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Investigating neuronal activity by SPYCODE multi-channel data analyzer

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ABSTRACT

Multi-channel acquisition from neuronal networks, either *in vivo* or *in vitro*, is becoming a standard in modern neuroscience in order to infer how cell assemblies communicate. In spite of the large diffusion of micro-electrode-array-based systems, researchers usually find it difficult to manage the huge quantity of data routinely recorded during the experimental sessions. In fact, many of the available open-source toolboxes still lack two fundamental requirements for treating multi-channel recordings: (i) a rich repertoire of algorithms for extracting information both at a single channel and at the whole network level; (ii) the capability of autonomously repeating the same set of computational operations to 'multiple' recording streams (also from different experiments) and without a manual intervention. The software package we are proposing, named SPYCODE, was mainly developed to respond to the above constraints and generally to offer the scientific community a 'smart' tool for multi-channel data processing.

1. Introduction

The brain is undoubtedly the most fascinating but still mysterious machine of the known universe. The Nobel laureate Santiago Ramon Y. Cajal used to say that 'the brain is a world consisting of a number of unexplored continents and great stretches of unknown territory'. After more than one hundred years, his sentence remains true but the progress in technological research makes modern neuroscientists optimistic about the possibility to open an access towards those 'unexplored' territories. Among others, recent advancements in micro-fabrication technologies enabled the introduction of devices for multi-channel recordings (i.e. Micro-Electrode Arrays, MEAs), giving the capability of investigating neural interdependency and computational properties of dynamically interacting cell assemblies (Hebb, 1949; Miller & Wilson, 2008; Quiroga & Panzeri, 2009).

Thanks to the pioneering work by Gross (Gross, Azzazy, Wu, & Rhodes, 1995; Gross, Rhoades, & Jordan, 1992; Gross, Rieske, Kreutzberg, & Meyer, 1977), who first demonstrated the possibility to use neuronal cultures coupled to MEAs as a cell-based biosensor, MEA-based devices are now a well-accepted electrophysiological technique for both *in vivo* (Blanche, Spacek, Hetke, & Swindale, 2005; Buzsaki, 2004) and *in vitro* (Egert, Heck, & Aertsen, 2002; Minerbi et al., 2009; Schneidman, Berry, Segev, & Bialek, 2006) measurements. Tens of micro-electrodes permanently in contact with electrogenic cells allow monitoring the electrophysiological activity of a cell population (i.e., multi-units recordings) for long periods of time (Bologna et al., 2010; Chiappalone, Bove, Vato, Tedesco, & Martinoia, 2006; Wagenaar, Pine, & Potter, 2006). Such a system represents a perfect candidate to routinely record and evaluate the patterns of spontaneous as well as stimulated network behavior (Bakkum, Chao, & Potter, 2008; Chiappalone, Massobrio, & Martinoia, 2008; Marom & Shahaf, 2002).

MEA-based systems are currently available on the market (Multi Channel Systems, Reutlingen, Germany; Panasonic, Osaka, Japan; Ayanda Biosystems, Lausanne, Switzerland; Plexon, Dallas TX, USA; Axion Biosystems, Atlanta, GA, USA) and find applications in many research fields, such as neuroscience, pharmacology, cardiac electrophysiology, neurorobotics and bidirectional brain machine interfaces (Nicolelis, 2003). Notwithstanding the widespread use of this technique, there is still a lack of efficient software to manage a large amount of electrophysiological data produced by such multi-site recordings (Nicolelis, 2001; Potter, 2001). Especially in the case of neuropharmacological experiments, tens of gigabytes of data are daily produced in order to characterize the response to specific drugs or to test the effects of unknown ones (Gramowski et al., 2006; Gramowski, Jugelt, Weiss, & Gross, 2004).

Among the general-purpose commercially available processing tools, often sold together with the acquisition system (e.g.

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MC_Rack by Multi Channel Systems, NeuroExplorer and Offline Sorter by Plexon, Conductor and Mobius by Alphamed), there are still no automated tools suitable to manage and support such a large amount of data and with the possibility to easily extend their functionalities. In other terms, no chance is given to the user to operate, speedily, a multi-channel analysis on more than one recorded stream at a time. Additionally, commercial software tends to have only limited possibilities to incorporate new tools or modify existing ones. For these reasons, new software tools for massive data management and signal processing have to be developed. Recently a number of scientists have started developing custom-made tools capable to analyze multi-electrode recorded data, such as Mea Tools (Egert, Knott et al., 2002), MeaBench (Wagenaar, DeMarse, & Potter, 2005), FIND (Meier, Egert, Aertsen, & Nawrot, 2008), BSMART (Cui, Xu, Bressler, Ding, & Liang, 2008). Unfortunately the cited tools do not provide the users with a large number of algorithms for data analysis and they are not able to manage massive quantities of data at a time. These are the main motivations that induced us to develop a new and innovative software package, named SPYCODE which aims at overcoming such limitations. SPYCODE provides a working environment able to perform efficient data management and processing since it incorporates a very rich repertoire of standard and advanced signal analysis tools. Furthermore, it includes the novel analyses published by our group in recent years (Chiappalone, Vato, Berdondini, Koudelka-Hep, & Martinoia, 2007; Garofalo, Nieus, Massobrio, & Martinoia, 2009; Maccione et al., 2009; Pasquale, Martinoia, & Chiappalone, 2009; Pasquale, Massobrio, Bologna, Chiappalone, & Martinoia, 2008). A few examples of "unconventional" analysis are given by information theory methods, extraction of connectivity maps, self-adapting burst and network burst detection. In the following, we will present the functionalities of our software and an example of application to data recorded from cortical cultures during a neuropharmacological study.

