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Identification of reliable spike templates in multi-unit extracellular recordings using fuzzy clustering

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Abstract

A method for extracting single-unit spike trains from extracellular recordings containing the activity of several simultaneously active cells is presented. The technique is particularly effective when spikes overlap temporally. It is capable of identifying the exact number of neurons contributing to a recording and of creating reliable spike templates. The procedure is based on fuzzy clustering and its performance is controlled by minimizing a cluster-validity index which optimizes the compactness and separation of the identified clusters. Application examples with synthetic spike trains generated from real spikes and segments of background noise show the advantage of the fuzzy method over conventional template-creation approaches in a wide range of signal-to-noise ratios. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Multi-unit spike train; Template extraction; Spike discrimination; Fuzzy clustering; Fuzzy K-means

1. Introduction

The study of anatomical connections and single-cell spatio-temporal interactions in small populations of neurons is essential when attempting to understand the mechanisms of information processing in biological neural networks, such as those found in the nervous system. Typically, extracellular recordings (obtained from a single electrode or an array of electrodes) comprise the activity of groups of adjacent neurons; thus, further analysis of such multi-unit signals is necessary to recover the contribution of individual neurons.

However, to accomplish this, it is necessary to know both the number of neurons contributing to a particular recording and their characteristic waveforms. In general, action potentials (also known as spikes) arising from different neurons have different shapes, depending on the type of neuron and its distance from the recording site, as well as on the specific characteristics of the electrode and the intervening medium. Moreover, the neuron-electrode interface has the attributes of a lowpass filter; thus, activity generated far away from the recording electrode has different characteristics than that generated close to it.

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Therefore, single-unit spike trains can be easily extracted from multi-unit recordings if the signal-to-noise ratio is high enough and if spikes originating from different neurons do not overlap in time (spikes generated by the same neuron cannot overlap due to the neuron's refractory period). However, spike separation becomes nontrivial when there is partial or complete overlap among spikes. For instance, when two neurons fire simultaneously, the recording electrode detects the summed activity and, as a result, the amplitude, duration, and (peak) latency of the detected spikes are changed. Fig. 1 shows an example of the summation effects on the recorded signal when a spike (spike 2) enters into the time window of another one (spike 1) Another complicating factor is the presence of noise. In this case, because of changes in their shape, some spikes may be misidentified as activity from additional neurons. Thus, the presence of overlapping activity and/or high-amplitude noise may result in erroneous identification of individual spikes.

Despite the large number of techniques developed in the past to analyze multi-unit spike trains (e.g. [1–3,6–9,12–16,18–22,24,25]) most methods do not deal with template creation (i.e. identification of the characteristic waveforms generated by the different neurons contributing to the spike train) but, rather, they concentrate only on spike discrimination (i.e. detection and classification of the spikes found in a spike train) assuming that the templates are known a priori. In the present study, however, we specifically focus on the (i) creation of reliable spike templates, one representative for each contributing neuron, and (ii) identification of the true number of neurons being recorded. Here we deal only with the problem of template creation; the issue of spike discrimination and spike-train reconstruction has been addressed in a companion paper [29]. To overcome the aforementioned problems, we follow a fuzzy clustering approach which, due to the inherent ability of fuzzy classifiers to deal with incomplete and ambiguous information, has the advantage of drastically reducing the effects of noise and of overlapping activity.

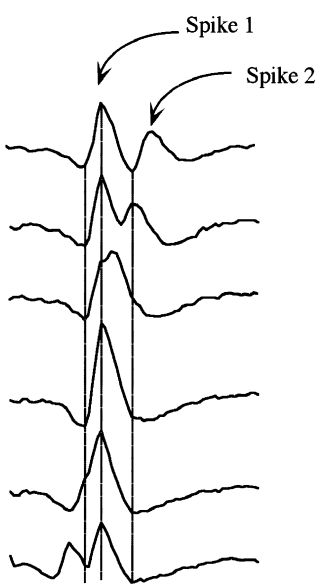


Fig. 1. Example of summation effects on the signal recorded when one spike (spike 2) enters into the time window of another one (spike 1).

The clustering procedure presented here employs a fuzzy K-means algorithm the performance of which is controlled by an optimization criterion. Short descriptions of the procedure have been previously presented in abstract form [27,28]. Here we give illustrative examples with synthetic spike trains generated from real spikes and segments of biological background noise. Extensive simulations allow to determine the optimum 'amount of fuzziness' for real-time processing and also, they clearly demonstrate the advantage of the fuzzy procedures in identifying the exact number of neurons in a recording and the corresponding exact templates. The performance of the proposed procedure is compared to conventional crisp clustering and its usefulness is demonstrated over a wide range of signal-to-noise ratios.

