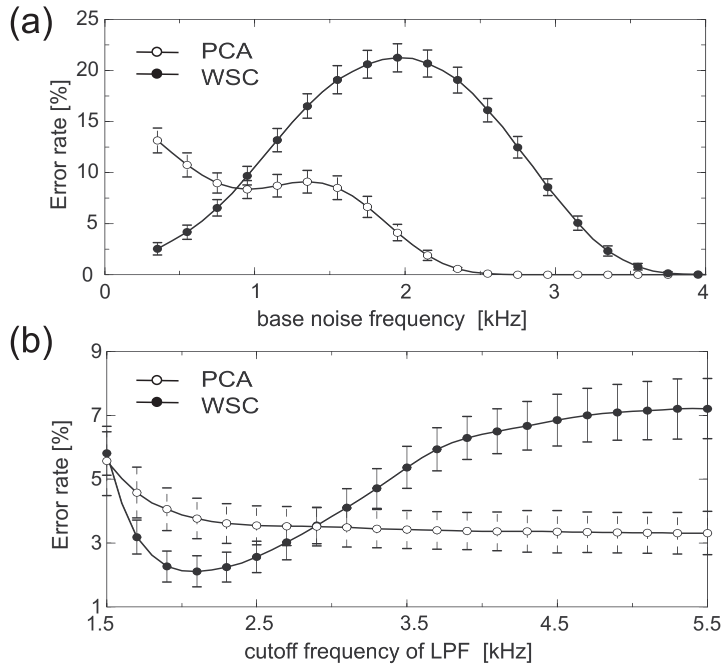

Figure 2. a) Error rate of spike sorting versus base the central frequency of the experimental noise (the noise band $\Delta f = 700$Hz). b) Spike sorting error rate after applying the low-pass filter to the recording.

where ξ_i are uncorrelated colored noise sources of the experiment that in the first approximation have the same statistics. In the most general form the continuous WT of the spike waveform $s_i(t)$ reads:

$$W_i(a,b) = \frac{1}{\sqrt{a}} \int_0^T s_i(t)\psi_{a,b}(t)\; dt, \tag{2}$$

where T is the spike duration (typically 1–3 ms), and $\psi_{a,b}(t) = \psi(\frac{t-b}{a})$ is a translated and scaled mother wavelet, with b and a characterizing time localization and scale. Applying the WT for a selected parameter set (a,b) to the recorded spikes we obtain:

$$W_i(a,b) = \eta_i + \left\{ \begin{array}{l} W_A,\;\; i = 1,2,...,N \\ W_B,\;\; i = N+1,...,N+M, \end{array} \right. \tag{3}$$

where we denoted

$$\eta_i(a,b) = \frac{1}{\sqrt{a}} \int_0^T \xi_i \psi_{a,b}\, dt, \tag{4}$$

$$W_{A,B}(a,b) = \frac{1}{\sqrt{a}} \int_0^T w_{A,B} \psi_{a,b}\, dt. \tag{5}$$

$\eta_i(a,b)$ represent a kind of measurement noise, and $W_{A,B}$ are the WT coefficients of the corresponding noise-free spikes.

The coefficients W_i now can be used for sorting. The aim is to separate them blindly into two clusters or groups with the lowest possible error rate. This has a sense if W_i exhibit a bi-modal distribution, otherwise when, e.g., the noise is too strong or parameters (a,b) are suboptimal and no bi-modal distribution exists, spike sorting is meaningless. In our case the sorting is achieved by selecting a threshold $W_{th} \in [W_A, W_B]$ and assigning spikes having $W_i < W_{th}$ to neuron A, while the others to neuron B (Fig. 3).

Let us now assume that the measurement noise is approximately Gaussian[9] with the standard deviation σ. The reason for this assumption arises from the central limit theorem, which, roughly speaking, asserts that a

