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Spike sorting: the overlapping spikes challenge

Abstract: When recording action potentials (spikes) from many neurons simultaneously via multichannel micro-electrodes the overlapping of spikes from different neurons is a demanding problem for detection and classification of spikes (spike sorting). Since multichannel electrodes provide better possibilities to separate the superimposed waveforms, we refined an algorithm for separation of overlapping spikes for the use on multichannel recordings and tested it on simulated data with different numbers of signal channels and with several signal parameters. We show that the larger the number of signal channels the better the separation that may be achieved, especially under demanding recording conditions.

Keywords: neural activity; extracellular recordings; multichannel electrodes; action potential; overlapping spikes; source separation

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1 Introduction

The study of brain activity relies on the fact that most neurons in the brain communicate by firing action potentials often referred to as spikes. The action potentials can be recorded extracellularly by microelectrodes implanted in the brain. It is possible to measure the action potentials of many neurons in a local area [1].

It is emanated that the shape of a spike arising from one neuron stays constant with time aside from special situations like bursts [2]. Based on these assumptions it is possible to discriminate spikes from background noise and to cluster spikes into units depending on their characteristic shapes according to the neuron they arose from, a process often referred to as spike sorting.

Common spike sorting methods are generally successful if spike waveforms are clearly distinguishable from background noise and from waveforms of other neurons. This case occurs if the neurons fire independently from each other. But a more demanding situation arises if two or more neurons fire at the same time. In this case the result is a linear superposition of the individual waveforms of two or more units, in the following referred to as overlapping spikes. Here, common spike sorting methods generally fail [3] because the superimposed spike waveform resembles usually none of the origin waveforms and thus cannot be assigned to the correct neurons.

Overlapping spikes usually only present the minor part of the detected spikes but inappropriate analysis of these overlapping waveforms may lead to wrong conclusions about neuronal synchrony and connectivity [5].

The idea for the applied algorithm bases on an overlap separation algorithm developed by Michael S. Lewicki that computes models for all temporal overlaps and all possible neuron combinations [6].

Over the last years the use of multi-channel recordings became more popular because they provide a distinct amplitude distribution for each neuron on the recording channels, also known as stereotrode effect [7]. The amplitude of a spike on a channel corresponds to the distance between neuron and electrode tip. This effect leads to a better spatial resolution (see Fig. 1).
To analyse the improvement of the separation results due to the stereotrode effect we adapted the algorithm of Lewicki to the use on multichannel recordings and tested it on simulated multichannel recordings with known spike times and waveforms.

## 2 Methods

### 2.1 Signal simulation

To generate data that represents the activity of neurons as realistic as possible, we used the simulation algorithm published in [8]. The algorithm bases on the simulation of individual interspike interval distributions given by an exponential distribution in combination with a Gaussian distribution to model spike-firing rates and refractory periods.

During the simulation the interspike intervals are generated from these distributions and are summed up to get the spike times for each neuron. At each spike time the waveform of the firing neuron is inserted and consequently in case of an overlap the voltage traces will be summed up to a new waveform. The waveforms were taken out of recorded data, kindly provided by Liana Melo-Thomas, Univ. Marburg.

Neurons were simulated as point sources and electrodes as point sinks. The distance between source and sink is then calculated as Euclidean distance and was used for the calculation of the characteristic amplitude distribution. For the testing we simulated heptodes, tetrodes, stereotrodes and monotrodes. A sketch of the geometry of recording electrodes is shown in Figure 1, stereotrodes consist of channels 4 and 7, monotrodes of channel 1.

**Figure 2:** Linear superposition (red) as sum of mean spike from unit 1 (blue) and mean spike from unit 2 (green) with a distinct shift against each other. The red curve is also a model for overlap separation.

For realistic incorporation of noise its amplitude distribution and spectrum were extracted from a real extracellular recording. Using these noise parameters sequences were generated and added randomly to the waveforms. The noise recording was kindly provided by the group of Frank Bremmer, Univ. Marburg.

**Figure 3:** Block diagram of the overlapping spike separation algorithm.

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### 2.2 Data preparation

For the application of the separation algorithm we assume perfect detection and classification results. Thus the application of a spike sorting algorithm can be left out and the knowledge about spike times and units can be taken from the simulation results. The simulated spikes were extracted from signal by means of the known spike times. All spikes were aligned to an overall mean spike. If an overlap occurs the waveform is excluded and the shifts and the contained units are saved for later comparison. It is also assumed that overlapping spikes are perfectly recognized as those by a clustering algorithm. The spikes which are already assigned to the right units are used for model calculation within the overlap separation algorithm.

### 2.3 The separation algorithm

Our separation algorithm calculates models for all possible neuron combinations and time shifts. But instead of using a probabilistic description to find the most appropri-
In an agreement model as in [6] we estimated the best fitting model by calculating cross-correlations of models and overlapping spikes.

