# Spike manager: a new tool for spontaneous and evoked neuronal networks activity characterization

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**Abstract**

Recent developments in the neuroengineering field and the widespread use of Micro Electrode Arrays (MEAs) for electrophysiological systems led to new investigation approaches in the study of large neuronal networks dynamics, both in in-vivo and in in-vitro conditions.

In spite of these new possibilities there is still lack of commercially available software tools that can help in the management and analysis of large amount of data coming from experimental sessions.

A new software tool, built on a Matlab© environment, was developed with the aim to offer a valuable help to the neuroscientific community for processing the electrophysiological signals.

In this paper we present the developed software tools and some examples of real applications related to spontaneous as well as evoked (i.e., electrically stimulated) electrophysiological neuronal network activity.

## 1. Introduction

During the last decade (Palsson, 2000; Fields, 2001), the progress in the neuroengineering field led to the possibility to develop new electrophysiological systems for studying simultaneous recorded activity of large neuronal networks. Microelectrode Arrays (MEAs), on which cells cultures can be grown and kept alive for a long time (from weeks, up to several months), are valuable devices to define distinct collective functional states of the network and to study the dynamics of a neuronal population (Gross et al., 1991; Jimbo and Kawana, 1992).

Despite the widespread use of such electrophysiological systems, there is a lack of commercially available tools for data analysis and visualization, easy to use and compatible within these emerging recording techniques. As appropriately pointed-out by others (Potter, 2001; Nicolelis, 2001), the lack of valuable software tools for on-line and off-line analysis of multichannels data is one of the major bottlenecks for a more rapid advance in this new field of neuroscience. Recently a small number of scientists have been started developing home made tool capable to analyze multi electrode recorded data (Egert et al., 2002).

Taking advantage from the long experience gained by our laboratory in managing and processing data coming from MEAs (Bove et al, 1997; Chiappalone et al., 2001) a new processing tool, named *Spike Manager* has been made up to analyze multi-channel signals obtained from multi-site electrophysiological recordings of neuronal population. The key point of our software is to produce a detailed statistical analysis of the electrophysiological recordings in a simple and fast way, allowing the management and the storage of large amount of data.

## 2. Experimental setup

The widespread use of Multi Electrode Arrays (MEAs) among neuroscientists promoted the birth of commercial systems based on this technology. One of the mainly utilized commercial system - more than fifty laboratories around the world are equipped with that - is the MCSystem (Multi Channel System, Reutlingen, Germany), adopted also in our laboratories. It allows to record the neuronal activity from up to 60 channels, with the possibility to stimulate the network through 8 electrodes simultaneously.

The data presented here refer to experiments performed with dissociated primary cultures of cortical neurons extracted from embryonic rats (E-18). Neurons were plated on the arrays, pre-coated with adhesion promoting molecules (Poly-D-lysine and laminin), and kept alive in healthy conditions (37 °C, 5% $CO_2$). After a week we started to record the electrophysiological activity (Fig. 1) to characterize the spontaneous behavior of the neuronal network just formed, then we used electrical pulses to modify it and to study the evoked activity.

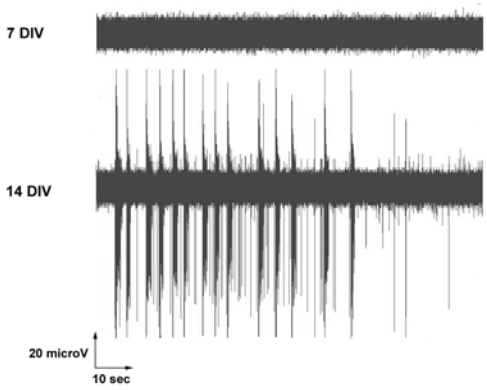


Fig. 1. Example of spontaneous activity (7 and 14 DIV) recorded from one electrode of the array.