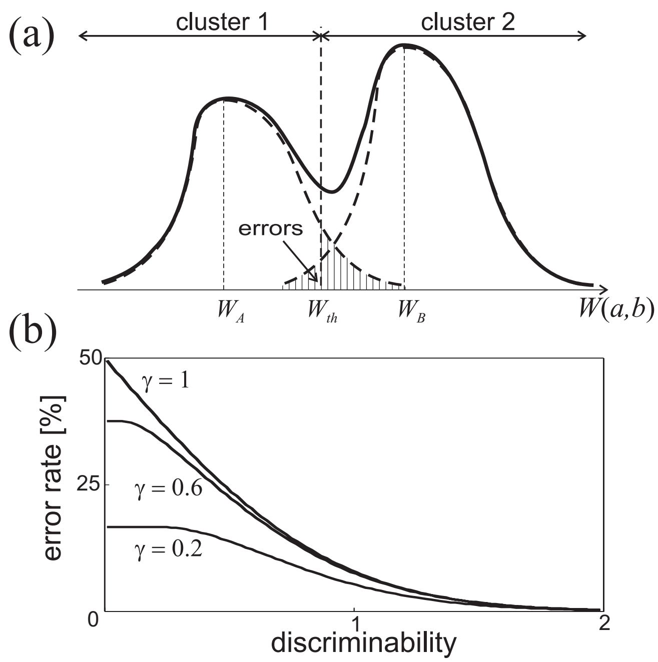

Figure 3. (a) Histogram of the distribution of the WT coefficients W_i describing noisy spikes of two neurons. Dashed curves depict the histograms of single neurons. Classification errors appear in the region of overlapping. (b) The minimal error rate given by Eq. (9) as a function of the discriminability for several different values of γ.

random variable composed of many independent, arbitrary distributed parts is Gaussian.[10] We denote the half distance between the noise-free spikes in the wavelet space as:

$$\widehat{W} = \frac{W_B - W_A}{2} = \frac{1}{2\sqrt{a}} \int_0^T (w_B - w_A)\psi_{a,b}\, dt. \tag{6}$$

Without loose of generality we can set $\widehat{W} \equiv W_B = -W_A$. Then the distribution of W_i reads:

$$h = \frac{M}{\sqrt{2\pi}\sigma} \left(\gamma e^{-\frac{(W+\widehat{W})^2}{2\sigma^2}} + e^{-\frac{(W-\widehat{W})^2}{2\sigma^2}} \right), \tag{7}$$

where $\gamma = N/M$ is the ratio of the numbers of spikes emitted by the neurons. Then the minimum of the total number of missclassified spikes is attained for:

$$W_{th} = \frac{\sigma^2}{2\widehat{W}} \ln \gamma. \tag{8}$$

Note that the optimal threshold value in general ($\gamma \neq 1$) does not correspond to the position of the minimum in the histogram. Finally, the theoretical minimum of the error rate is given by:

$$R_{\min} = \frac{\gamma \,\mathrm{erfc}\left(\Delta + \frac{\ln\gamma}{4\Delta}\right) + \mathrm{erfc}\left(\Delta - \frac{\ln\gamma}{4\Delta}\right)}{2(1+\gamma)}, \tag{9}$$

where erfc is the complimentary error function, and $\Delta = \widehat{W}/\sqrt{2}\sigma$ is the *discriminability* coefficient. Accordingly, the error rate is a two parametric function of γ and Δ that decays with an increase of Δ (Fig. 3b). The ratio of the spike numbers, γ, is fixed by the experiment, hence we are left to play only with the discriminability Δ.

Let us now explore the ways to improve the discriminability. Selecting appropriately (a, b) we can maximize $\widehat{W}$, that for a constant σ increases Δ. However, as we show further scaling parameter a affects nontrivially the noise standard deviation, σ, and consequently Δ.

The experimental noise $\xi(t)$ of a limited frequency band Ω_{noise} can be presented as a sum of harmonics:

$$\xi_i = \sum_{\Omega_{noise}} A(\omega_k)\cos(\omega_k t + \phi_{ki}), \tag{10}$$

where ω_k and ϕ_{ki} are the frequency and random phase of the corresponding harmonic, and $A(\omega)$ defines the noise power spectrum. Using the Haar wavelet advocated for spike sorting[6] we obtain the WT of the experimental noise (10):

$$\eta_i = -\frac{4}{\sqrt{a}} \sum_k \frac{A(\omega_k)}{\omega_k} \sin\phi_{ki} \sin^2 \frac{a\omega_k}{4}. \tag{11}$$

Note, that the statistical properties of η do not depend on the localization parameter b. Then the standard deviation of the measurement noise reads:

$$\sigma^2(a, \Omega_{noise}) = \frac{8}{a} \sum_k \frac{A^2(\omega_k)}{\omega_k^2} \sin^4 \frac{a\omega_k}{4}. \tag{12}$$

