

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

A. Vato¹, L. Bonzano¹, M. Chiappalone¹, S. Cicero², F. Morabito², A. Novellino¹, G. Stillo²

¹ Neuroengineering and Bio-nano Technologies- NBT, Department of Biophysical and Electronic Engineering-DIBE, University of Genoa, Italy

² Telecom Italia- Learning Services S.P.A., Rome, Italy

Abstract

Recent developments in the neuroengineering field and the widespread use of Micro Electrode Arrays (MEAs) for electrophysiological investigations led to new 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 several experimental sessions. A new software tool, built in Matlab[®] environment, was developed with the aim to offer a valuable help to the neuroscientific community for processing multi-channel electrophysiological signals.

In this paper we present the developed software tool and some examples of real applications on spontaneous as well as evoked (i.e., electrically stimulated) electrophysiological neuronal network activity from cortical cultures.

Keywords: neural networks; multi-electrode array; wavelet decomposition; spike detection; burst detection

1. Introduction

During the last decade [7,13], 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 [10,17].

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 [15,18], 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 tools capable to analyze multi electrode recorded data [6].

Taking advantage from the long experience gained by our group in managing and processing data coming from MEAs [3,4,12] a new processing tool, named *Spike Manager* has been developed to analyze multi-channel signals obtained from multi-site electrophysiological recordings of neuronal population. The key point of our software is to realize 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 MCSsystem (Multi Channel System, Reutlingen, Germany, www.multichannelsystems.com), 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 and independently.

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% CO₂). After a week we started

to record the electrophysiological activity (Fig. 1) trying to characterize the spontaneous behavior of the just formed neuronal network, then we delivered electrical pulses to modify it and to study the evoked activity.

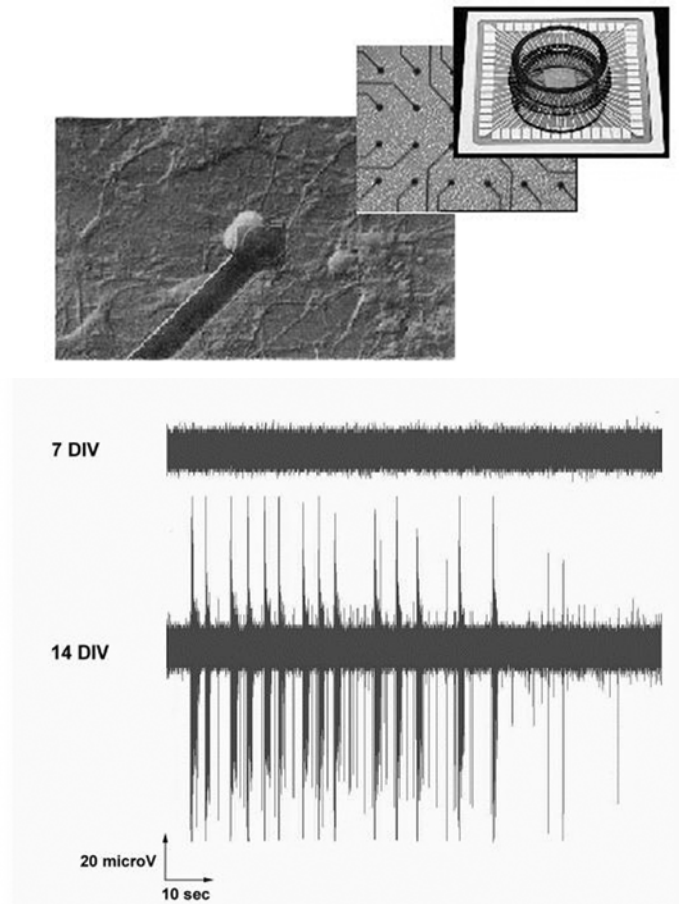


Fig. 1. Cortical neural network grown on a MEA (Multi Electrode Array) (top) and an example of spontaneous activity (7 and 14 DIV) recorded from one electrode of the array (bottom).

