IEEE Int. Conf. Neural Networks & Signal Processing
Nanjing, China, December 14-17, 2003

SPIKE SORTING IN MULTI-CHANNEL EXTRACELLULAR RECORDINGS OF RETINAS*

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ABSTRACT

Spike sorting is a prerequisite for studying the multi-channel extracellular recordings. A new method is developed for spike sorting in multi-channel extracellular recordings of retinas. Based on the assumption that noise follows Gaussian distribution, spike templates are constructed based on principal component analysis and subtractive clustering, then a template matching procedure is applied to choose the best-fit template or templates combination for overlapping spikes so that after subtracting the template or templates combination from the spike event, the sum of squared residue follows Chi-square distribution. Artificial spike trains are used to assess the performance of the proposed method.

1. INTRODUCTION

A fundamental question in neuroscience is how the information relevant to behavior is presented in the activity of neurons [1]. The visual system, especially the retina, offers some advantage to explore the neural code owing to its explicitly layered structure and relatively simple neuron types [2]. A series of studies carried out by Meister et al. suggested that the nerve impulses in the population of retinal ganglion cells collectively encode the visual stimulus [3]. Thus, in order to understand coding, decoding and information processing that occur in the retina, multi-channel extracellular electrode arrays have been employed [3,4].

Since the multi-channel extracellular recordings contain the spike streams of several neurons adjacent to each electrode, spikes from different neurons may overlap temporally and produce novel waveforms, and noise inevitably distorts spike waveforms, it is necessary to decipher the recordings to know the number of neurons contributing to each electrode recording, their characteristic waveforms (templates) and spike temporal sequence of each neuron.

This spike-sorting problem has received intense attention and a large number of techniques have been developed [5,6,7]. But most methods concentrate on detection and classification of the spikes found in a spike train by assuming that the templates are known a priori, and only a few methods deal with templates creation and spike temporal sequence reconstruction [8,9,10].

Here we present a new spike sorting method. Firstly use principal component analysis (PCA) and subtractive clustering techniques to create spike templates, then perform template matching procedure on the recording data set together with the Chi-square test to sort spikes and reconstruct spike temporal sequence of each neuron.

2. METHODS

The assumptions in the methods are: 1) The spike waveforms generated by a neuron are time-invariant [11]; 2) The noise follows Gaussian distribution [9]; 3) The spike events and the noise are statistically independent [12]; 4) The overlapping spikes are added linearly by single spikes [12].

2.1. Spike events detection

The first step is to extract the events that could represent spikes from the recording. One of the simplest and most common ways to detect spikes is threshold detection. Spikes of retinal ganglion cells are usually characterized by an initial sharp negative trough, so we choose a threshold of negative peaks to detect the spike events.

Once a negative peak exceeds the predetermined threshold, a fixed length of waveform is extracted from the original recording $X(t)$ as one spike event. In this study, the fixed length is 40 sampling points (16 points before, 23 points after the peak). When sampling frequency is 20k Hz, 40 sampling points refer to 2 milliseconds. Define the ith spike event as y_i, $y_i \hat{I} R^{40}$. All of the detected spike events produce a data set as $\boldsymbol{Y}$, $\boldsymbol{Y} \hat{I} R^{n\acute{}40}$, where n is the number of spike events.

* This research was supported by the grant from Shanghai Science and Technology Development Funding (No. 02JC14008).

2.2. Spike events features extraction

PCA is a method for choosing features from the data set $\boldsymbol{Y}$. The basic idea in PCA is to find an ordered set of orthogonal basis vectors that capture the directions in the data of largest variation. PCA effectively decomposes $\boldsymbol{Y}$ as

$$\boldsymbol{Y} = \boldsymbol{S}\boldsymbol{L}^T = \sum_{i=1}^{n} s_i l_i^T \quad (1)$$

where $\boldsymbol{L}$ is the principal component loadings matrix and $\boldsymbol{S}$ is the principal component scores matrix. Information on the clustering of the spike events is obtained from the scores. In this study, the scores of the first two components serve as features for spike events classification.

2.3. Spike events classification and templates construction

The scatter plot of the scores of the first two principal components is analyzed by subtractive clustering method [13] to reveal classification of spike events.

