Statistical long-term correlations in dissociated cortical neuron recordings

Federico Esposti, Student Member, IEEE, Maria G. Signorini, Steve M. Potter, Sergio Cerutti, Senior Member, IEEE

Abstract—The study of nonlinear long-term correlations in neuronal signals is a central topic for advanced neuronal signal processing. In particular, the existence of long-term correlations in neuronal signals recorded via multi-electrode array (MEA) could provide interesting information about changes in inter-neuron communications. In this study we propose a new method for long-term correlation analysis of neuronal burst activity based on the periodogram and a slope estimation of the MEA signal. We applied our method to recordings taken from cultured networks of dissociated rat cortical neurons. We show the effectiveness of the method in analyzing the activity changes as well as the temporal dynamics that take place during the development of such cultures. Results demonstrate that the α parameter is able to divide the network development in three well-defined stages, showing pronounced variations in the long-term correlation among bursts.

Index Terms—Long-term correlations, MEA, periodogram

I. INTRODUCTION

In last decade the mammalian cortex has been deeply studied in vitro in the form of dissociated neuro-glial cultures implanted on multi-electrode arrays (MEA) [1-10]. One of the fundamental features of neuronal networks that is often investigated through MEA devices is “spontaneous activity” [11-13]. It refers to the activity that the network shows in absence of any external stimulation. It is well-known that this behavior, also reported in in-vivo studies, is characterized by periodic synchronization episodes, usually called bursts (as reported, e.g., in [14-17]). Moreover it was observed that both single electrode [18] and multi-electrode [19-21] neuronal recordings exhibit non-linear long-term characteristics [22].

The study of nonlinear long-term correlations in neuronal signals is a central topic for advanced MEA signal processing. In particular, the existence of long-term correlations (LTC) in such a signal could provide interesting information about changes in inter-neuron communications (e.g. LTP or LTD) correlated with the administration of neuroactive drugs or with the development of the network itself.

In this study we propose a method based on a calculation of α coefficient in the power-law fitting of the experimental data in long-term correlation analysis of neuronal burst activity of dissociated cortical neurons studied through MEA recordings. Further, we study the changes in the LTC behavior induced by the network development.

II. MATERIALS AND METHODS

A. Cell cultures, MEA recordings and spike sorting

In the present study we employed MEA data collected and described by Potter and colleagues in [17]. In particular, we employed 12 thirty-minute-long recordings classified as “dense” and 12 classified as “small” in [17]. In brief, these recordings were performed on cultures of E18 rat cortical neurons (plus glia) with a density of 2.5 ± 1.5 × 10³ cells/mm² and 1.6 ± 0.6 × 10³ cells/mm² at the first day in vitro (div), respectively, recorded longitudinally from the 6th to the 35th div with 59 electrode MEAs with a diameter of 30 μm, purchased from MultiChannel Systems® (Reutlingen, Germany). The electrodes were organized in a square grid with the corners missing, spaced 200 μm center-to-center. Spike sorting was performed with the MEABench software [23], using a threshold based detector. Spikes were detected as upward or downward excursions beyond 4.5*(estimated RMS noise), as described in [17]; in the same reference, details about dissections, cultures, recordings and spike detection, may be found.

B. Analysis procedure

As expected, the recordings showed spontaneous activity characterized by periodic synchronization episodes, usually called population bursts. In order to approach an intra-burst analysis, we proposed and employed a frequency-based burst...
detector algorithm. Fig.1 represents how the algorithm works: all MEA channels are merged forming a single series, showing on the x axis the recording time and on the y axis the spiking activity of all channels. Along this series the starting point of a burst is detected comparing the merged series with a frequency threshold fixed at 10 Hz. Frequency is computed as the inverse of the inter-spike interval (ISI) of the merged series. The reference duration of a burst is chosen to be 200ms. Such a parameters were chosen by training the algorithm on the experimental dataset. 10Hz demonstrated as the most performing frequency threshold for the burst detection algorithm and 200ms was the average maximal length of the burst we detected in the recordings.

![REAL SPIKE SERIES](image1)

![MERGED SPIKE SERIES](image2)

![SPIKING FREQUENCY](image3)

Fig. 1. Graphical representation of the burst detection algorithm (for simplicity three channels only are represented in figure).

Some controls are implemented in order to verify the presence of a real burst. First, the number of channels involved has to be more than the 15% of the total channel number. A channel is considered as “firing” if it spikes at least three times in the 200ms time window. Moreover, two detected bursts can not be closer than 600ms.

