RELIABILITY OF STATISTICAL FEATURES DESCRIBING NEURAL SPIKE TRAINS IN THE PRESENCE OF CLASSIFICATION ERRORS

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Abstract: In order to investigate functioning of the brain processes, it is important to have reliable processing of neural activity. For precise tracking of local neural network processes, reliable clustering of single neurons' action potentials (spikes) is necessary. So far, it was common to keep the signals of high quality and discard the others. This work examines the possibility of extracting reliable information from bad quality signals, in the presence of spike classification errors. We tested the robustness and information capacity of several statistical parameters used to describe firing patterns of spike trains using simulated signals mimicking most common cases in nature. Although complete reconstruction of firing patterns is not always possible, we show that the approximation of the mean firing frequency as well as the detection of bursting processes can still be quantified successfully, thereby paving the way for future applications.

1 INTRODUCTION

To extract the useful information about the condition and changes in functioning of a region in the brain, appropriate processing of neuronal signal recordings is a crucial step (Chan et al., 2010).

Neurons are the foundation of our nervous system. They are the transmitters of all the information in our nervous system through electrical and chemical signaling. Information processed in the brain is embedded in neuronal spikes which are all-or-none binary processes. By observing "firing" patterns of some neurons by means of extracellular recordings, we are able to get a glimpse on general conditions in the observed area. It is common practice to keep the signals of high quality in terms of signal-to-noise ratio (SNR) and discard the others. However, more high quality signals usually implies more recording places, which means bigger damage of the tissue during electrode placement and so on. It is therefore useful to maximize the value of extracted information if possible by processing even low quality signals.

We investigate the robustness of certain statistical parameters used to describe firing patterns of neurons when the quality of the signal and the spike classification is low. In order to detect and assign spikes to their firing neurons, we apply the well-known Wave_clus spike clustering algorithm (Quiroga et al., 2004). We then continue and generalize our approach so that arbitrary clustering algorithm can be used. We assume and vary a certain percentage of wrongly classified spike appearance times (timestamps) and observe the results in terms of errors of statistical parameters used to describe spike trains.

Using artificial signals with realistic distributions, with known underlying values of parameters, we show how to assess the information carrying capacity of each of them as well as their robustness. Standard parameters (mean, median, burst coefficient, coefficient of variation, skewness, kurtosis etc.) are used. In addition new parameters are also introduced.

2 METHODOLOGY

2.1 Artificially generated distributions and signals

Since real data's underlying distribution of spike timestamps is unknown, artificially generated

signals were used in this study. These served as input to the clustering algorithm and for later statistical parameter estimation. In this way, the true values of parameters describing the underlying distribution can be compared to those obtained after detection and clustering.

We used spike shapes obtained from real recordings, some of them available on the internet (Rutishauer, 2011) and some obtained from IMEC - Belgium recordings. Some examples used in this research are shown in Figure 1.

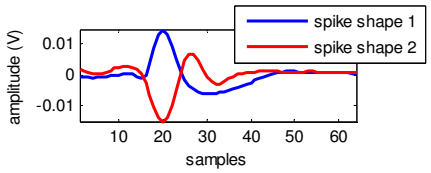

Figure 1: Two examples of spike shapes used here.

Matlab was used to generate normally and Poisson distributed timestamps. These are reported to be realistic models to describe the firing pattern of a neuron (e.g. Dayan and Abbott, 2005). Also, a simulated distribution of a firing pattern containing bursts (fast consecutive neural firing) was created. A neuron is bursting if it is firing consecutive spikes with very small intermediate break intervals (< 3 ms). Signals are created by adding spikes whenever indicated by created timestamps. Noise is also added to form a realistic artificial neuronal signal. This noise is a mixture of white noise and background noise. The latter consists of a large number of randomly selected and scaled waveforms which were added to mimic far away neurons (Rutishauser et al., 2006). The SNR is differed among those artificial signals (calculated as in formula 1 with n the number of samples of a selected spike). To create different low quality signals, this random noise trace can be rescaled to obtain signals with a pre-specified SNR. Overall, around 120 signals were used in this study.

$$\text{SNR}=\frac{\text{signal power}}{\text{noise power}}=\frac{\sqrt{\sum_{i=1}^{n} x_i^2}}{\sqrt{n*(\text{std}(noise))^2}} \qquad (1)$$

A more general and computationally faster procedure avoiding the need for the clustering is also applied. This procedure simulates classification errors by mixing timestamps of multiple spike trains. A certain percentage of these timestamps, imposed by the user, is correctly classified; accordingly, misclassified (assigned to a wrong cluster) timestamps are added as well. In addition, a certain percentage of timestamps is left undetected to imitate the realistic case, where some spikes evade detection because of low SNR. The computational gain is achieved by mimicking the clustering results without applying the actual procedure. After assigning timestamps to clusters, robustness of parameters is tested.

2.2 Robustness of parameters in the presence of classification errors

Reported values of statistical features for neuronal clusters, like mean firing frequency or coefficient of variation are often taken for granted. However, one should be aware of deviations caused by clustering errors. Figure 2 shows one example of a cluster associated with a single neuron. It is obvious that variations around the mean spike shape are large, indicating a possible mixture of more spike shapes.

