Statistical analysis of neural spike trains for evaluation of functional differences in brain activity

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***Abstract* — In order to investigate connectivity and functioning of the brain, it is very important to have reliable processing of neural activity. Detection and clustering of single neurons' extracellular activity potentials (spikes) is a necessary precondition for this analysis. Neural spikes extracted from single channel recordings were used to determine if there is a difference between two groups of rodents: one subjected to the SIP (schedule-induced polydipsia) model, imitating obsessive-compulsive disorder or OCD, and a control (CON) group. Several statistical parameters used to describe probability density functions of spike trains are introduced. The goal of this study was to estimate the discriminatory power of these parameters. A number of them (mainly variability) showed to distinguish between these groups, whereas other commonly used ones (like mean frequency), did not show significant difference. A later pilot study we performed with a simulated signal revealed that this result is most likely connected to low robustness of some parameters to errors in detection and clustering of neuronal spikes.**

***Index Terms*—Neuronal clustering, spike trains, statistics, deep brain recordings**

I. INTRODUCTION

Local field potentials (LFPs), representing common activity of a particular tissue volume, are often used to make conclusions about the group activity of a particular brain region [1], [2]. However, the drawback of LFPs is that they treat equally tissue volume containing many neuronal cells, which themselves, can have different properties that would evade observation [3].

Alternatively, analysis of single neural cell activity and their evoked extracellular potentials (spikes) can be performed. In this way specific local properties of the neural network communication in the brain region where these neurons are located can be revealed. These properties are embedded in the statistics of spike trains.

Neural spikes (supertreshold evoked responses) are binary signals produced by neurons. Information they hold lies in the time of their occurrence. Detection of neural spikes and then clustering them by the neuron which "fired" them provides us with spike trains. We then obtain time instances of successive appearances (and corresponding interspike intervals - ISIs) for each individual spike train (cluster). Analyzing unknown distribution functions of interspike intervals (each cluster has its own distribution) with statistical parameters helps us to describe the spike trains.

In this study we test the hypothesis that the two groups of rats, one (SIP – schedule induced polydipsia), subjected to conditions resembling obsessive-compulsive disorders (OCD), and the control (CON) one, have different tendencies of individual neuronal cells' functioning, provided they are recorded in the same brain region suspected to be affected by the disorder. In order to investigate this, we analyzed several statistical parameters of the functioning patterns of these cells and we observed the alteration of their activity (as a group).

We extracted a number of parameters from spike trains, several known (variability, skewness, kurtosis, etc.) and additional, newly proposed ones. Features we extract should give us information about distribution functions of the firing of each neuron. For instance, we need to detect bursting activity – fast successive firing of the same neuron, mean firing frequency of a neuron, etc.

The new parameters were introduced because of their robustness to errors in detection and classification of spikes, as well as their information carrying potential.

Extracted parameters were used to observe global change between the groups in functioning of neurons. Although not fully understood, since single neurons are just part of global networks, combined statistics of their spike trains might be used to make conclusions about condition and functioning of the brain region where they are located.

In this paper, we introduce statistical parameters to describe neural spike trains and test the mentioned hypothesis in the OCD experiment. We then evaluate them in terms of robustness to clustering errors using simulated spike trains.

II. METHODS

Data

All recordings were performed by the medical partners with rat subjects under anesthetized conditions. Animal experiments and procedures were approved by the ethical committee of the K.U.Leuven, Belgium.

The recording intracranial depth was varied inside the marked target region itself, resulting into 5-minute recordings for each depth. The entire dataset was composed of 400 of these recordings. Filtering was adjusted to the spike detection range (500Hz-5kHz), with 24kHz sampling frequency. A Medtronic LeadPoint device and a commercial FHC electrode were used.

The third group of rats that was identified prior to recordings was the resistant (RES) one, consisting of subjects who were subjected to the OCD model [4] but their behavior was not qualified as obsessive.

Analysis

The Wave_clus algorithm [5] based on superparamagnetic clustering [6] was applied in all cases for detection and clustering of neural spikes from the recorded signals.

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The output (formed spike clusters and corresponding timestamps – time instances of appearance of each spike) was analyzed with custom Matlab code to enable extraction and analysis of the statistical parameters.