## 3. Software development

The software tool *Spike Manager*, was developed using the Matlab© platform that provides several mathematical libraries, based on matrix structures, very useful to manage the data streams recorded from the MEA-system. We developed a software package that provides a set of functions and graphic tools for multi-channel acquired data, building an user-friendly interface and a modular open-structure easy to be updated with new functionalities.

### *3.1 Matlab© - available format conversion*

Considering the widespread use of the MCSystem, our software tool includes a reliable and automatic routine for the conversion of multichannels binary data (proprietary format) into Matlab© data type. The MCRack software, included in MCSystem, stores the recorded data on a single multiplexed file that reaches very high dimensions (about 400 Mbytes for five minutes of recording).

The Spike Manager can handle electrophysiological signals recorded with MCRack thanks to the MCSteream.dll, a dynamic link library that supports MCSystem data type file access. It was used as the core of an external tool, developed in Visual Basic environment, to convert the multiplexed data format file into N binary files (where $N \leq 60$ is the number of recording channels) containing signals recorded from a single electrode each. An algorithm named *managedat* converts the binary files into Matlab© data type using mono-dimensional matrices containing the samples of the signals. These functionalities make files ready to be processed in order to extract significant parameters.

### *3.2 Signal processing*

The study and extraction of relevant parameters from neural populations involve long-term measurements (from minutes to hours); therefore several Gigabytes of data are the result of a single trial experiment: we usually record more than one file for each experimental phase. *Spike Manager* was thought to reduce this huge amount of data, without loosing the peculiar information, that is stored in the spike timing and in the delay between two consecutive spikes. In this framework, with the aim of studying the development and the synchronization of the network, the first step in the analysis process consists in the detection of spikes (single or organized in bursts) characterizing the electrophysiological activity.

#### *3.2.1 Peak Detection*

To detect the presence of spikes, as already reported in the literature (Perkel et al., 1967) an algorithm based on a peak-to-peak threshold was developed. The threshold value is calculated as a multiple of the standard deviation (7*STD) of the biological noise (Jimbo et al., 1999; DeMarse et al., 2001), sampled from each electrode during the spontaneous activity phase. A window, sized to handle at most a single spike, is shifted over the signal; when the difference between the maximum and the minimum in the current window matches the threshold criterion, the corresponding spike time is stored. Setting only two parameters (window length and peak-to-peak threshold) the described algorithm follows the biological variability of the signal and extracts relevant features useful for further

processing procedures. In order to estimate the network activity level, the algorithm extracts the mean firing rate. The spike occurrences and the mean firing rate values are stored in files saved as *sparse matrix*.

### *3.2.2 Burst detection*

Besides spiking activity, cortical neuronal networks show also bursting behaviors. To focus the attention on this firing mode, parameters, such as the bursting frequency and the burst length, are extracted to characterize the network. For this aim two different approaches for the burst detection are proposed: one based on the timing and the amplitude of spikes, one on the wavelet transformation.

In the first algorithm, a burst is found when the amplitudes (peak-to-peak values) sum of spikes, handled by the mobile window, is over a threshold set, in order to ignore isolated action potentials. This method identifies both bursts composed of many "weak" spikes, and bursts of few "strong" spikes.

The second method is an innovative approach based on the auto-similarity or fractal property of the recorded signals. Self-similarity is a common property of many natural phenomena, explained by the simple following relationship: given the temporal process X(nT) (a continuous process X(t) sampled at T), if the aggregated process satisfies the following property

$$Z \approx a^{-H} X \text{ with } Z(mK) = \sum_{n=mK}^{(m+1)K} X(nT)$$

then the process X(nT) can be defined as *strictly self similar*, where $H$ is the Hurst parameter containing the degree of auto-similarity of the process ( $H \in [0.1]$, $H = 1$ completely auto - similar process ).

Observing that the fractal-likeness of the signal increases during the "bursting" active state, it is possible to characterize the burst activity by evaluating a simple estimator E, based on wavelet decomposition, of the Hurst parameter H in a variable step window.

