Characterization of Spontaneous Electrical Activity Arising from Inactive Neuronal Cultures

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Abstract — This article analyses electrical signals of neuronal cultures from which measurements taken by multielectrode arrays (MEAs) present very low amplitudes and spike rates, so that no connections among neurons and microelectrodes seem to be established. Such information may be useful to characterize MEA instrumentation noise, which influences subsequent signal processing associated with spike and burst detection. A simple estimator of the probability density function of the signal amplitude is calculated, pointing out that this random variable is Gaussian. This conclusion is also attested by normality tests, as well as by high-order moment analysis. Average values for the mean and variance of the noise amplitude are provided.

Keywords – probability function estimation; multielectrode array; gaussianity.

I. INTRODUCTION

One of the most relevant frontiers of the research on Brain-Machine Interfaces aims at the development of neural prosthesis for clinical applications, regarding pathologies of the central nervous system [1,2]. For example, one could point out epilepsy, which involves the anomalous and synchronized activity of large groups of neurons within the human cortex [3]. In consequence of previous discussions, several efforts are currently deployed in order to develop implantable neural prosthesis that are capable of communicating in a bidirectional way with the cortex [4,5]. Although several problems still remain unsolved, the literature presents interesting results in terms of some prototypes applied to *in-vivo* experiments. For example, in [6], the authors describe basic principles of implantable prosthesis, reporting fruitful preliminary results of electrostimulation on animal models, by means of nanotechnological devices such as Microelectrode Arrays (MEAs).

Signals from neuronal cultures are composed of basic unities called "spikes" [7]. Since the classical analysis of spikes [7] does not take into account the biological noise segments [5], then it leads to a loss of biological information. In fact, although current neurophysiological knowledge focus on spikes as the source of the most important biological information [5], one should not forget that biological noise plays a very important role, as pointed out by several works devoted to its analysis within the nervous system [8]. In addition, it should be pointed out that the first operation of any signal processing applied to MEA data correspond to spike detection, which is subsequently used to generate the Interspike-Interval Time Series (ISI), to perform burst analysis and to estimate associated histograms. Such detection is mainly influenced by instrumentation noise, which is a quite complex issue in the context of extracellular recordings, due to its several possible sources [9].

Classical spike signal processing [7] always supposes the gaussianity of this noise, particularly for intracellular data, in order to enable simple mathematical treatment. However, very few works in the literature [9,10] are focused on the background noise disturbing extracellular recordings. In [10], authors provide a simple illustration of the noise gaussianity based on real signals, supposing this hypothesis for deriving a new spike detection technique, as well as its variance within the range (0.06 – 0.08) $(\mu V)^2$. Article [9] considered the noise variance within the range (0.286 – 0.091) $(\mu V)^2$ for testing a new proposition, without formal justification for this choice.

This article is devoted to analyze background noise, which is experimentally studied by means of the concept of "inactive neuronal culture" , to be discussed below.

The underlying process leading to functional connections between cultured neurons and microelectrodes is not completely clear [11]. Just after the tissue deposition on the planar MEA surface, the ensemble is stored in an incubator. In general, eight or

ten days after the deposition, which are labeled as 8thDIV ("Day-In-Vitro") and 10thDIV respectively, the culture is connected to the acquisition system, in order to check if functional or anatomical connections between the cells and the microelectrodes have taken place. In this case, signal amplitudes may attain at least 100 μV during spike activity, firing rates are at least greater than 0.1 spikes/second [12], and the culture is called "active". Otherwise, it is called "inactive".

To the best of authors' knowledge, few works in the literature are devoted to culture inactivation, with the exception of [12], which presents some remarks and discussions, without experiments. In general, signals from these cultures are not considered as "useful information", being not processed by researchers in the field.

II. MATERIALS AND METHODS

A. Signal Acquisition

Extracellular electrophysiological signals were recorded by means of a planar sixty-electrode MEA system (*MultichannelSystems, Reutlingen, Germany*). Dissociated primary cultures of cortical neurons were prepared after tissue extraction from eighteen-day rat embryos, which were previously anesthetized. All procedures required by Genova University Animal Ethics Commission were employed to ensure the necessary care towards the rats. Further details regarding culture preparation are presented in [13]. MEA microelectrodes are distributed in an 8x8 array, each of them presenting a diameter of 30 μm and separated by 200 μm from each other. Microelectrodes located in all four external edges of the device were not active. Sampling frequency was 10 kHz.