Based on visual inspection, clusters of too low quality (obvious clustering errors, see Discussion) and those where the signal-to-noise ratio (SNR) was below 1.7 (value which we derived empirically) were discarded and their statistics were not included in the study.

The SNR for each spike cluster was calculated as proposed in [7], i.e. the root-mean square (RMS) of a mean spike shape divided by the standard deviation of a signal noise estimate obtained as in [5].

A. Parameters for statistical analysis

The parameters computed on the spike trains are listed and described bellow:

- Mean frequency – total number of spikes in each cluster divided by the recording time in seconds (300*s*)
- Variability – standard deviation of ISIs divided by mean ISI [8]
- Frequency variability – newly derived parameter describing variation of the mean frequency (f) measured for each of the 5 minutes separately; Used formula: $$f_\mathrm{var} = \frac{(\max(f) - \min(f)) * 100}{\max(f)}$$
- Skewness – parameter often used to describe symmetry (or lack of it) of the observed values; e.g. for a normal distribution (fully symmetric), the skewness equals zero. Used formula: $skewness = \frac{\sum_{i=1}^{N}\left(Y_i - \overline{Y}\right)^3}{(N-1)\sigma^3}$, Y_i, $\overline{Y}$, N, σ are: observed value, the mean, number of obtained values and standard deviation of ISI respectively
- Kurtosis – measure indicating whether the data are peaked or flat relative to a normal distribution. Used formula: $kurtosis = \frac{\sum_{i=1}^{N}\left(Y_i - \overline{Y}\right)^4}{(N-1)\sigma^4}$
- Modality burst – newly derived, unitless parameter; ratio of the number of interspike intervals (ISIs) below 10ms to the number of those above 10ms
- Pause index – ratio of the number of ISIs above 50ms to the number of ISIs below; indicator of pauses in firing [9]
- Pause ratio – similar to pause index, the difference is that it adds durations of ISIs above 50ms and divides this figure by added durations of ISIs below 50ms [9]
- Variability variance – ratio of the variance of ISIs to the mean ISI; unitless
- Burst index – ratio of the number of bursting to the number of non-bursting spikes; a spike is classified as a bursting one if it appears less than 3ms after the previous one (from the same neuron)
- Burst-like behavior – newly derived parameter meant to describe fast successive firings which does not necessarily fall into the category of bursts; defined as the percentage of 100ms (non-overlapping) windows with more than 5 spikes (empirical measure)
- "f>0.8*mean" – parameter defined as the percentage of one second (non-overlapping) windows in which the firing frequency is larger than 80% of the overall mean for the particular cluster. This parameter is newly derived and should allow us to assess the stability of the neuron firing

B. Noise artifacts

The data were affected with 70Hz artifact noise of unknown origin which induced shapes largely resembling spikes. More details are given in [10]. These artifacts were often detected by the clustering algorithm as spikes.

In our semiautomatic post-processing approach, we relied on visual observation of spike clusters and their interspike interval (ISI) histograms. When the sharp peaks in those histograms (which are not biologically justifiable) appeared and/or the spikes were too narrow, the cluster was discarded as being noise.

C. Statistical analysis for group discrimination

After the parameters extraction, each spike cluster was assigned to the group of rats it originated from (SIP, RES or CON). The extracted parameters (mean frequency, variability, bursting, skewness, kurtosis, etc.) were then fed into Wilcoxon rank sum test and *p*-values were observed. All neurons from all the recordings were treated in the same way and no a priori separation criterion (same/different rat, recording depth, etc.) was applied.

Simulation study

In order to investigate the robustness of the used parameters to noise and errors induced by clustering imperfections, we used an artificial signal. We examined the error of estimated parameters compared to true values when noise level was varied. A simulated signal described in [7] and freely available online at [11] was used. This signal contained 3 spike clusters, with spike shapes shown in Figure 2, each cluster having respectively 470, 706 and 392 spikes. The duration of the signal was 100*s*. All 3 clusters in the simulation signal exhibited the same distribution of their interspike intervals (ISIs), (Poisson-like distributed) with mean value 212.9 ms and standard deviation 210.5ms.