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 MCSsystem, 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 MCSsystem, 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 MCStream.dll, a dynamic link library that supports MCSsystem 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 losing 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 [14] an algorithm based on a peak-to-peak threshold was developed. The threshold value is calculated as a multiple of the standard deviation ($7 \cdot \text{STD}$) of the biological noise [11,5], 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. By setting only two parameters (spikes latency 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, while the tool is performing the spike detection, the mean firing rate is computed. 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 behaviours (see the Fig.3 top panel). To focus the attention on this network firing mode, parameters, such as the bursting frequency and the burst length, are extracted. To this end, two different approaches for the burst detection are proposed: one based on the timing and the amplitude of spikes, one based on the wavelet transformation.

In the former algorithm, a burst is found when the sum of spikes amplitudes (peak-to-peak values), handled by the inspecting mobile window, is over a noise 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.

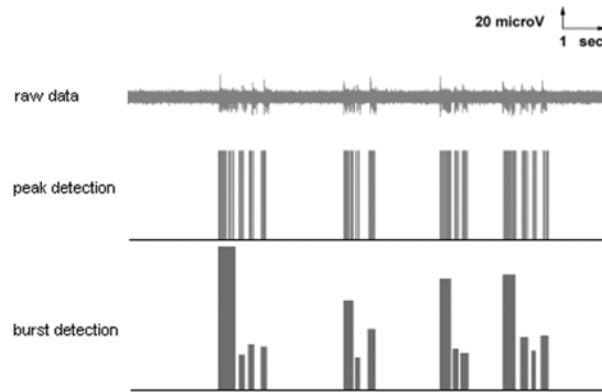


Fig. 2. An example of result of the peak detection and the burst detection algorithm.

The latter method is an innovative approach based on the auto-similarity or fractal property of the recorded signals [8]. 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 [2,9], 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.

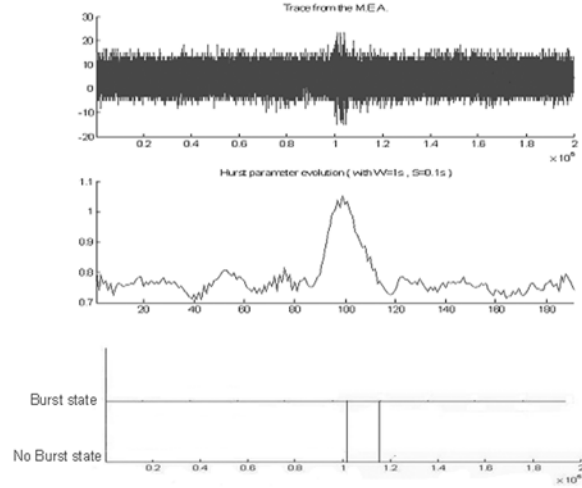


Fig. 3. (top) Stream of data (20 sec) showing a typical burst pattern, (middle) Hurst parameter estimation and (bottom) result of the Burst Detection

Figure 3 shows 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:

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

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

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 and to detect significant changes due to delivered external electrical stimulations, algorithms to implement classical statistical functions were written.

Available classical algorithms, 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) allow to estimate the synchronization concerning the spike activity.

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 algorithm [1] 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. Preliminary experimental 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 was easy and fast to extract several parameters in order to characterize network dynamics. 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 sessions.

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.

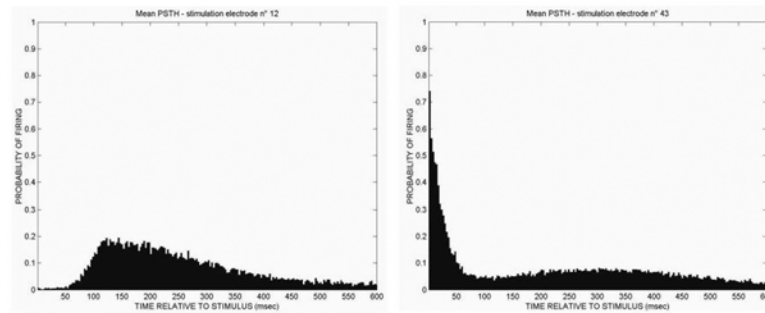


Fig.4: The graphs show the PSTHs built using the signals recorded from all the electrodes after stimulation from electrodes 12 and 43. The example shows how PSTH shape is modulated simply changing the stimulation site.