Two neuronal cultures were monitored, and they are identified by C358 and C359. As long as it was possible, one complete experiment of each culture was performed during a twenty-minute recording time. In brief, our database is composed of fifteen data files or sessions, each of them lasting five minutes. Culture inactivation was "spontaneous" in the sense that no special biological or external procedure was performed to assure such condition.

B. Probability Density Function Estimation

The "naïf estimator" [14] supposes that a random process *X*(n) is divided up into Q amplitude intervals $\Delta x(n)$, which will be called "amplitude resolution"; n denoting the discrete time. X(n) represents the signal recorded at one microelectrode (or channel) of the MEA. The random process is stationary during a short time slot of L*T seconds, where L is the total amount of signal samples within this slot, and T is the sampling period. Then, the probability of the amplitude taking values within the interval $\Delta x(n)$ may be estimated by its relative frequency throughout the time slot under analysis as:

$$P\ (x(n) \le X(n) \le x(n) + \Delta x(n)) \cong q_X(n) / L \quad (1)$$

Wherein $P\ (x(n) \le X(n) \le x(n) + \Delta x(n))$ is the probability of the random variable X(n) lay within the interval of $\left(x(n) + \Delta x(n)\right)$ and $q_{X(n)}$ is the number of times that the signal amplitude X(n) takes values within the range $\left(x(n) + \Delta x(n)\right)$ μV, during the analogue time interval (0,L.T) seconds.

Consider vector $\boldsymbol{x}_k$ containing samples of the extracellular electrical activity amplitudes [μV] of one single channel or microelectrode k (k = 1,2,…60), during five minutes. In order to cope with nonstationary issues, each vector $\boldsymbol{x}_k$ was then divided into 300 segments, each of them lasting one second. In fact, previous results achieved by classical spike-detection techniques [4,7] suggest that these segments should not be longer than 1-2 seconds. For each segment, a simple estimation of its histogram was performed according to (1). The average statistical behaviour of one channel was assessed, by means of averaging all its 300 histograms, which leads to the "Single-Channel Single-Session Histogram" (SCSSH). This same procedure was repeated for all 60 channels of the session, thus generating 60 SCSSHs.

The previous paragraph described a procedure applied to data associated with one single session (five-minute recording). Since the database is composed of fifteen sessions, all steps were repeated for each digital file. Finally, by averaging all these results, it was possible to generate one single histogram for each MEA channel, which will be called "Single-Channel All-Session Histogram" (SCASH). The last one finally characterizes the average statistical behaviour of one single channel, considering all the fifteen sessions. Another possible feature is the "All-Channel All-Session Histogram" (ACASH), which is obtained by averaging the sixty SCASHs, thus providing an overall statistical behaviour of the signal, considering the whole set of microelectrodes throughout all the fifteen recording sessions.

C. High-Order Moments of Particular Channels (SCASHs)

High-order moments associated with the SCASH of one single channel were calculated according to the definitions presented below [14].

$$\hat{M}_{1X} \cong \sum_{i=1}^{Q} x_i p(x_i);\ Q = 30 \quad (2)$$

$$\hat{\sigma}^2 = \hat{M}_{2X} \cong \sum_{i=1}^{Q} \left(x_i - \hat{M}_{1X}\right)^2 p(x_i);\ Q = 30 \quad (3)$$

$$\hat{M}_{jX} \cong \sum_{i=1}^{Q} \left(x_i - \hat{M}_{1X}\right)^j p(x_i);\ Q = 30;\ j = 3,4 \quad (4)$$

Wherein $p(x_i)$ represents the probability of the signal amplitude attains the value x_i .

In addition, the following statistical quantities were also estimated.

$$S_k = \hat{M}_{3X} / \hat{\sigma}^3 \; ; \; K = (\hat{M}_{4X} / \hat{\sigma}^4) - 3 \quad (5)$$

Where Sk is the "skewness" of the SCASH [14], which characterizes the symmetry of the estimated probability density with respect to its mean value; and K is the "kurtosis" of the SCASH, providing information on the general aspect of the density plot, as it is compared to the normal distribution. If Sk = 0, then the random variable may be considered completely symmetric. If K = 0, the probability density is indeed Gaussian. For K < 0, the histogram is called "platykurtic", otherwise, for K > 0, the histogram is called "leptokurtic".