The algorithm consists in the following steps:

1. Storage of the samples;
2. Windowing of the samples through variable lasting windows;
3. H estimation over these windows with the wavelet estimator;
4. Generation of the temporary estimator E;
5. Thresholding over E;
6. Variable step window sliding.

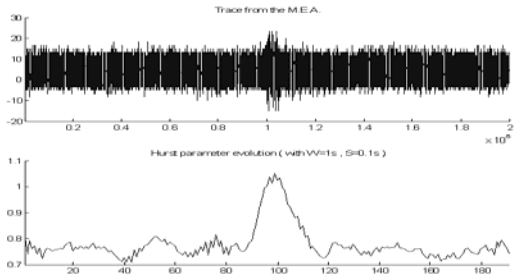


Figure 2: Recorded signal and Hurst parameter estimation

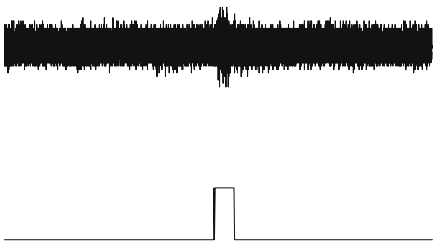

Figure 3: Recorded signal and Burst detection

Figure 2,3 show the result of the algorithm applied on a raw signal: it is important to remark that the approach rejects automatically the noise through aggregation, being noise a chaotic process. The analysis is robust to the presence of noise in the signals, being the white noise typically short long dependence (his correlation is almost zero). The estimator is expressed by the following:

$$\hat{H} = \frac{1+M}{2};$$

with: $$\log_2\left(\frac{1}{n_j}\sum_k \left|d_x(j,k)^2\right|\right) = \log_2\left(\mathrm{var}\left(d_j\right)\right) = Mj + C$$

where $d_x(j,k)$ is the **k**-th coefficient of the detail of level **j**-th derived from the wavelet decomposition of the signal. This first evaluation produces an estimate of H for each temporal window. Then the algorithm evaluates a second estimator introduced to simplify the thresholding operation, expressed by:

$$E = \frac{1}{(\max H - H) + \left(\frac{1}{\max est}\right)}$$

where *max(H)* is to be considered 1 and *max est* is the maximum allowed value for E. A burst is founded when the amplitudes of estimator E is over a threshold.

### *3.2.3 Statistical tools*

To study the synchronization level of the network activity or to detect significant changes due to external electrical stimulations, algorithms to implement classical statistical functions were written.

Regards the spike activity mode available algorithms allow to estimate the synchronization with the Inter Spike Interval Histogram (ISIH), the autocorrelation function with the Joint ISI Histogram (JISIH) and the conditional cross-correlation function with the Cross ISI Histogram (CISIH).

Changing the time scale, it is possible to extract similar information from the burst activity mode using the Inter Burst Interval Histogram (IBIH), the Cross IBIH and the Joint IBIH.

The Spike Manager was developed to offer useful displaying utilities where Matlab© environment provides great availability of graphic functionalities and this is very useful to help the dynamics interpretation. In this framework the simplest utility consists in the showing recorded raw data. Once displayed on the GUI, various options are available to analyze the electrophysiological activity focusing attention both on global behavior (range of seconds), and on a selected and zoomed window of neuronal signal (up to few milliseconds).

### *3.2.4 Post Stimulus Time Histogram (PSTH)*

Besides the study of the network development, that requires spontaneous activity recordings performed at different DIVs, MEA allows to build experimental protocols using electrical stimulation where the electrodes are the input channels to communicate with the neurons.

Stimuli consist of biphasic current or voltage pulses and the response of the network is studied analyzing the PSTH (Rieke et al., 1997) algorithm results in the close proximity to delivered pulses.