To additionally assess discrimination power of parameters that showed lowest errors the following was done: a control sample of ISIs having a slightly different distribution was created. 500 normally distributed random samples, with the same mean value (212.9ms) but a twice smaller standard deviation (105.25 ms) were generated. This case corresponds to a more regular neuron firing at the same mean frequency.

We then compared values of variability, skewness and kurtosis (which previously showed the lowest errors) for these 2 distributions (relative comparison to the values from simulation signal).

III. RESULTS

OCD experiment

Out of the initial group of 400 5-minute recordings, signals containing no spikes were discarded. Eliminating clusters with low SNR, clustering errors, and the ones being classified as noise artifacts, left us with 392 clusters, out of which 192 originated from the recording target region. From these 29 proved to belong to SIP, 114 to RES and 53 to the CON group. Most of the kept clusters were in the SNR range of 1.7-2.2.

Table 1 shows how significantly (expressed by the *p*-values of the Wilcoxon rank sum test) the 6 parameters discriminate between the group pairs (SIP-CON, SIP-RES and RES-CON). Statistically significant group separation was observed for the parameters variability, frequency variability, skewness, kurtosis and burst-like bahaviour parameters (*p*-value being below 0.05). However, only the first three yielded *p*-values below 0.01.

Table 2 depicts mean values ± standard deviation for the same group of parameters.

TABLE I

p-values for 6 parameters: comparison with all group pairs

Parameter	SIP-CON	SIP-RES	RES-CON
variability	2.86E-08	6.43E-07	0.0617
frequency variability	1.33E-04	0.0017	0.0792
f>0.8*mean	0.0962	0.0015	0.0401
skewness	1.61E-04	0.0665	0.0042
kurtosis	0.0017	0.6209	0.0016
Burst-like index	0.0130	0.0647	0.2023

TABLE II

Group wise values (mean±standard deviation) for 6 parameters

Parameter	SIP	RES	CON
variability	0.82±0.28	1.29±0.54	1.38±0.71
frequency variability	26.64±17.60	41.53±24.57	48.58±24.64
f>0.8*mean	70.36±21.50	54.18±22.21	61.54±21.28
skewness	2.16±0.70	3.29±3.21	4.22±3.87
kurtosis	11.75±5.75	38.12±131.63	47.45±103.8
Burst-like index	2.45±6.27	1.11±3.77	1.81±3.62

Simulations

The SNR in the simulation study was varied between 1.7 and 3.7. Large and unpredictable errors were noticed for almost all the variables except variability and to some extent skewness, kurtosis and f>0.8*mean (Table 3). Mean frequency had error in the range of (0.1-65%) and showed to be unpredictable and very dependent on clustering errors. Pause index and pause ratio provided errors in the range of (1.5-over 100%) and (0.2-over 100%) respectively. It is worth mentioning that particular spike trains did not model bursting activity, and therefore those parameters could not be tested. More details are given in the Discussion section. We also found that the real SNR (calculated from the template), compared to the estimated one varied in the range (-7.85-11%).

For SNR levels below 1.6 we observed cluster merging and/or even complete misdetection.

Table 4 displays the relative difference between control normal distribution sample and ISI distribution of the simulated signal. Compared parameters are variability, skewness and kurtosis.

IV. DISCUSSION

We analyzed spike trains in order to investigate functional differences in brain activity in more detail. The Wave_clus algorithm [5] was used to extract individual spike trains. The template matching Osort algorithm [7] was also considered but Wave_clus was chosen because of its better computational efficiency and due to the fact that superparamagnetic clustering relies on comparing “closest neighbors”. This enables to properly assign even slightly different spike shapes (for instance different amplitudes) to the same cluster. This is particularly the case when having a longer recording (as in the OCD experiment) where shape differences could be induced for example by occasional electrode drift.

In Table 1 we have shown clear separation between SIP and CON but also between SIP and RES and RES and CON groups on several parameters (mostly on variability, skewness and frequency variability).

Table 2 shows that SIP compared to CON group has lower variability and skewness which points out a bigger symmetry of ISI distributions. Together with lower values of frequency variability and higher values of the “f>0.8*mean” parameter, this indicates a more regular firing tendency.

Apart from that, some “inter-group” observations (significant differences between all three groups) were noticed as well (Table 1, f>0.8*mean criteria).