2. Methods

2.1. Fuzzy clustering

Clustering is a mathematical tool that attempts to assess the relationships among several objects in a data set (often referred to as patterns) by organizing the patterns into clusters (i.e. groups,

or classes), so that patterns within a cluster are more similar (according to a predefined measure of similarity) to each other than the patterns in the rest of the clusters. In the present study, a pattern is a spike, and the measure of similarity is the Euclidean distance between two spikes. Thus, after clustering, the distance between two spikes from the same group is smaller that the distance between two spikes from two different groups.

In conventional clustering, a spike can either belong or not belong to a particular class (in the former case, its degree of membership to that class equals one, in the latter case, it equals zero). However, overlapping events are composed of more than one spike and, therefore, they may have partial characteristics from several classes. Thus, these procedures cannot handle correctly hybrid activity, because they artificially create clear-cut boundaries between classes.

In fuzzy clustering, on the other hand, every spike belongs to all possible classes, but with different degrees of membership. Membership values are allowed to vary continuously from zero to one. The higher the membership of a spike to a given class, the more 'typical' the spike for that class. Additionally, during estimation of a class representative (referred to as template), 'typical' spikes contribute more to the template, whereas spikes that are less typical contribute less, depending on their membership values. Thus, the resulting templates are more reliable representatives of their respective classes. The use of fuzzy methods is justified by the very nature of the data that often are mixed due to the simultaneous activation of several neurons that contribute to the recorded activity.

2.2. Fuzzy K-means algorithm

As mentioned earlier, in our case a pattern is a spike, one of the N spikes contained in the whole data set under analysis. Each spike is composed of M time samples, thus the j-th pattern can be indicated as $X_j(t)$, with $j = 1,2,\ldots,N$ and $t = 1,2,\ldots,M$. However, to simplify notation, the time index t will be dropped in the following analyses and the j-th pattern $X_j(t)$ will be simply denoted with X_j.

Assume that a set of N patterns (spikes) X_j, $j = 1,2,\ldots,N$, each having M components, is to be divided into K classes C_i with centroids V_i. where $i = 1,2,\ldots,K$, respectively. In conventional set theory, the membership of a pattern X_j to a particular class C_i can be represented by a characteristic (membership) function, μ_{ij} that may take on only one of two values, 0 or 1. That is,

$$\mu_{ij} = \begin{cases} 1, & \text{if} \quad X_j \in C_i \\ 0, & \text{if} \quad X_j \notin C_i \end{cases} \tag{1}$$

However, if the values of this function are extended to cover the whole range of the closed interval [0,1], then μ_{ij} represents the degree of membership of pattern X_j to fuzzy class C_i. The membership function can be computed from the following formula [4],

$$\mu_{ij} = \frac{\left[\dfrac{1}{d^2(X_j, V_l)}\right]^{\frac{1}{q-1}}}{\sum_{l-1}^{K}\left[\dfrac{1}{d^2(X_j, V_l)}\right]^{\frac{1}{q-1}}} \tag{2}$$

where

$$d^2(X_j, V_i) = \sum_{t=1}^{M} [X_j(t) - V_i(t)]^2 \tag{3}$$

is the usual Euclidean distance and q is the fuzziness index. The value of q is to be determined empirically (typically $q > 1$ since $q \to 1$ gives hard clustering).

Among the many procedures devised to accomplish such data partitioning is the K-means algorithm in its crisp [23] and fuzzy [4] forms. The iterative version of the fuzzy K-means [5] has been shown to find local minima and is executed in the following steps:

1. Initialize with random membership functions μ_{ij} such that

$$\sum_{i=1}^{K} \mu ij = 1 \quad \text{for} \quad j = 1,2,\ldots,N \tag{4}$$

2. Compute fuzzy centroids V_i, $i = 1, 2,\ldots,K$, through the relation

$$V_i = \frac{\sum_{j=1}^{N} (\mu_{ij})^q X_j}{\sum_{j=1}^{N} (\mu_{ij})^q} \tag{5}$$

3. Use Eq. (2) and Eq. (5) to obtain updated fuzzy memberships and centroids, μ_{ij}^* and V_i^*, respectively.

4. Compute the total error E_t using

$$E_t = \sum_{i=1}^{K} \| V_i - V_i^* \| \tag{6}$$

where $\| V_i - V_i^* \|$ is the Euclidean distance between V_i and V_i^*.