Stimulus artifacts are detected automatically through a similar criterion used in spike detection, where the threshold magnitude is increased to match the delivered pulses instead of action potentials. Details of the PSTH histogram are defined by setting the maximal time to handle after the artifact and the bin width.

The analysis of a waste amount of data, recorded in several different phases from all the 60 MEA electrodes, was automated through sequential processing logical step. In this way the Spike Manager is able to drastically reduce the time spent for data processing and makes results available so fast that can be even reused to drive the next phases of the experimental session.

## 4. Experimental preliminary results

By using commercially available stimulator systems, it is possible to investigate whether in the network we can find embedded distinct neural synaptic pathways or, more generally, if the electrophysiological activity can be modulated by stimulating the network from different sites. Preliminary experiments were performed using an electrical stimulation protocol according to which seventy bipolar pulses (amplitude ± 2.5 V, semi duration 100ms) are delivered asynchronously in about 5 minutes.

Thanks to the developed software it is easy and fast to extract several parameters in order to characterize the network dynamic. In this context our attention was focused on the analysis of the Inter Spike Interval (ISI), the Inter Burst Interval (IBI), the mean duration of bursts and of the Post Stimulus Time Histogram (PSTH).

Induced changes in the neuronal network activity are identified as changes of these parameters among the different experimental phases.

Examples of PSTHs calculated by signals recorded from different electrodes are shown (Fig. 4). Events occurring in the 600 milliseconds after the stimulus are used to build PSTHs. Bins are set at 3 milliseconds, in order to catch at most one spike in the selected window.

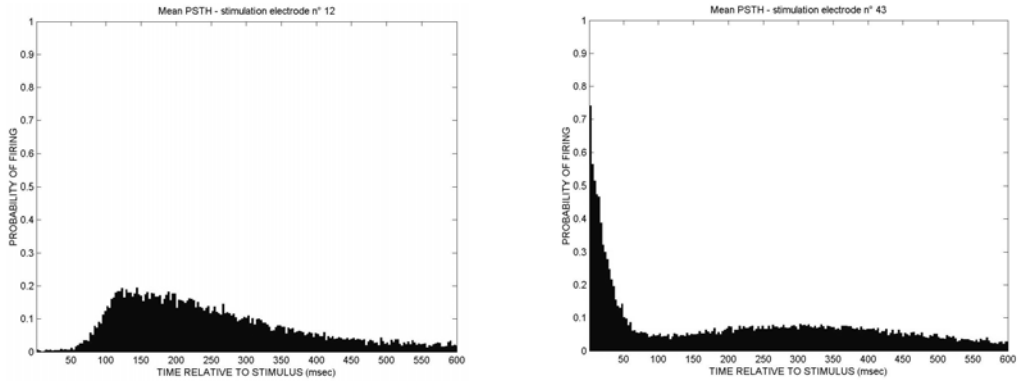


Fig.4: The graphs show the PSTHs built using the signals recorded from all the electrodes after stimulation from electrodes 12 and 43. The example shows how PSTH shape is modulated simply changing the stimulation site.

Figure 4 (on the left) shows PSTH with a delayed response, after about 100 ms, which consists of a bursting activity lasting about 300ms; it could be interpreted as the propagation of the electrically - induced activity from the stimulating site to the entire network. By changing the stimulation site, the early response together with the delayed one are observed in the same recording electrodes (Fig. 4, on the right). In other words, in all the recording electrodes is found the same behavior, which could be modulated simply by changing the stimulation site. These results, obtained by ad hoc algorithms (such as the proposed PSTH), show how different network pathways can be used in neurons activation and signal propagation.

Our next goal is to tune the transmission capability of these neural pathways by using stimulation protocols aimed at modifying the synaptic connections among the neurons of the network, such as in the induction of long term phenomena (LTP and LTD). Monitoring the extracted parameters, in subsequent experimental sessions from the same culture, we can try to estimate the occurrence of phenomena of adaptation and plastic changes (Shahaf and Marom, 2002).

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