There are n data points in the scatter plot referring to n spike events. In subtractive clustering method, rough estimates of the local population densities are used to determine cluster centers in a serial fashion. The density $D(z_i)$ at data point z_i is

$$D(z_i) = \sum_{j=1}^{n} \exp\left\{-a\left\|z_i - z_j\right\|^2\right\} \quad (2)$$

The data point with the highest local density is considered to be the first cluster center. Let z_1^* denote the first cluster center and let $D(z_1^*)$ denote the density at z_1^*. In the next step, this cluster is removed from the data set by discounting the contribution due to the cluster center to the density at every point according to

$$D(z_i)^{new} = D(z_i)^{old} - D(z_1^*)\exp\left\{-b\left\|z_i - z_1^*\right\|^2\right\} \quad (3)$$

The two parameters, a and b, specify the size of the cluster in each of the data dimensions. At this point, the next highest density location is found, and this process is repeated until the density of left points is small than a predetermined threshold.

In this way, the number of neurons contributing to the recording is identified as the number of clusters. Define the number is M. Then the template of each neuron is constructed as the average waveform of the spike events belonging to each cluster.

2.4. Template matching

In extracellular recordings, the noise can be approximated as Gaussian noise. Suppose the variance of noise is s^2 and the mean is zero. s^2 can be estimated from idle (nonspiking) periods of the recording [8].

The template matching criterion is that choosing the best-fit template or templates combination for overlapping spikes so that after subtracting the template or templates combination from the spike event, the sum of squared residue follows Chi-square distribution. Because if the spike event is generated by the template or templates combination, after subtracting the template or templates combination from the spike event, the residue is only composed of noise, and then the unbiased estimate variance of the residue v^2 must be the same as s^2. Define this hypothesis-testing problem as follows

H_0: $v^2 = s^2$ Against H_1: $v^2 \neq s^2$

$$\text{If } H_0 \text{ is true, } \frac{(W-1)v^2}{s^2} \sim c^2(W-1) \quad (4)$$

where W is the length of residue. Given the significance level γ of rejection of H_0, H_0 is accepted if and only if

$$c^2_{1-\gamma/2}(W-1) < \frac{(W-1)v^2}{s^2} < c^2_{\gamma/2}(W-1) \quad (5)$$

We choose the width of the window as 80 sampling points, i.e., $W = 80$.

For each spike event, the template matching procedure starts from one-template-matching. Slide each template in the spike event window to find the best-fit template M_{one}^* at the best position t_{one}^*, then process Chi-square test to the spike event residue. If H_0 is accepted, it means this spike event is formed by M_{one}^* at the position t_{one}^*. If H_0 is rejected, then continue to process two-template-matching. Slide every two templates in the spike event window to find the best-fit templates M_{two1}^* and M_{two2}^* at the best positions t_{two1}^* and t_{two2}^*, then process Chi-square test to the spike event residue. If H_0 is accepted, it means that this spike event is formed by M_{two1}^* and M_{two2}^* at the position t_{two1}^* and t_{two2}^*. If H_0 is rejected, then continue to process three-template-matching until the H_0 is accepted. If H_0 is still rejected after all of the M templates have been combined to do the matching, the spike event should be forced to be sorted as the template or templates combinations that let the residue variance be the smallest.

Consequently, the spike temporal sequence of each neuron is reconstructed according to template matching results.

3. RESULTS

The proposed method is tested on an artificial spike train composed by three spike templates and noise. The spike train lasts 1 second (20000 sampling points when the sampling frequency is 20k Hz). The spike templates are shown in Fig. 1, which imitate the extracellular recording spikes of retinal ganglion cells. Thirty instances of each template are randomly inserted to the noise background, and the temporal sequence of each template is a Poisson train. The noise follows Gaussian distribution with zero

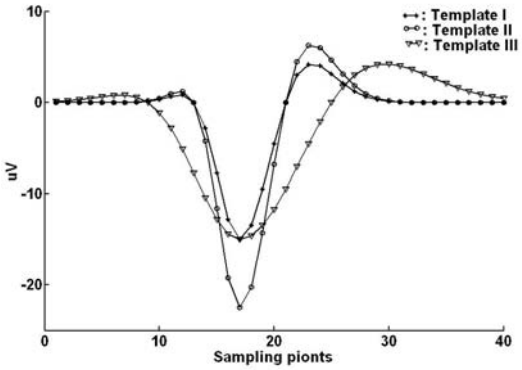

Fig. 1. Standard templates.