The models are computed with help of the mean spikes from each neuron. The mean spikes are shifted over a distinct period against each other and summed up to obtain a linear superposition (see Fig. 2).

The model with the highest correlation will be assigned to the observed overlapping spike. The active neurons (units) and the spike times forming the overlapping spike will then be defined as the units and the spike times which are included in the model which fits best.

The calculation of the number of required models leads to a combinatorial problem that can be described as the number of ways to choose \( k \) overlapping units out of a set of \( n \) units. The number of possible combinations \( c \) can be calculated with the binomial coefficient.

\[
c = \sum_{k=2}^{m} \binom{n}{k}
\]  

(1)

In Eq. 1 \( n \) is the number of units included in the signal and \( k \) is the number of units that can be included in one overlap thus cannot be smaller than two. If really all theoretically possible combinations ought to be considered it must be \( m = n \) with \( m \) the maximum number of units that can be contained in an overlap. All combinations of units from two up to \( m \) have to be considered, \( m \) can be chosen smaller than \( n \), hence \( 2 \leq k \leq m \leq n \).

In the second step it must be considered that the waveforms forming an overlapping spike can occur within different intervals. Thus the degree of overlap has to be considered (see Fig. 3 (C)).

Since the real spike times are usually not known and spike alignment introduces additional temporal shifts, several time shifts have to be introduced into the model calculation. The number of considered shifts \( s \) is set to the number of samples within a spike waveform. The number of models \( M \) is then

\[
M = \sum_{k=2}^{m} \binom{n}{k}s^k.
\]  

(2)

To find the model which resembles the overlapping spike as good as possible, the cross-correlation is applied to each model and each overlapping spike.

Seeing that the spike alignment, which is applied during a common spike sorting, modifies the position of the waveforms within the extracted window, we incorporate the coefficients from shifts of \( \pm s/2 \) between model and overlapping spike for the selection of the best fitting model (see Fig. 3 (D and E)).

3 Results

3.1 Influence of noise level

We evaluated the success of the overlapping spike separation in percent of correctly separated overlapping spikes for different noise amplitudes. The number of included neurons in the signal \( n \) was set to 3 as well as the maximum number of spikes contained in an overlap model \( m \). So for the testing conditions we set \( n = m \). For each amount of noise 30 datasets have been analysed and as a result the mean percentage value has been plotted in Figure 4.

![Figure 4: Separation results for different noise amplitudes and different numbers of signal channels. Each bar shows the standard error of the mean.](image)

Figure 4: Separation results for different noise amplitudes and different numbers of signal channels. Each bar shows the standard error of the mean.

Results indicate that the performance of the algorithm increases with higher numbers of used signal channels. With low noise amplitudes the algorithm achieves reliable results for all numbers of signal channels. With rising noise level, the results of the heptode and the tetrode only
decline slowly, while the results of the monotrode fall off even with little rise of noise. The improvement even the stereotrode achieves in comparison to the monotrode is clearly visible in Figure 4.

3.2 Influence of the number of neurons

Further we evaluated the success of the separation for different numbers of neurons simulated in the signal. The standard deviation of noise was kept constantly at 0.03. The number of included neurons in the signal \( n \) varied from 3 to 6. The maximum number of spikes contained in an overlap model \( m \) was set to 3. For each number of simulated units 13 datasets have been analysed and as result the mean percentage value has been plotted in Figure 5.

The results of the monotrode decline rapidly when more neurons are enclosed in the simulation, while the results of the heptode and the tetrode stay reasonably constant. Once again, already the use of a stereotrode leads to a significant improvement compared to the monotrode.

4 Discussion

We refined an overlapping spike separation algorithm published by Lewicki in [6] for the use on multichannel recordings and evaluated the performance on simulated data.

Our results indicate that the performance increases with rising number of signal channels especially under conditions with high noise amplitudes and a high number of neurons. Due to the fact that neurons produce spikes with stereotypic shapes the waveforms can be quite similar. The use of multichannel electrodes leads to a greater dissimilarity due to the characteristic amplitude distribution and consequently provides a better separability. The runtime and the computational effort increase with rising number of channels. However, the optimal number of channels is yet unknown.

The runtime increases massively with rising number of neurons in the signal as a consequence of the increasing number of models (cf. 2). Additionally, the runtime increases if more neurons are simulated because of the rising amount of overlapping spikes.

Another limitation is due to the so called overfitting problem, which occurs when an overlapping spike can be represented by different models and when the model with the highest component number but (partially) wrong model spikes fits best. This problem is often intensified in the case of very similar spike waves.

Nevertheless is an overlapping spike separation algorithm unavoidable especially when recordings containing many neurons with high firing rates have to be analysed.

The next steps will be the integration of our separation algorithm in a spike sorting algorithm which includes a classification of overlapping spikes. Then it can be tested on real recordings.

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Author’s Statement

Conflict of interest: Authors state no conflict of interest.

Material and Methods: Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors’ institutional review board or equivalent committee.

References