III. RESULTS AND DISCUSSION

A. *Probability Density of all Channels*

The "All-Channel All-Session Histograms" (ACASHs) of cultures C358 and C359 were estimated. Fig. 1 presents the average histogram obtained from these two ones, depicting the overall statistical behavior of the whole set of signals from sixty microelectrodes, throughout all the fifteen recording sessions. Clearly, the plot resembles a Gaussian distribution.

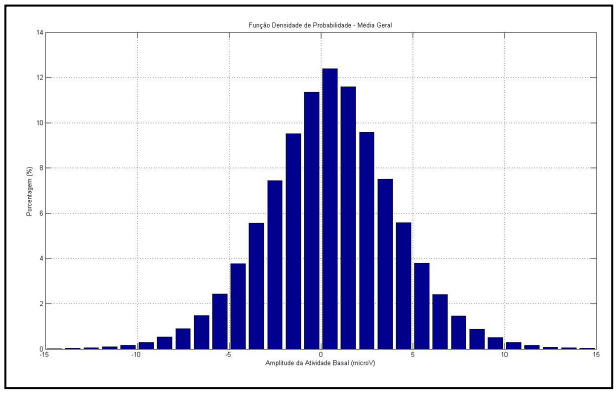

Figure 1. Histogram obtained after averaging the ACASH of C358 and the ACASH of C359. Vertical axis is expressed in percentage [%], whereas horizontal axis is associated to the instrumentation noise amplitude.

Table I presents estimated values for the mean and variance of the final histogram in Fig. 1.

TABLE I. GAUSSIAN-DISTRIBUTION PARAMETER ESTIMATION, CONSIDERING HISTOGRAM OF FIG. 1

Parameter	*Estimate*	*95%-Confidence Interval*
Mean value $\hat{M}_{1X}$ [μV]	0,497	(0,497; 0,567)
Variance $\hat{\sigma}^2[(\mu V)^2]$	12,711	(12,366; 13,070)

Since values in Table 1 approach the zero-mean Gaussian, Shapiro-Wilk statistical test was also performed for both the mean value and for the variance, supposing the following hypothesis: $H_0 : \hat{M}_{1X} = 0$ and $H_1 : \hat{\sigma}^2 = 1(\mu V)^2$. Both hypothese were rejected with 5% of significance, thus pointing out that the general density of Fig 1 is not associated with a zero-mean and unitary-variance Gaussian distribution.

B. *High-Order Moment Analysis and Particular Channel Behaviour*

Analysis of the sixty SCASHs by means of equations (2)-(5) was performed, and results are presented in Fig. 2 and Table II.

Fig. 2 depicts the overall variance of each MEA channel respectively, considering the average performed on all fifteen sessions. The upper part of Fig. 2 represents the bottom view of the planar MEA device, wherein little square subdivisions are associated with the spatial location of particular microelectrodes. Variance amplitudes are depicted in color scales. The second part (lower one) introduces the color scales used for amplitude representation, and the scale ranges are written in the figure title.

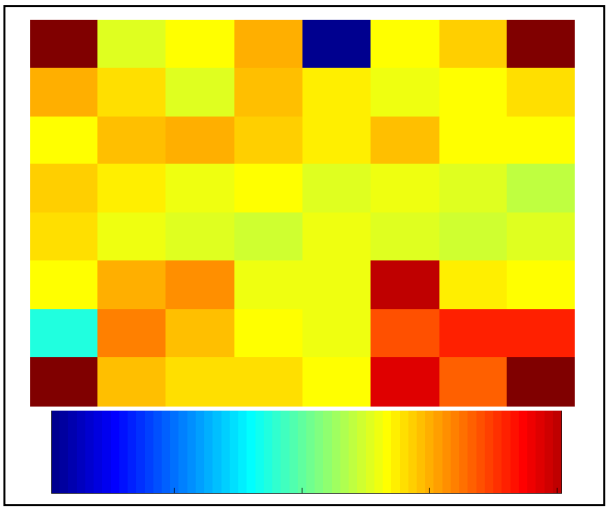

Figure 2. Variance amplitudes $\hat{\sigma}^2$ for all channels. Scale ranges 1.94 $(\mu V)^2$ for dark blue ; 17.74 $(\mu V)^2$ for dark red.