Thus the discriminability may depend nontrivially on: the parameters (a, b), spike waveforms, and the spectral characteristics of the experimental noise. A natural way to change the noise spectrum is to filter the signal. Denoting by f_c the cutoff frequency of the filter we finally reduce the problem of the optimal spike sorting to *seaking the parameter set* (a, b, f_c) *maximizing the discriminability.* Note that our problem statement is more general than the conventional methods relaying on a search of the best parameter set of the WT only. Including spike filtering in the problem of spike sorting we account for the specific noise of the individual experiment and potentially may provide the best possible spike classification. Besides, up to date known methods of the WT parameter selection[3,5,6] are based on the empirical analysis of the experimental distribution of coefficients of the WT, whereas our method is parametric.

4. SPIKE SORTING WITH THE PWAF TECHNIQUE

In the experimental conditions we have no a priory knowledge neither on the noise-free spikes nor on the spectrum of the experimental noise. To estimate them and optimally sort spikes we propose the following algorithm consisting of 5 steps:

1. *Estimate the noise-free spike waveforms.* Applying a conventional algorithm, e.g. PCA, we find peaks of the distribution of spike features and average spikes waveforms in a vicinity of each peak thus estimating $w_{A,B}$.

2. *Estimate the spectrum of the experimental noise* $P(\omega)$. In the simplest case, the spectrum of the whole extracellular signal can be considered as an approximation of $P(\omega)$.

3. *Find an optimal parameter set* (a^*, b^*, f_c^*) *maximizing the discriminability.* For a given (a, b, f_c): i) filter signal representing the waveform difference $(w_B - w_A)$ and evaluate $\widehat{W}$; ii) evaluate $A^2(\omega) = P(\omega)H^2(\omega)$, [$H$ is the filter magnitude response] and then σ; iii) evaluate the discriminabilty, $\widehat{W}/\sqrt{2}\sigma$. Find maximum of $\Delta(a, b, f_c)$.

4. *Filter spikes with* f_c^* *and calculate* $W_i(a^*, b^*)$.

5. *Sort spikes accordingly to the coefficients* W_i.

Note that the proposed method can be very efficient for big data sets*, and the algorithm allows the use of more than one feature set for sorting†. It allows essential improving of sorting results if standard techniques show bi-modal distributions of spike features. In the case when more than two neurons are presented, the method gives an opportunity to improve resolution between any two overlapped clouds of points in the feature space of standard approaches.

To test the algorithm we employ simulated data sets differ by noise statistics and spike waveforms. Figure 4 shows an example of application of the algorithm to the data set #1. The PCA method has strong overlapping

*Steps 1–3 do not depend on the number of spikes and the WT of the whole data set is evaluated only once.

†At step 3 we can obtain more than one extrema, and then perform step 4 for all of them this way describing each spike with more than one feature (wavelet coefficient) and can use them together for spike sorting at step 5.