2. Methods

In this section we present a concise review of the theoretical background of the signal processing algorithms implemented in SPYCODE.

2.1. Spike analysis

2.1.1. Spike detection

Spike timing is the first information to extract from raw data. Since typical signal to noise ratios are much larger than one, the most used method to identify the spikes is a threshold-based algorithm (Maeda, Robinson, & Kawana, 1995) resulting in a point process (e.g. spike train, ST) in which each element represents the position in time of a spike.

$$ST(t) = \sum_{s=1}^{N} \delta(t - t_s). \tag{1}$$

Eq. (1) reports the formal definition of a spike train, where t_s is the timing of a spike, N is the number of recognized spikes and $\delta(t)$ is the Kronecker delta function.

After obtaining the spike trains and before proceeding with further analysis, it is important to take into consideration the issue of stationarity, which plays a central role when dealing with neuronal signals. Indeed, experiments performed through non-implantable MEAs (either involving slices or dissociated cultures) generally do not present the non-stationarity typical of in vivo experiments, neither when spontaneous activity is recorded nor stimulation protocols are applied. Hence, the main assumption upon which SPYCODE is based and so data analyzed is the stationarity of recorded neuronal signals, at least for specific time intervals. Furthermore, the possibility to split or join recordings into chunks of desired time length (cf. Section 3.1.1) and the fine tuning of analysis parameters can help detecting possible non-stationary "anomalous" activity periods (e.g. occurring after moving MEAs devices (Wagenaar et al., 2006)) and discard them from the analysis. In order to perform the latter selection, and depending on individual user's needs, also additional Matlab toolbox explicitly dealing with stationarity analysis (e.g. GARCH toolbox, the Mathworks) can be used.

2.1.2. Firing rate

Once spikes have been identified, the easiest and most direct way to characterize the level of activity of a cell is computing its Firing Rate (FR). According to Adrian's definition (Adrian, 1928), the firing rate is the number of spikes in a rather large time window and it can be measured from just one representation of the neural activity, as follows (Eq. (2)):

$$\mathrm{FR} = \frac{\int_0^T \left(\sum_{s=1}^{N} \delta(t - t_s) \right) \mathrm{d}t}{T} = \frac{N}{T} \tag{2}$$

with T representing the duration of the recording and N the number of spikes occurring at time t_s. If we count the spikes in a small window of size Δt, centered at $(t_a - t_b)/2$ and we divide by the bin width, we compute the Instantaneous Firing Rate (IFR) (Rieke, Warland, de Ruyter van Steveninck, & Bialek, 1997).

Dealing with MEAs, tens of channels are likely to be involved during an experimental session. For this reason, it is useful to compute the FR or the IFR of the whole culture and see how it changes given the delivered stimulation. These quantities are simply obtained by computing the FR and the IFR of each single channel and then averaging among all the active electrodes of the MEA, obtaining the Mean Firing Rate (MFR) and the Average Firing Rate (AFR) of the network.

2.1.3. Inter-spike interval histogram

The ISI distribution is the probability density of time intervals between consecutive spikes and it is a useful statistics for describing spike trains (Dayan & Abbott, 2001). The formula to calculate the ISI histogram is reported below (Tam & Gross, 1994):

$$\mathrm{ISI}(\tau) = \sum_{s=1}^{N-1} \delta(t_{s+1} - t_s - \tau) \tag{3}$$

where τ represents the ISI. As reported by the literature (Perkel, Gerstein, & Moore, 1967), for finite samples of data, such as the observed neuronal spike trains, the ISI histogram (ISIH) serve as an estimator of the actual probability density function. Different shapes of the ISIH give an estimate about the synchronization of the neural network, and each shape denotes a set of timing with shared properties. For this reason, the ISIH can provide a detailed way to classify the dynamic pattern of neurons, e.g. 'spiking' or 'bursting', since bursting neurons usually display "bimodal" ISI histograms (Cocater-Zilgien & Delcomyn, 1992; Tateno, Kawana, & Jimbo, 2002). However, it has been recently demonstrated (Selinger, Kulagina, O'Shaughnessy, Ma, & Pancrazio, 2007) that plotting histograms of logarithmic ISI instead of linear ISI can be useful in better discriminating between intra-burst and inter-burst intervals (Pasquale et al., 2009). For the above reason, the possibility to compute and plot logISIH has been implemented within SPYCODE.

2.2. Burst analysis

2.2.1. Burst detection

Highly non-uniform spike timing, or spontaneous bursting, is observed in a wide range of in vitro neuronal preparations and developing organisms (Ben-Ari, 2001). Non-uniformity is present at different time scales (Corner, 2008; Wagenaar et al., 2006) and as