5. If $E_t < \varepsilon$ stop; else repeat steps 3 and 4.

2.3. Cluster validity

In order to determine the optimum number K of clusters C_i, with $i = 1,2,\ldots,K$, present in a data set some measure of cluster validity must be considered. For hard clustering, a well-established validity criterion is the separation index D_1 defined as [10]

$$D_1 = \frac{\min\limits_{1 \le i \le K-1} \left\{ \min\limits_{X_i \in C_i,\, X_j \in C_j} \| X_i - X_j \| \right\}}{\max\limits_{1 \le k \le K} \left\{ \max\limits_{X_1\, X_j \in C_k} \| X_i - X_j \| \right\}} \tag{7}$$

where $\| \cdot \|$ indicates the usual Euclidean norm. It assumes that the most valid K-partition is the one that yields maximum D_1. For fuzzy clustering, among several other validity criteria, the fuzzy clustering validity function S, in its original [26] or modified [17] version, is expressed as the ratio of the compactness to the separation of the clusters and can be explicitly written as

$$S = \frac{\sum_{i=1}^{K} \sum_{j=1}^{N} \mu_{ij}^q \| V_i - X_j \|^2}{N \min\limits_{ij} \| V_i - V_j \|^2} \tag{8}$$

This criterion assumes that the most valid data partition is the one corresponding to minimum S, since smaller S means a more compact and more separate partition. When the number of clusters becomes very large, this criterion has a tendency to decrease, but in practical applications, it is sufficient [26] that $K_{\max} \leqslant N/3$.

2.4. Defuzzification

At the end of fuzzy clustering, the algorithm provides for each class a fuzzy template, which is obtained by averaging all patterns in the data set—each weighted by its membership value to that class. These templates represent hypothetical 'typical' spikes and, by construction, provide maximum separation of the classes. However, they cannot be used when exact values of amplitude and latency are needed for individual peaks in a waveform. It is therefore necessary to convert fuzzy partitions into hard (crisp) ones.

One such approach involves the use of a maximum membership defuzzification function $\mu_{mm,\,ij}$ defined as [4]

$$\mu_{mm,\,ij} = \begin{cases} 1, & \text{if} \quad \mu_{ij} > \mu_{sj}, \quad \forall \quad i \neq s \\ 0, & \text{otherwise} \end{cases} \tag{9}$$

The above function assigns each pattern to one class only, the class to which the pattern has maximum membership. That is, the ith crisp template is computed by averaging over all patterns j for which $\mu_{ij} > \mu_{sj}$.

2.5. Data and analysis procedure

Synthetic spike trains simulating extracellularly recorded activity were generated, using four well-defined spikes and chunks of background biological noise obtained from crayfish data [11]. The time of spike occurrence was approximated by a Poisson random process, which allowed several spikes to totally or partially overlap. The signal-to-noise ratio was controlled by changing the variance of the superimposed noise. Fig. 2 shows an example of a synthetic spike train construction.

The fuzzy K-means algorithm described above was employed to group individual spikes into K clusters, with $K = 2,\ldots,10$. The number of spikes N used in a single run was approximately 1000. Each spike was composed of 64 time samples corresponding to a total duration of 1.6 ms. Thus the dimension M of the feature space was 64. The value of the fuzziness index q was set equal to 1.8, which was empirically determined to give the best performance. Clustering is typically performed on features extracted from the data; our analysis used all of the time samples of a spike as the clustering features. The noise variance σ could

