Burst detection algorithms for the analysis of spatio-temporal patterns in cortical networks of neurons

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Abstract

Cortical neurons extracted from the developing Central Nervous System are spontaneously active and, after a few days in culture, display a typical electrophysiological pattern ranging from stochastic spiking to organized bursting. Using microelectrode arrays (MEA), on which dissociated cultures can be grown for long-term measurements, we recorded the electrophysiological activity of cortical networks during development, in order to monitor their responses at different stages of the maturation process.

Employing new algorithms for burst analysis and statistical procedures we were able to extract relevant parameters useful for describing the neural dynamics changes at different stages of the developmental process.

Keywords: Neuronal networks, Microelectrode array, Burst Detection, Network Burst

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1. Introduction

Experimental investigation of the electrophysiological behavior shown by neural ensembles is a fundamental step towards understanding how the brain works. Dissociated cortical networks maintained *in-vitro* represent a reduced neurobiological system where the strategies employed by the nervous system to represent and process information can be approached and basic physiological mechanisms can be quantitatively characterized [10, 15].

Taking advantage of the potentiality offered by the use of substrate-embedded multielectrode-array technology [2, 3, 10], experiments on cortical preparations over arrays of 60 planar microelectrodes were performed in order to monitor the electrophysiological properties of the neural preparation both from spatial and temporal point of view [6, 11]. Investigations in these relatively large neural populations (i.e. from 10^4 – 10^5 cells) usually focus on the identification that an action potential (i.e. a spike) has occurred at a given time in a

given area of the network, since neural cells are supposed to store information in the spike rate or in the spike timing. However this is not enough, at a different level of analysis, the information is also spread in the collective dynamics, the so called "bursting" behavior [7, 16, 17]. It was suggested [1] that synchronized activity plays an important role in the strategy followed by the developing brains to finally reach an active condition that possesses a highly diversified range of electrical signals and billions of selective synapses. For these reasons we are convinced that burst analysis is one of the main issue to address the problem of characterizing the spontaneous neural dynamics.

2. Materials and Methods

2.1. Cell Culture and Electrophysiological Recordings

Neuronal cultures were taken from cerebral cortices of embryonic Wistar rats at embryonic day 18 (E18). The cerebral cortex was dissociated using Trypsin .Cells were plated on a 60-channels Micro Electrode Arrays - MEAs (MultichannelSystems, Reutlingen, Germany), pre-coated with adhesion promoting molecules (Poly-D-Lysine and Laminin), at the final density of $6\text{-}8\cdot10^4$ cells/device and maintained in Neurobasal medium (Sigma) supplemented with 2% B-27 and 1% Glutamax-I. Measurements were carried out in physiological medium (NaCl 150 mM, $CaCl_2$ 1.3 mM, $MgCl_2$ 0.7 mM, KCl 2.8 mM, Glucose 10 mM, HEPES buffer 10 mM) at 5 different stages of the network maturation process: 7, 14, 21, 28 and 35 Days In Vitro (DIV).

The electrophysiological signals (Fig. 1) were recorded using a standard commercially available experimental set-up for extracellular measurements (MultichannelSystems). Each channel was sampled at a frequency of 10kHz.

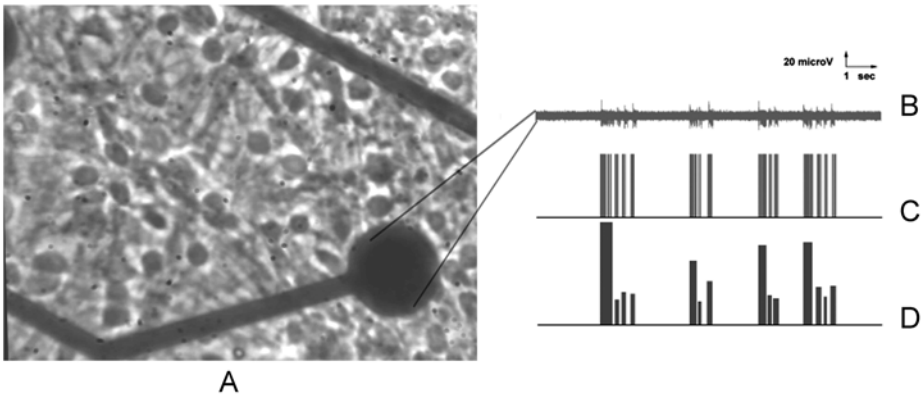

Fig. 1. (A) Dissociated cortical neurons on MEA surrounding a planar microelectrode (30 μm diameter). (B) Electrophysiological activity from one recording electrode; (C) spike detection result; (D) single-channel burst detection.

2.2. Spike detection technique

Extracellularly recorded spikes are usually embedded in biological and thermal noise ranging from 10μV up to 40μV peak-to-peak and they can be detected using a threshold based algorithm [4, 13]. Briefly, a sliding window, sized to contain at most one single spike (i.e. 3-4 msec), is shifted over the signal until the difference between the maximum and the minimum within the window is below the peak-to-peak threshold and, when the difference exceeds the threshold, a spike is found and its time-stamp is saved. The threshold (34.4 ± 5.0μV), calculated as a multiple of the standard deviation (7*SD) of the biological noise [4, 9], is separately defined for each recording channel.