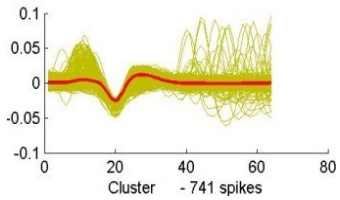

Figure 2: Example of bad classification (clustering) of spikes.

2.3 Statistical parameters

Statistical parameters are introduced to describe the firing patterns of neurons, often observed through the so called interspike interval histogram (ISIH). This is a distribution of the observed time intervals between successive spikes collected in bins of fixed width.

Standard parameters are used to compare different models. They are applied to describe individual clusters. **Mean** corresponds to the average interspike interval (ISI), whereas the **median** is the middle value of a finite ordered list of these ISIs. **Skewness** and **kurtosis** are also used, which are measurements for the asymmetry and the peakiness of the ISIH respectively (NIST, 2010). **Coefficient of variation C_V** and **spiking randomness** (Kostal et al., 2007) are also included in the study. C_V is the ratio of the standard deviation divided by the mean, and represents spiking variability. Spiking randomness is a mathematical measure based on the entropy in the signal. Roughly speaking, this entropy increases with the larger variability of different ISIs and with more freedom in the serial ordering of the ISIs in the

spike train (Kostal et al., 2007).

To detect bursting activity, some new parameters are included as well (Gligorijevic et al., 2010). **Pause_index** is the ratio of the number of ISIs longer than 50 ms over the number of ISIs shorter than 50 ms, whereas the **pause_ratio** is similarly defined using instead the sum of these interval lengths to calculate the ratio. **Mod_burst** is the ratio of the number of ISIs less than 10 ms to the ISIs longer than 10 ms. Finally, to quantify fast activity yet formally not bursting, we define the "**Percentage window > 5 spikes**" as the percentage of fixed windows (here 100 ms is used) in which at least 5 spikes appear.

2.4 Calculation of errors

We can compare values and errors of specified parameters at three different moments of the spike train analysis based on the following three models:

- Continuous underlying distribution.
- Sampled distribution after taking values conforming to the continuous distribution for the interspike intervals (Figure 3a).
- Distribution after clustering (Figure 3b).

We extracted the described parameters from the computed ISIHs. These parameters assist us in describing the firing model of a neuron.

To compare the three different models, the errors on used statistical features have to be calculated. For example, the error between the mean of the sampled distribution and the distribution after clustering is calculated as in formula 2. The error shows how this value deviates after clustering compared to the one before. This deviation error could therefore be larger than 100% in contrast to the classification errors, which could potentially reach up to 100% (when all the spikes are assigned to the wrong cluster).

$$error\ (\%) = \left| \frac{mean_{clustered} - mean_{sampled}}{mean_{sampled}} \right| * 100 \qquad (2)$$

The behaviour of the parameters is examined for different distributions and different SNRs in the sense of their robustness (to errors) and information that they carry. Also, after clustering, some of the spikes are not detected by the algorithm, which can be observed as peaks on multiples of mean firing frequency (Figure 3b).

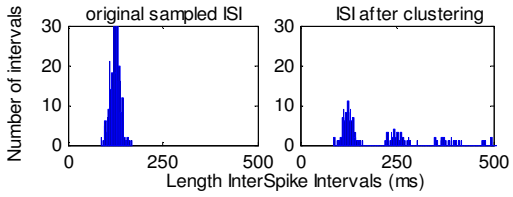

Figure 3: (a) Sampled normal distribution provides ISIs for generation of the artificial neuronal signal. (b) ISIH after clustering (using a spike sorting algorithm).

2.5 Overclustering

Sometimes the clustering outcome provides clusters with similar shapes and it remains unknown if it is a result of "overclustering" of a single neuron activity. To investigate this case and its reflection on values of parameters, the following approach was adopted. Two distributions of interspike intervals can be compared after splitting the underlying distribution. More specific, a certain percentage of the total of ISIs constructing the underlying distribution is assigned to one cluster. The second cluster consists of the remaining ISIs of the same underlying distribution. It was investigated if these separate clusters have similar enough values for certain parameters. If so, this could indicate the need to merge them.

3 RESULTS AND DISCUSSION

3.1 Parameter estimation

The goal we set was to investigate statistical parameters and their information capacity for 'low' quality clusters. More than 120 signals were examined with different timestamp distributions, different low SNRs and different spike shapes. The median was found to be a better feature to approximate the mean frequency of the underlying distribution than the extracted mean of the reconstructed distribution after clustering. Indeed, the calculated errors between the values of the median describing the two sampled models - before and after clustering - are in all of our simulations smaller compared to the errors of the mean. These errors reduce when the SNR increases, so the estimation of the mean firing frequency becomes better. An example is shown in Figure 4 for signals with different low SNRs.