Fig. 2 provides information on the particular statistical behaviour of each channel. In fact, it points out that the variance attains quite different amplitudes as one moves little distances within the device surface. Such amplitudes are high, specially for those microelectrodes located at the right lower part.

TABLE II. ESTIMATION OF THE MEAN-VALUE AND THE VARIANCE FOR EACH HIGH-ORDER MOMENT ASSOCIATED WITH SCASHS, CALCULATED CONSIDERING THE AVERAGE PERFORMDED ON ALL SIXTY MEA CHANNELS

	Mean-value $\hat{M}_{1X}$	*Variance* $\hat{\sigma}^2$	*Skewness Sk*	*Kurtosis K*
Mean value	0,4981	12,6993	-0,0213	0.3903
Variance	$9{,}4300\times10^{-5}$	3,9151	$5{,}152\times10^{-4}$	0,0374

Conclusions of the previous paragraph may be clearly attested by the third column of Table II, that presents the mean-value and the variance of the several moments, after averaging all the characteristic parameters over the sixty channels. Notice that the mean-value, the skewness and the kurtosis present a quite low variance, which means that they do not vary too much throughout all the channels. In addition, notice also that amplitudes of the mean-value, the skewness and the kurtosis are very low.

In consequence, major differences between the statistical behaviors of the MEA data are due to different signal powers (variances) at the microelectrodes. In addition, skewness and kurtosis present very low average amplitudes, which clearly characterizes the gaussianity of the random variable under analysis. This conclusion also attests the previous results of Table 1.

IV. Conclusion and Future Work

This article analyzed two topics very few studied in the literature: inactive cultures and MEA instrumentation noise. Signals arising from these cultures were considered as a possible experimental framework in order to develop a statistical analysis of instrumentation noise. It should be pointed out that only absolute-amplitude signals have been processed, since it is not possible to derive the interspike-interval time series, due to very low signal amplitudes and spiking frequency. In this context, the random character of this stochastic process was supposed to be tied to the signal amplitudes. Particular attention was devoted to the nonstationary issues that are instrinic to biological signals. For this purpose, data segmentation was performed based on a fixed-length strategy, followed by the application of simple theoretical tools, such as the "naïf estimator" and high-order moments for analyzing the probability density function.

From the point of view established in the previous paragraph, it was possible to conclude that the MEA instrumentation noise follows indeed a Gaussian distribution, which was attested by visual inspection, and from high-order moment analysis. This distribution, as an overall trend for all the sixty channels and recording sessions, presents an average mean of 0.497 μV and an average power of $\hat{\sigma}^2 = 12.711(\mu V)^2$. When these experimental results are compared to those generally used in the literature devoted to spike-detection techniques and MEA signal simulation [9,10,15], discussed in Section 1, one could ask whether the last ones are realistic, since our results point out that noise is neither zero-mean, nor uncorrelated. In addition, its variance is much higher than values currently used in the literature. Regarding the statistical behavior of the several MEA channels, they present almost no differences in terms of mean values, kurtosis and skewness. In consequence, variance is the major statistical characteristic of MEA instrumentation noise at different microelectrodes, which is specially high for channels at the right lower part of the device.

This article presents an experimental confirmation of a hypothesis that is used in papers related to extracellular recording simulation [9,10,15], which devotes very few attention to the noise and to the nonstationary issues associated with biological signals under analysis. The results are useful for deriving more accurate statistical models for MEA instrumentation noise, so that to contribute for the development of more efficient spike-detection methods, as well as for the synthetic generation of MEA data. However, care should be taken regarding the statistical analysis performed in this paper. Histogram averaging, although necessary due to the signal nonstationarity, may lead itself to gaussianity, in view of the Central Limit theorem. In addition, as future work, more significant statistical tools such as adherence tests should be applied to single segments of the signals under analysis, in order to provide a more rigorous assesment of the signal gaussianity.

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