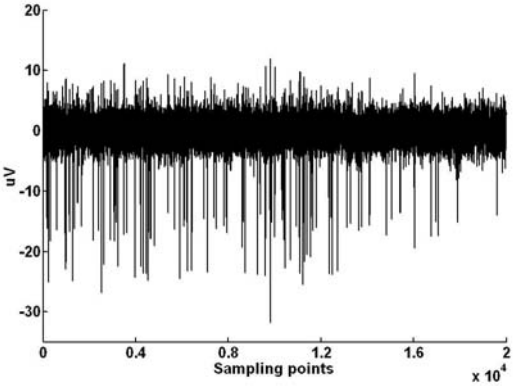

Fig. 2. Artificial spike train.

mean and variance $s^2 = 4$. The resulting spike train shown in Fig. 2 imitates three neurons firing independently and contains 5 overlappings with a delay of less than 1 millisecond (20 sampling points) between peaks.

Setting the threshold of the peak value as –12.0 μV, there are 85 spike events after checking the spike train and they produce the spike events data set $\boldsymbol{Y}\hat{I}\ R^{85\acute{}40}$.

The data set $\boldsymbol{Y}$ is processed by PCA and subtractive clustering method, and the result is shown in Fig. 3. There are 3 clusters, which means the number of neurons contributing to the spike train is 3. Those points unclassified to none of the three clusters in Fig. 3 are bias, which may be noise, overlappings, or badly distorted spikes. The constructed templates are shown in Fig. 4.

Then the proposed template matching procedure proceeds step by step. When proceeding Chi-square test we choose the significance level $\gamma = 0.80$. One example is shown in Fig. 5. In this artificial spike train, all of the spike events are sorted well.

To evaluate the spike sorting results, define the degree of misalignment of each correctly sorted constructed-template as follows:

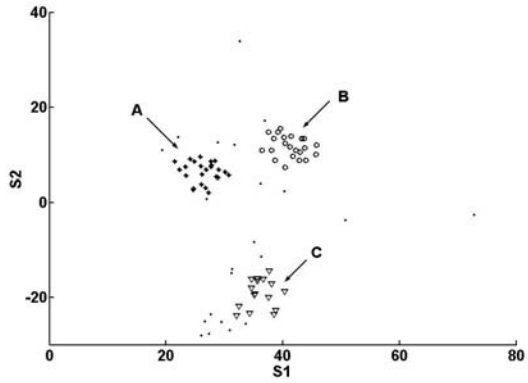

Fig. 3. Scatter plot of scores of the first two principal components and clustering results.

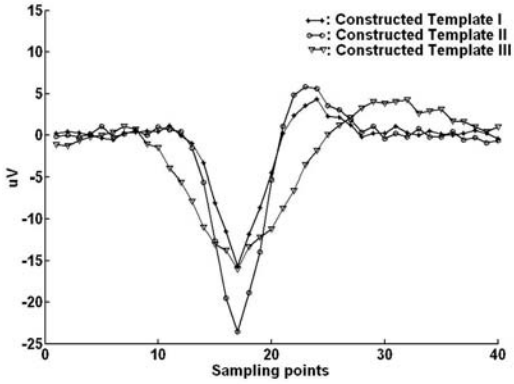

Fig. 4. Templates construction results.

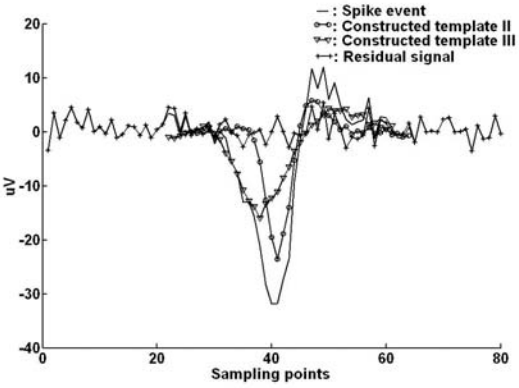

Fig. 5. Example of spike sorting results. This spike event is sorted as the combination of constructed template II and III.

$$D = \frac{\sum_{i=1}^{M}\sum_{j=1}^{m_i}\left|t_{i,j}-\hat{t}_{i,j}\right|}{\sum_{i=1}^{M} m_i} \tag{6}$$