Due to the above procedure we extracted the time instants in which bursts took place in the recordings. The application of complex nonlinear signal processing methods for long-term correlation estimation requires one to work with one-dimensional data. As a matter of fact, most nonlinear signal processing methods are designed for one-dimensional data only. For this reason we processed MEA recordings (in particular, bursting epochs of the MEA raster plots, extracted thanks to the burst detection algorithm) in order to obtain 1D signals. We applied the Space Amplitude Transform (SAT), originally introduced in our paper \[24\], in order to perform such a conversion.

The Space-Amplitude Transform, \( A(s(\cdot), r) \), is a geometric transform that executes a projection from a 2D domain set \( s(x,y,t) \), e.g. the usual Raster plot, to a 1D image set \( I(r,t) \), exploiting an Arrangement table \( r \) as Fig.2 describes in detail.

In the domain set \( s(x,y,t) \), i.e. in the Raster plot, a spike is coded in terms of 0 or 1 event. Each spike is characterized by spatiotemporal coordinates \((x,y,t)\); \((x,y)\) correspond to a specific MEA channel and \((t)\) identifies the time instant.

The \( A(s(\cdot), r) \) transform codes the MEA channels thanks to the arrangement table \( r \), that is a “correspondence table” in which different amplitude values are univocally assigned to each channel of \( s(x,y,t) \). In this way, an amplitude value corresponds to just one channel, and vice versa. The output of \( A(s(\cdot), r) \) is a lookup table in which an amplitude value is assigned to each spike of the raster plot, as function of the channel in which the spike takes place. Finally, a 1D signal \( I(r,t) \) is created; this signal combines the amplitude information that derives from the above lookup table and the temporal information that is preserved from the starting raster plot \( s(x,y,t) \).

![Graphical example of the role of the arrangement algorithm \( r \) and the Space-Amplitude Transform method, \( A(s(\cdot), r) \), at work with a 9 channel raster plot. The transform joins together the raster plot, \( s(x,y,t) \), and the arrangement vector, \( r \), creating a 1D signal, \( I(r,t) \), by exploiting an arrangement table \( r \) as Fig.2 describes in detail.](image4)

\[
A(s(\cdot), r) \leftrightarrow s(x,y,t)
\]

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Thanks to the above procedure we extracted the time instants in which bursts took place in the recordings. The application of complex nonlinear signal processing methods for long-term correlation estimation requires one to work with one-dimensional data. As a matter of fact, most nonlinear signal processing methods are designed for one-dimensional data only. For this reason we processed MEA recordings (in particular, bursting epochs of the MEA raster plots, extracted thanks to the burst detection algorithm) in order to obtain 1D signals. We applied the Space Amplitude Transform (SAT), originally introduced in our paper [24], in order to perform such a conversion.
The final output of the transformation, \(I(r,t)\), is a representation of the spatial and temporal activity of the whole network (Fig.3). In this process, no spatial or temporal information is lost with respect to the starting raster plot. The possibility to completely reconstruct the topological structure of the activity is granted by the biunivocal correspondence of the Space-Amplitude transform.

The method allows to approach an intrinsically 2D plus time dataset, i.e., the time recording deriving from a 2D electrode array, as a 1D plus time signal. This transform speeds up and makes simpler the data analysis allowing, as said, the application of non-linear signal processing techniques.

C. Long-term correlation estimation

A signal displaying a power law spectral density near the origin is called “one-over-\(f\)” noise. Such signals are commonly observed in many different systems, including physical, biological, physiological, economic, technological and sociological ones [22]. In particular, many classical observations reported these kind of dynamics in neurophysiological signals, such as [18, 25-29]. When \(P(f)\sim 1/f^\alpha\), with \(P(f)\) the signal power spectral density (PSD), for \(f\to 0\) and some \(\alpha >0\), it is often possible to define some sort of generalized correlation function (such as, e.g., the autocorrelation function) which is found to decay very slowly (hyperbolically).

The slow decay signifies that the current value of the series is affected not only by its most recent values but also by its past values. For this reason, such processes are often referred to as “long-memory” or “long-range dependence” processes [22]. In this specific case, we are interested in evaluating the presence of long-term correlations in the global activity of the neuronal network. The SAT signal, representing with no distortion the spatial and temporal information of the raster plot, is a very good candidate in showing long-term correlations.

As a matter of fact, other works report, while employing different methods, the presence of such a dynamics in neurophysiologic recordings (see, e.g., [19, 20, 30]).