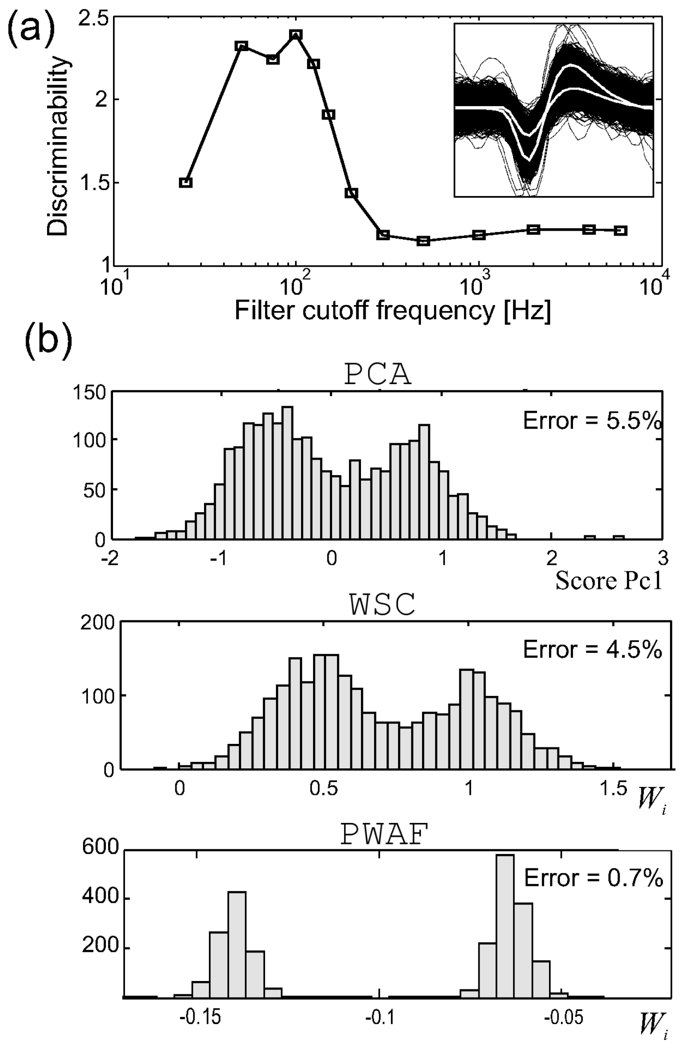

Figure 4. Sorting of data set #1. a) Discriminability, Δ, vs the cutoff frequency of a LPF. For the maximal $\Delta = 2.39$ the theoretical minimum of the error rate is 0.3%. Inset shows superposed experimental spike waveforms and noise free spikes (white). b) Distributions of spike features for different methods: PCA, WSC and PWAF.

Data Set	PCA	WSC	PWAF
Simulated #1	5.5%	4.5%	0.7%
Simulated #2	28.0%	5.5%	1.7%
Experim. #1	11.1%	7.0%	3.4%
Experim. #2	12.2%	10.1%	6.8%

Table 1. Error rate for spike sorting by different algorithms with simulated and experimental data sets.

of the spikes of two neurons and resulting error rate at 5.5%. The WSC-approach gives a bit better classification achieving 4.5% error (Fig. 4). The PWAF method is much superior with the error rate at 0.7%, which is quite close to the theoretical minimum. This confirms our hypothesis that an intelligent filtering is essential for wavelet methods. Note, that unlike the commonly used approach to filtering we consider the filter's cutoff frequency as the most important parameter being tuned individually for each extracellular signal within the PWAF-technique. We performed the same procedure for another data set (Table 1) that has been selected to exhibit differences between noise free spikes at small time scales. This is a case when the wavelet technique has an advantage over the PCA method. Indeed PWAF provides much better classification than PCA and WSC.

We now test our method on real measurements. Extracellular recordings were made using tetrode electrodes whose design permits recording of the same neuron by two electrode tips (for details see[11]). In exceptional cases two channels besides a considerable activity of many different neurons can show simultaneous, sufficiently profound spikes belonging to one neuron. Among many experimental recordings we have selected two data sets where these conditions were satisfied. For these data sets relating voltage traces of the two channels we manually sort spikes with a good fidelity. Then using this information we estimate the error rate of the automatic methods. Table 1 summarizes the results showing that the PWAF method again is superior.

5. CONCLUSIONS

Spike separation is a basic prerequisite for analyzing of the cooperative neural behavior and neural code when registering extracellularly. Current extracellular experiments provide recordings of multi-unitary activity, where several neurons nearby to the electrode tip produce short lasting electrical pulses, spikes, of different amplitudes and shapes. Consequently, extracting useful information from these measurements relies on the ability of separating the recorded firing events into groups or clusters. Ideally each cluster should contain all spikes emitted by only one neuron. Errors occur when spikes belonging to different neurons are grouped together (false positive) or when some spikes emitted by a single neuron are not included into the group (false negative). The performance of the spike sorting procedure defines the final quality and reliability of any bio-physical results obtained upon the analysis of spike timings. However, the quality of the spike separation by a human operator is significantly below the estimated optimum. Besides, amount of the data generated by modern experimental setups is really huge, thus there is a big demand for automatic separation techniques. In this work we have shown that the performance of the WT method can be significantly improved incorporating the filtering step into the problem of selection of the optimal feature set. We have presented a novel parametric method for sorting of neural spikes (Parametric Wavelet sorting with Advanced Filtering – PWAF). The method includes spike filtering in the problem of an optimal parameter selection. Testing the method on simulated and real measurements we have demonstrated its advantage.

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