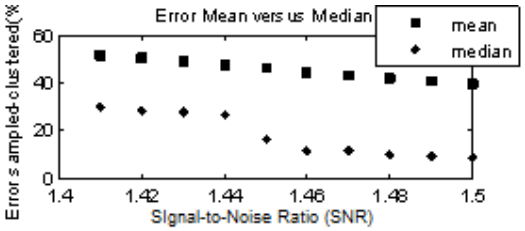

Figure 4: Error mean versus error median for signals with different low SNRs.

C_V parameter was found to be informative and reasonably accurate (some examples in Table 1). C_V>1 was reported as an indicator for bursting activity (Kostal et al., 2007). In our simulations this feature has higher values for signals with bursts, approximating 1 or higher after clustering substantiating this claim. As a consequence of low SNR many spikes are not detected, hence the change of the standard deviation and mean will lead to the large deviations of the Cv. Nevertheless, it has informative capacity indicating main features of distribution (Table 1).

Table 1: Example of two normal distributions (with and without bursts) corresponding to the two active neurons recorded in one signal. C_V is calculated for two models - before and after clustering. The second column is a repeated simulation with other values for the means of the underlying distributions.

Mean (ms)	83,33 +bursts	125,00	75,00 + bursts	133,33
Std (ms)	12,50	20,00	12,50	20,00
Cv,before_cl	0,60	0,17	0,45	0,15
Cv,after_cl	1,63	0,52	1,86	0,53

Spiking randomness indicates the variety of spiking patterns. However, it showed large and unpredictable errors, indicating little practical usefulness. The mean error was 401,67 (±403,22) %.

Burst parameters can reveal the presence or absence of bursts (Table 2). In this case both features mod_burst and `percentage window > 5 spikes' are larger than zero. On the other hand, the values for pause_ratio and pause_index are smaller than those values for signals without bursts. If all these conditions are true, even modest bursting activity of a neuron is always detected in our simulations.

Table 2: Examples of two normal timestamp distributions (with and without bursts), selected for generating an artificial signal. Bursting parameters are calculated for two models - before (b_cl) and after clustering (a_cl).

Mean (ms)	100 +bursts	108,33	91,67 + bursts	116,67
Std (ms)	12,50	20,00	12,50	20,00
Mod_burst, b_cl	0,31	0,00	0,22	0,00
Mod_burst, a_cl	0,29	0,02	0,22	0,02
Pause_index, b_cl	2,74	249,00	3,60	249,00
Pause_index_a_cl	2,22	13,93	2,57	10,79
Pause_ratio, b_cl	51,57	709,15	42,41	637,41
Pause_ratio, a_cl	27,04	79,18	21,88	55,57
Perc>5spikes, b_cl	3,96	0,00	3,30	0,00
Perc>5spikes, a_cl	3,30	0,00	2,82	0,00

3.2 Overclustering

The mean and median proved to be significantly different for the two clusters. Many missing timestamps resulted in longer interspike intervals (Figure 5), hence higher values for mean and median compared to those of the underlying distribution.

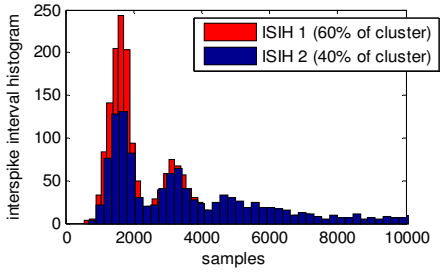

Figure 5: Two overlaid ISIHs, originally assumed as belonging to different clusters but having the same underlying distribution; dominant peaks matching almost perfectly.

Although not accurate in cases of individual clusters, skewness and kurtosis proved to be good indicators of overclustering. Values for the two clusters are similar (example in Table 3), with respectively differences of 15,61% and 18,98%. As a comparison, these differences are at least twice as large in cases of different distributions.

Fitting the ISIHs with analytical functions after clustering could be another condition to decide if the two clusters should be merged.

Table 3: To simulate overclustering, one original normal distribution (orig_distr) is randomly split up into two clusters. A certain percentage of timestamps is assigned to the first cluster and the other timestamps to the second cluster. Values for skewness and kurtosis are calculated and compared. Two examples are given.

Mean (Std) (ms)	66,67 (12.5)		66.67 (12.5)	
% cluster 1/ cluster 2	70	30	80	20
skewness, orig_distr	0.09		0.09	
skewness, after_cl	2,17	1,81	2,24	2,02
kurtosis, orig_distr	3,19		3,19	
kurtosis, after_cl	9,12	7,43	9,38	9,97

4 CONCLUSIONS

This research examined the possibility of reliable information extraction from neural clusters of bad quality. It showed that features like the mean firing frequency and burst detection can still be successfully extracted.

In the future, existing as well as newly derived parameters could be tested, possibly circumventing the problems of missed spikes and thus adding robustness and increasing the usefulness of the extracted spike trains.

These strategies could be implemented in the future as a tool that would help include previously discarded information coming from more distant neurons or signals corrupted in other ways, thus greatly increasing the possibilities for observation of brain conditioning. Initial results showed the potential for keeping the signals of lower quality while providing the trustworthy analysis, indicating the possibility of their future implementation.

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