In the course of our study, we encountered the following clustering problems: over and subclustering – over and underestimation of the number of neurons, and misclassification – assigning spikes to wrong clusters (often in combination with previous types of errors).

Clustering errors as such are not discussed here since we were mainly interested in the robustness of our parameters. For a more detailed study on clustering errors (with respect to SNR) for the same simulated spike train the reader is further referred to [7].

To assess how low SNR (and thus clustering errors as well) affects the study and estimated parameters, we used the simulation signal. This helped us to select the most robust features. In Table 3 we show how these parameters with lowest percentage of errors are only weakly dependent on SNR and clustering errors (even though the standard deviation is high, error limits are acceptable).

To further check how these features discriminate objectively different distributions of ISIs, we compared their values with those computed from a similar, normally distributed control sample (Table 4). It was found that they are not only robust,

but also informative since for various distributions their difference grows significantly.

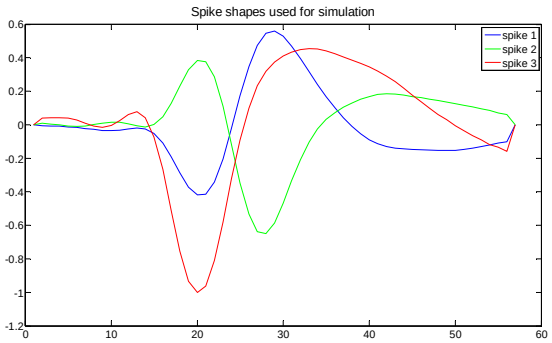


Figure 2: spike shapes

TABLE III

Percentage of errors (mean ± standard deviation) in estimating on different parameters for different SNR values of the simulated signal

Parameter	SNR range		
	1.7-2	2-3	3-3.7
variability	(2.81±2.48)%	(3.25±2.25)%	(2.82±3.22)%
skewness	(7.75±7.52)%	(12.97±10.63)%	(12.34±12.04)%
kurtosis	(16.56±14.74)%	(21.50±16.12)%	(20.96±18.80)%
f>0.8*mean	(11.43±8.60)%	(11.57±12.42)%	(7.02±3.51)%

TABLE IV

Comparison between similar spike trains' parameters

Parameter	Spiketrain 1	Spiketrain 2 (altered)	Relative difference (%)
variability	0.99	0.44	-55.56
skewness	2.09	0.20	-90.43
kurtosis	9.49	2.78	-70.71

We observed that newly derived fast firing parameters ("burst-like behavior" and "f>0.8*mean") are very informative and robust. For the simple bursting event, the probability of assigning a spike to a burst is a conditional probability of detecting and classifying correctly preceding and observed spike (in order to classify it as bursting). Contrary to that with "burst-like behavior" this comes down to probability of detecting and classifying correctly any 5 spikes in taken time window, thus increasing robustness.

The fact that we did not achieve good separation on this criterion indicates that, in this case, bursting events are not good indicators of the OCD condition that we are inducing.

We observed from the simulation signal as well as our study, that similarity of mean spike shapes affects the quality of clustering greatly, making solely SNR based criteria for data evaluation insufficient. Hence, apart from a high SNR the observed cluster must be sufficiently different from the other clusters to enable a reliable cluster analysis.

V. Conclusion

We have shown that it is possible to distinguish and to detect a brain tendency indicating the disorder (in this case OCD), by observing single neuron cells and their activity. However, the final conclusions on the validity of the study are yet to be made by clinicians.

Several features (variability, skewness, kurtosis, "burst-like behavior") extracted from distribution of ISIs, showed to be robust to errors even at low SNR, and thus are recommended by authors.

On the other hand, however, some of the well established parameters, like pause index, pause ratio and even mean firing frequency, become very unreliable when the signal is of low SNR quality. Furthermore, when the observed signal contains similar spike shapes coming from different neurons, errors in clustering increase and lead to errors in observed parameters.

The open question remains: what is the minimal number of neurons to analyze in order to be able to make a reliable conclusion about altered activity of observed brain region. We intend to address this problem in one of our future studies. Based on our insight in this paper, we suggest including neural spike analysis can offer more accurate insight than only observing the LFPs.

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