Fig.4 summarizes the different physical properties that long-term correlated signals exhibit as a function of the \(\alpha\) slope value their PSD assumes. \(\alpha\) near 0 indicates the presence of a stationary process with zero correlation, i.e. a white noise. An \(\alpha\) between 0 and 1 indicates a long-range positively correlated stationary signal. An \(\alpha\) between 1 and 2 indicates a long-range negatively correlated non-stationary signal and, finally, an \(\alpha\) between 2 and 3 indicates a long-range positively correlated non-stationary signal. If \(\alpha = 2\), the process is classified as Brownian motion.

We recall that, both white noise and Brownian motion signals are characterized by zero correlations among samples, while white noise is a stationary signal (hence produces oscillations around a fix mean value), Brownian motion is a non-stationary signal, resulting in strong trends. One of the simplest method for the \(\alpha\) estimation is the log-periodogram regression, or simply, the periodogram analysis [22, 31, 32]. This method estimates \(\alpha\) as the linear slope of the periodogram (a discrete Fourier transform spectrum), in a log-log plot, close to the zero frequency axis. As a matter of fact, being \(P(f)\sim 1/f^\alpha\), for \(f\to 0\) and some \(\alpha >0\), \(\log(P(f))\approx -\alpha \log(f)\).

Fig.5 shows an example of the \(\alpha\) coefficient estimation in the periodogram of a burst taken from the experimental dataset (estimated on \(N = 400\) points). The range of periodogram plot regression was chosen in the two lowest decades of frequencies (2·10\(^{-4}\) - 10\(^2\) Hz), i.e., considering samples occurring from one to every 200ms to one to every 10ms, considering a sampling resolution of 0.1ms. These values were chosen by considering as minimal frequency value (1 event over 200ms) the maximal window length considered for a single burst (see Analysis procedure) and, as minimal value (1 event over 10ms), a signal window that, on average, could contain at least 25 spikes (evaluated on the experimental dataset).

![Example of the projection obtained by the Space Amplitude Transform (SAT) on a real Burst.](image)

Fig.3. Example of the projection obtained by the Space Amplitude Transform (SAT) on a real Burst. (a) The raster plot of a real burst displayed thanks to the NeuroExplorer® commercial software (Next Technologies, MA, USA). On the x axis is reported the absolute time of the recording (seconds); on the y axis are listed the channel names. (b) The 1D signal resulting from the SAT (displayed in Matlab®). The Mathworks, MA, USA). On the x axis is reported the frame number of the burst (being 0-time the burst onset and 0.1ms the inter-frame time, hence, e.g., 200 = 20ms); on the y axis is the amplitude of the obtained SAT signal (arbitrary chosen scale).
We applied the above described methods (burst detection, SAT and Periodogram analysis) to 12 “dense” and 12 “small” longitudinal recordings from [17], ranging from the 6th to the 35th div. Each recording contained hundreds of bursts.

III. RESULTS

We obtained 12 plots for each one of the two datasets (“dense” and “small”) showing, on the y axis, the mean $\alpha$ value ($\pm$ std) of all bursts vs. the number of days in vitro (on the x axis). All plots showed a similar trend, exemplified in Fig.6. The average trends, obtained as averaging of all the 12 cultures for the two populations, are reported in Tab.1. We found that the $\alpha$ coefficient is close to 0.5 in the first two weeks in vitro (wiv) (indicating a positive long-term correlation in a non-stationary time series). $\alpha$ suddenly grows to 1.5 circa around the 17th div ($\pm$ 2div) and then irregularly grows till 2 in the following 20 days. The same trend is advisable in both the datasets.

![Graphical representation of the physical meaning of the $\alpha$ parameter.](image)

Fig.4. Graphical representation of the physical meaning of the $\alpha$ parameter.

IV. DISCUSSION

The behavior of the $\alpha$ exponent across the culture development can be divided in three stages, which are well represented in Fig.6. The first stage essentially coincides with the first two wiv. In this period $\alpha$ is $\leq$ 0.5, representing a condition in which the SAT signal is stationary (over the 200ms long burst time window) and presents a positive correlation. It is known that in the first one or two wiv the network is poorly bursting and the spiking activity can be classified as “global”, i.e., bursts usually involve almost the whole neuronal network [17, 33-35].

The presence of slow trends in the SAT-transformed signal that derives from the global structure of the activity, accounts for this topological characteristic.