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 [16].

Acknowledgments

Bonzano's Ph.D. fellowship is sponsored by Telecom Italia S.P.A. Novellino's Ph.D. fellowship is sponsored by eTT s.r.l. (<http://www.ettolutions.com>). This work was supported by the NeuroBit project IST-2001-33564, "A bioartificial brain with an artificial body: training a cultured neural tissue to support the purposive of an artificial body".

References

- [1] F. Rieke, D. Warland, R. de Ruyter van Stevenick, W. Bialek, *Spikes: Exploring the Neural Code*, The MIT Press (Cambridge, Massachusetts 1997).
- [2] P. Abry, D. Veitch, Wavelet Analysis of Long-Range-Dependent Traffic, *Trans. on Information Theory*, 44 (1998).
- [3] M. Bove, M. Grattarola, G. Verreschi, In vitro 2D networks of neurons characterized by processing the signals recorded with a planar microtransducer array, *IEEE Trans. Biomed. Eng.*, 44 (1997) 964 – 977.
- [4] M. Chiappalone, A. Vato, M.T. Tedesco, M. Marcoli, F. Davide, S. Martinoia, Networks of neurons coupled to microelectrode arrays: a neuronal sensory system for pharmacological applications, *Biosensors & bioelectronics* 18 (2003) 627-634.
- [5] T.B. DeMarse, D.A. Wagenaar, A.W. Blau, S.M. Potter, The Neurally Controlled Animat: Biological Brains Acting with Simulated Bodies, *Autonomous Robots* 11 (2001) 305-310.
- [6] U. Egert, Th. Knott, C. Schwarz, M. Nawrot, A. Brandt, S. Rotter, M. Diesmann, MEA-Tools: an open source toolbox for the analysis of multielectrode-data with Matlab, *J. Neurosc. Methods* 117 (2002) 33-42.
- [7] S. Fields, The interplay of biology and technology, *Proc. Acad. Sci.* 98 (2001) 10051-10054.
- [8] W.E. Leland, M.S. Taqqu, W. Willinger, D.V. Wilson, On the Self-Similar Nature of Ethernet Traffic (Extended Version), *Transactions on Networking*, 2 (1994).
- [9] S.G. Mallat, A Theory for Multiresolution signal decomposition: the Wavelet Representation, *Transactions on pattern analysis and machine intelligence*, 11 (1989).
- [10] Y. Jimbo, A. Kawana, Electrical stimulation and recording from cultured neurons using a planar array, *Bioelectrochem. Bioeng.*, 40 (1992) 193-204.
- [11] Y. Jimbo, T. Tateno, H. P. C. Robinson, Simultaneous Induction of Pathway-Specific Potentiation and Depression in Networks of Cortical Neurons, *Biophysical Journal*, 76 (1999) 670-678.
- [12] A. Novellino, M. Chiappalone, A. Vato, M. Bove, M.T. Tedesco, S. Martinoia, Behaviors from an electrically stimulated spinal cord neuronal network cultured on microelectrode arrays, *Neurocomputing*, 52-54 (2003) 661-669.
- [13] B. Palsson, The challenges of in silico biology, *Nature Biotechnology*, 18 (2000) 1147-1150.
- [14] D.H. Perkel, G.L. Gerstein, G.P. Moore, Neuronal Spike train and stochastic point processes, *Biophysical Journal*, 7 (1967) 391-418.
- [15] S. M. Potter, Distributed processing in cultured neuronal networks, *Progress In Brain Research*, 130 (2001) 49-62.
- [16] G. Shahaf, S. Marom, Learning in Networks of Cortical Neurons, *The Journal of Neuroscience*, 21 (2001) 8782-8788.
- [17] G.W. Gross, J.M. Kowalski, Experimental and theoretical analyses of random networks dynamics, In P. Antognetti, V. Milutinovic, ed., *Neural Networks, Concepts, Application and Implementation*, Vol. 4. (Prentice Hall, NJ, 1991) 47-110.
- [18] M.A.L. Nicolelis, Advances in Neural Population Coding, in *Progress in Brain Research*, ed., MAL editor, Vol. 130 (2001) 49-62.