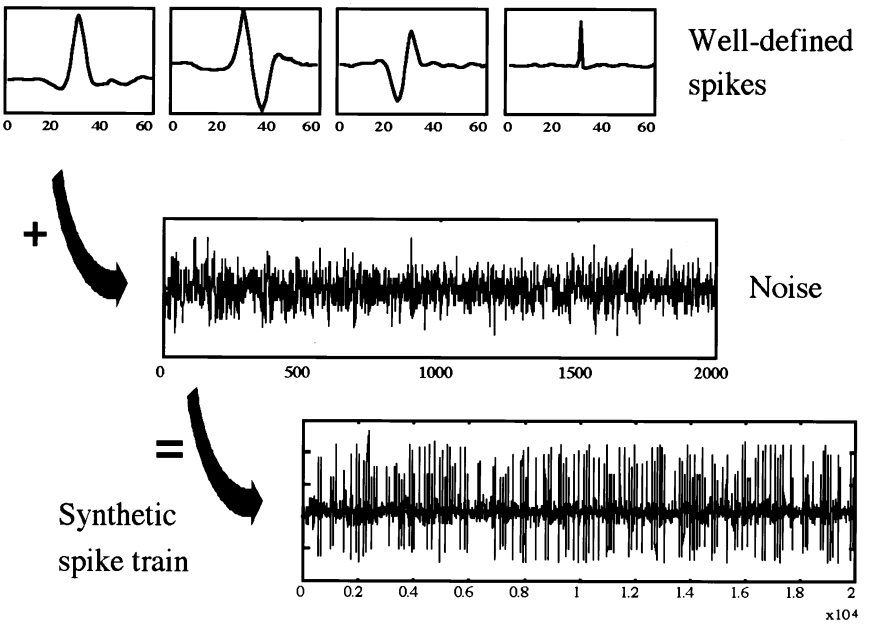


Fig. 2. Example of generating a synthetic spike train simulating extracellularly recorded multineuron activity.

take on one of the following values: 1, 2, 5, 10, or 20. The value of the criterion to terminate the iteration was set equal to a small number ($\varepsilon = 0.0001$). The clustering procedure was repeated for all combinations of σ and K, and each time, the cluster validity criterion function S was computed. To improve performance, for each value of K the fuzzy K-means was repeated 20 times, and the partition giving the smallest S was considered optimal for that value of K. At the end of the entire procedure, the value of K corresponding to the overall minimum value of S was considered to represent the optimum number of clusters present in the data.

For comparison purposes, the exact same data were analyzed following a 'classical' hard-clustering approach, again with $K = 2,\ldots,10$ and 20 repetitions of the hard K-means algorithm for each value of K. In this case, optimal partitions and the optimum number of clusters were determined by the maximum value of D_1.

Furthermore, the root-mean-square (RMS) error between the real and the estimated templates was also computed for all values of the noise variance for both the fuzzy and the crisp algorithms. This RMS error can be regarded as a measure of similarity between real and estimated templates during the template creation process (this error is different from the percentage of correctly classified spikes during the discrimination process).

3. Results

The optimal number of neurons estimated by the proposed fuzzy procedure can be determined from Fig. 3 which depicts the values of the criterion S as a function of the number of clusters K and the noise variance σ (for clarity, large values of S have been truncated to 3.0). It can be seen that S has a minimum at $K = 4$ for all values of σ, indicating that only four neurons were contributing to the recording. This is in agreement with the number of distinct spikes used to create the synthetic spike trains.

The corresponding four templates estimated through fuzzy clustering are shown in Fig. 4(b),

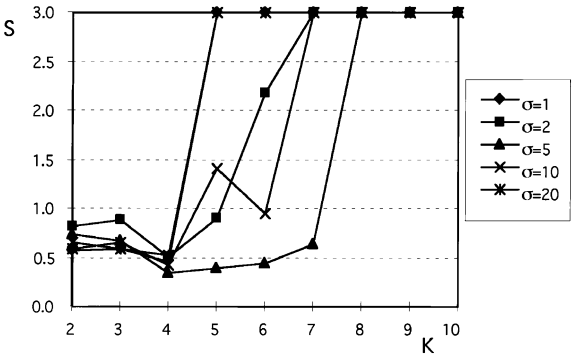


Fig. 3. The optimal number of neurons estimated with fuzzy clustering is given by the minimum value of the criterion function S plotted here for various values of the noise variance σ.

along with the original spikes (Fig. 4(a)) and the templates obtained after defuzzification (Fig. 4(c)). These figures show that the estimated and the actual templates are practically identical.

The optimal number of neurons identified by the crisp clustering algorithm can be determined from the values of the criterion D_1 computed for several values of the number of clusters K and the noise variance σ. Since there are only four distinct spikes in the synthetic spike trains, the function D_1 should have a maximum at $K=4$, indicating the presence of four clusters. However, in this case, the suggested optimal number of clusters was inconsistent for the various values of K and σ, and mostly incorrect.

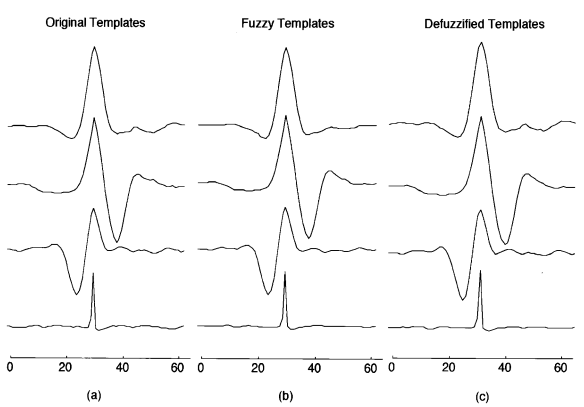


Fig. 4. Original spikes (a) and estimated templates obtained from fuzzy clustering before (b) and after (c) defuzzification.