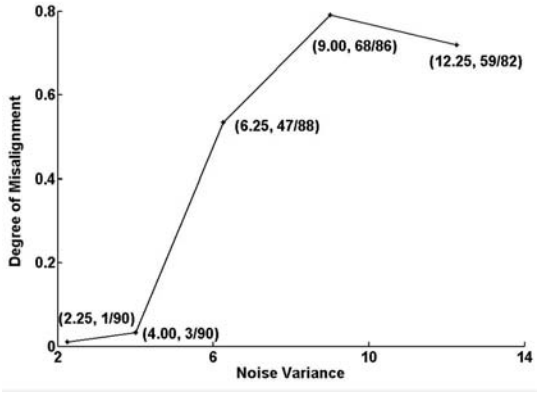

Fig. 6. Sorting results of spike trains with different noise variance.

where $\hat{t}_{i,j}$ is the peak time of the *i*th correctly sorted constructed-template at the *j*th firing time; $t_{i,j}$ is the true peak time of the corresponding *i*th template at the *j*th firing time; m_i is the number of the *i*th correctly sorted constructed-template firing. Here, $m_i = 30$. In this artificial spike train, D = 3/90.

Since the templates are constructed from the spike events that are extracted from the spike train, the constructed templates are sensitive to noise. The templates reliability decreases as the variance of noise becomes larger. Artificial spike trains are generated with five different noise variances, and the spike sorting results are shown in Fig. 6. When the variance of noise is larger, the signal-to-noise ratio is smaller, the degree of misalignment is larger, and misclassification and over-fit also occur. So when the signal-to-noise ratio is too low, the spike sorting results based on the constructed templates become unreliable.

4. CONCLUSION

The whole proposed procedure is done without a priori knowledge, such as the standard spike waveforms, the firing time, and so on. It is a blind source separation method. Template matching is performed together with Chi-square test, so we do not need to compare all possible combinations of the templates. In this way, the computation effort is reduced, especially for higher numbers of overlaps.

However, noise level has some impact on the performance of this method. The templates' reliability decreases as the variance of noise becomes larger as shown in Fig. 6. If reliable templates of spikes can be obtained using some other robust way such as single-unit recording and so on, the sorting procedure will produce much more reliable results.

5. REFERENCES

[1] W.M. Usrey, and R.C. Reid, "Synchronous activity in the visual system", *Annu Rev Physiol*, 61, pp. 435-456, 1999.

[2] D.M. Dacey, "Circuitry for color coding in the primate retina", *Proc Natl Acad Sci USA*, 93, pp. 582-588, 1996.

[3] M. Meister, J. Pine, and D.A. Baylor, "Multi-neuronal signals from the retina: acquisition and analysis", *J Neurosci Methods*, 51, pp. 95-106, 1994.

[4] A.H. Chen, Y. Zhou, H.Q. Gong, and P.J. Liang, "Chicken retinal ganglion cells response characteristics: multi-channel electrode recording study", *Science in China (Series C)*, 33, pp. 82-88, 2003.

[5] G. Zouridakis, and D.C. Tam, "Multi-unit spike discrimination using wavelet transforms", *Computers in Biology and Medicine*, 27, pp. 9-18, 1997.

[6] M.S. Lewicki, "A review of methods for spike sorting: the detection and classification of neural action potentials", *Network*, 9, pp. R53-R78, 1998.

[7] J.C. Letelier, and P.P. Weber, "Spike sorting based on discrete wavelet transform coefficients", *J Neurosci Methods*, 101, pp. 93-106, 2000.

[8] A.F. Atiya, "Recognition of multiunit neural signals", *IEEE Trans Biomed Eng*, 39, pp. 723-729, 1992.

[9] M.S. Lewicki, "Bayesian modeling and classification of neural signals", *Neural Computation*, 6, pp. 1005-30, 1994.

[10] G. Zouridakis, C.T David, "Identification of reliable spike templates in multi-unit extracellular recordings using fuzzy clustering", Computer Methods and Programs in Biomedicine, 61, pp. 91-98, 2000.

[11] K.D. Harris, D.A. Henze, J. Csicsvari, H. Hirase, and G. Buzsaki, "Accuracy of tetrode spike separation as determined by simultaneous intracellular and extracellular measurements", *J Neurophysiol*, 84, pp. 401-414, 2000.

[12] C. Pouzat, O. Mazor, and G. Laurent, "Using noise signature to optimize spike-sorting and to assess neuronal classification quality", *J Neurosci methods*, 122, pp. 43-57, 2002.

[13] R.N. Davé, and R. Krishnapuram, "Robust Clustering Methods: A Unified View", *IEEE Trans Fuzzy Systems*, 5, pp. 270-293, 1997.