The second stage is represented by a sudden change in the $\alpha$ exponent around the 17th div. As known from literature, in a period comprised between the 6th and the 18th div, the network reorganizes its synaptic connectivity through a process called “pruning” [17, 34, 36, 37]. Through this remodeling, the network sacrifices useless synapses and optimizes neuronal links. At the same time, from the spiking activity viewpoint, the network begins to produce a higher number of bursts characterized by a localized activity (i.e., bursts involve a limited number of neurons). This is a typical sign of the creation of sub-networks [34, 37]. In this stage, $\alpha$ suddenly raises to a value close to 1.5 and remains at this value for 2 – 8 div. Such an $\alpha$ indicates the presence of a non-stationary signal with a strong negative correlation.

![Example of $\alpha$ estimation results from a single 24-div burst Periodogram.](image)

Fig.5. Example of $\alpha$ estimation from a single 24-div burst Periodogram. The straight line represents the interpolation used for the estimation of the $\alpha$ slope parameter. (Periodogram estimated on N=400 samples from 2x10$^{-4}$-1x10$^{-2}$Hz). The same estimation is performed for all the bursts present in each one of the recordings.

The presence of localized activity produces pronounced discontinuities in the SAT signal, confirming the signal as a non-stationary negatively correlated.

![An example of $\alpha$ estimation results in a longitudinal “dense” rat cortical neuronal MEA recording.](image)

Fig.6. An example of $\alpha$ estimation results in a longitudinal “dense” rat cortical neuronal MEA recording. The x axis represents the number of day in-vitro (div); the y axis shows the corresponding $\alpha$ value. The scatter bars represent the $\alpha$ variations (mean+std) among all the bursts that take place in a recording at a specific div.

The last stage involves (at least) the 15 div following the previous stage. This period is characterized by a slow $\alpha$
increment toward $\alpha = 2$. This condition corresponds to a non-stationary signal that exhibits a trend similar to a Brownian motion. This state testifies an energetically stable system and this is probably the reason why the activity in form of random oscillations can be observed in neuronal sub-networks [17].

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<th>18</th>
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<td>1.681</td>
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<td></td>
<td>±0.335</td>
<td>±0.230</td>
<td>±0.264</td>
<td>±0.268</td>
<td>±0.153</td>
<td>±0.213</td>
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Tab. 1. Average $\alpha$ trend, represented div by div, obtained as averaging of all the 12 “dense” cultures (top row) and the 12 “small” cultures (bottom row): avg ± std.

These observations are supported by the morphological and temporal considerations obtained through the direct observation of regular bursts [17]. The same results were obtained by analyzing the less dense cultures classified as “small” in [17].

As it can be seen by comparing the presented results with literature, e.g., [19, 38], the proposed simple method it is able to obtain an estimation of the $\alpha$ value identical to the one proposed by that authors ($\alpha \approx 1.5$ for a mature network) but using a very limited burst population.

The request of a very small number of bursts allowed us to obtain an $\alpha$ estimation also for the scarcely-bursting immature networks (less than 8 div) that is precluded to other methods. This allowed to observe, for the first time, the sudden $\alpha$ value increment which is typical of numerous events that involve the whole neuronal network. The future development of our work is to show the usefulness of this kind of approach in analyzing other aspects of neuronal

Finally, we point out that the kind of analysis we employed in this context seems to be able to highlight activity modifications that involve the whole neuronal network. The future development of our work is to show the usefulness of this kind of approach in analyzing other aspects of neuronal

V. CONCLUSIONS

The results presented in this work suggest a simple method for the analysis of the statistical characteristics of neuronal network activity. This approach has the advantage to use a well-standardised signal processing methods, such as periodogram, for finally obtaining a power-law fitting with the experimental data from the whole-network-activity. Previously, the whole-network-activity nonlinear analyses required the creation of ad hoc laborious and less repeatable methods.

Concerning the biological results, we were able to divide the network development in three stages. A first stage (between the 6th and the 16th div circa) was characterized by a signal stationarity together with a positive long-term correlation. The second stage, corresponding to the network pruning, was characterized by a sudden $\alpha$ increment which indicates the non-stationarity of the signal endowed with a long-term correlation behavior characterized by negative correlations. This could be probably the consequence of sub-networks creation, which is typical of the development of self-assembling neuronal networks. Finally, network maturity was accompanied by a progressive $\alpha$ increment toward $\alpha \approx 2$, i.e. toward a Brownian motion-like process. In general, this analysis suggests that networks spontaneously produce long-term correlated activity; a sudden transition between white-noise-like behavior and Brownian-motion-like behavior was noticed, while avoiding the energetically unstable condition $\alpha = 1$, typical of numerous in-vivo recordings.

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MEA data described in [17].

REFERENCES


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