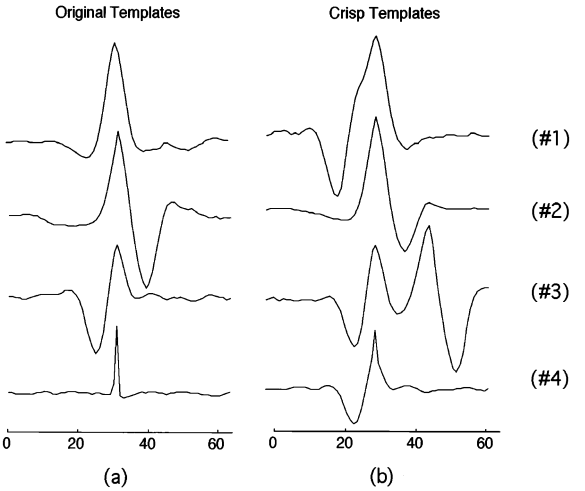


Fig. 5. Original spikes (a) and estimated templates (b) obtained from crisp clustering.

Furthermore, the optimal templates estimated by hard clustering in the case of $K=4$ are shown in Fig. 5. It can be seen that only one template (# 2) is similar to the actual spike used in the synthetic spike train, whereas the other three templates have completely new shapes. It is obvious that the classifier created templates to account for overlaps and this resulted in spikes with double peaks (# 3) and/or distorted amplitude (# 1 and # 4).

The RMS error between real and estimated templates when the number of clusters was four is shown in Fig. 6 for both crisp and fuzzy clustering. Inspection of this figure shows that the error

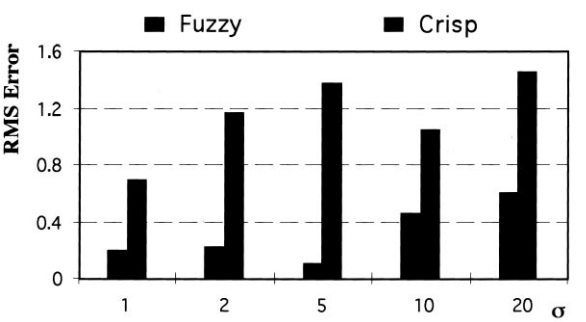


Fig. 6. RMS error between the real and the estimated templates obtained from crisp and fuzzy clustering for various values of the noise variance σ.

in the case of the crisp approach is much larger than the fuzzy method: in some cases, this error was as much as ten times larger than the one corresponding to the fuzzy classifier (see, for example, the case with $\sigma = 5$).

4. Conclusions

A method has been presented that addresses the specific problem of estimating the number of neurons present in a single channel of multineuron activity and of creating the corresponding spike templates reliably. This information is used in a subsequent stage of spike discrimination, whereby the algorithm starts with the multineuron recording and reconstructs several spike trains, each containing the activity of only one neuron [29]. The method described in this paper is based on fuzzy clustering and can successfully deal with the problem of overlapping activity and data variability due to the presence of biological noise. Indeed, as illustrated by the previous examples, in the case that, for instance, two spikes overlap to produce an unknown pattern, the algorithm would not create a third class to accommodate the seemingly new shape; rather, it would allow the pattern to have partial membership to both classes. However, for the same data, the crisp clustering algorithm would erroneously create a new class to accommodate the overlapping activity.

Another advantage of the fuzzy method is that it can inherently account for variability in the shape of spikes; thus it can identify templates more accurately even in the presence of noise. Indeed, the computation of the final templates is controlled by a defuzzification function, where the user has the option to select the degree of spike variability allowed in the estimates of the final templates. For instance, one could allow only spikes with high membership values, say $\mu \geqslant 0.8$, to be considered, while the more ambiguous ones, i.e. those heavily corrupted by noise and having small membership ($\mu < 0.8$) values, could be ignored.

The simulations presented in the previous paragraphs clearly demonstrate the ability of the fuzzy procedure to identify the exact number of neurons in a recording and the exact templates over a wide range of signal-to-noise ratios. The use of the proposed method is not limited to spike-train analysis only, but it has been applied successfully to other fields of waveform recognition, such the EEG and evoked potential analysis in normal [31] and pathological [30] cases.

Acknowledgements

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