Usually, the recording electrodes are surrounded by few cell bodies and electrical activity is often picked up from more thank one source (i.e., up to 3-5 neurons). In this study no attempt was made to discriminate and sort spikes collected by one channel, considering each signal as the time-spatial summation of the responses from a small ensemble and providing information of the "micro" network related to that recording site.

3. Burst Detection Algorithms

In order to analyze what is referred as rhythmic, oscillatory, clustered electrophysiological activity of neurons, several approaches have been proposed in literature. Quantitative analysis has been performed since the '60, when Rodieck and colleagues [14] studied non rhythmic bursts characterizing the distribution of the Inter Spike Interval. To analyze electrophysiological behaviors in anesthetized, paralyzed and awake animals Guido and Weyand [8, 20] defined a bursting criterion method based on the probability that the spikes within a burst were separated by an interval less than 4ms and the packages are separated by at least 100ms of quiescence. Dekhuijzen and Bagust proposed a burst analysis through an alternative quantitative analysis of clusters in the Poincaré maps [5], and evidenced that characterization is strongly affected by spike detection threshold. Barbieri et al. analyzed spiking and bursting activity of their non-Poisson stimulus-response models by using Q-Q [21] and K-S [12] plots. Recently Keefer and colleagues derived burst patterns from spike integration using a well assessed technique [10].

The above cited methods can be applied to some extent to extracellular electrophysiological recordings of dissociated cortical neurons. Most of them were defined to investigate single neurons activity, therefore we decided to develop new methods to identify and characterize the bursting activity recorded extracellularly by means of multi electrode arrays.

3.1. Theoretical definitions

Let $ST(t)$ be the spike train recorded from a single electrode [13, 16, 17]. It can be defined as:

$$ST(t) = \sum_{n=1}^{N} \delta(t - t_n) \qquad (1)$$

where N is the total number of spikes, t_n is the occurrence time of the n-th spike and $\delta(t)$ is a delta function denoting the occurrence of a spike at time $t = t_n$.

The Inter Spike Interval (ISI) is defined as the time interval between two consecutive spikes in the spike train:

$$ISI_n = t_n - t_{n-1} \quad (5)$$

Most neuroscientists usually adopt a qualitative definition of burst [16], defining its duration as the sum of the inter spike intervals within the burst itself. Two bursts are separated by an interval, called as Inter Burst Interval (IBI) relatively long compared to the burst duration.

Let the burst train $BT(t)$, with a total number of M bursts, be described as:

$$BT(t) = \sum_{m=1}^{M} \left(A_m \Pi \left(\frac{t - t_m}{\tau_m} \right) \right) \quad (6)$$

where t_m denotes the starting time of the m-th burst in the $BT(t)$, $\Pi\left(\frac{t}{\tau}\right)$ is the rectangular function denoting the occurrence of a burst at time $t = t_m$ and lasting τ. and A_m is the burst amplitude.

The generic burst amplitude A_i can be calculated as:

$$A_i = \frac{1}{\tau_i} \int_{\tau_i} \sum_{n=1}^{N_{\tau_i}} \delta(t - t_n)\, dt = \frac{N_{\tau_i}}{\tau_i} \quad (7)$$

where τ_i is the burst time duration, N_{τ_i} is the number of spikes within that burst. It follows that the amplitude of a burst is given by the spike rate inside the burst.

3.2. Single - channel Burst

To detect a channel burst, the spike train is analyzed through a shifting time window, sized as the minimum expected inter burst interval or, equivalently, the maximum expected Inter Spike Interval. Two thresholds are

fixed: the first one is based on the statistical distribution of the spike train and it is defined as the maximum Inter Spike Interval (maxISI, set at the value of 100msec) for spikes within a burst; the second one is defined as the minimum number of consecutive spikes belonging to a burst (minSpikes, set at the value of 10 spikes).

In the spike sequence, spikes are accounted to be part of a burst event if the time delay between two adjacent pulses is less than the maxISI and, if the total number of spikes accounted for is more than the defined threshold (minSpikes), a burst is detected.

The result of the burst detection is a sequence of rectangular functions, each of them representing a burst, whose amplitude is the spike rate within that burst, as reported in Fig. 1 D.

3.3. Network Burst

Network bursts are characterized not only by increased firing rates at individual sites, but also by an increase in the number of active sites. In the case of multi-electrode recordings this property can explicitly be used for improving the algorithm for network burst detection. To this end we have followed a pragmatic approach by calculating per time bin of 25 msec the product of the number of active sites and the total number of spikes at these sites. In the case of uncorrelated firing among the sites and not too high firing rates this product will not significantly differ from the total spike count itself. However, when during network bursts more sites become activated and activity becomes more synchronized it will sharply raise the product, clearly distinguishing network bursts events in the multi-site spike trains. The chosen value for the bin width of 25 msec turned out to give good results. A too high value will smear out the clustering and synchronization effect too much, while a too small value will not capture anymore the synchronization effect. The approach is illustrated in Fig. 2. The time point at which the product within a network burst attains its maximal value is further be used to indicate the center of the network burst. These burst center time points can further be used as a time alignment when subsequently detected network burst are being averaged in order to obtain averaged estimates of the firing rate profiles within networks bursts, both for the total network activity and for the individual sites. Applications of this method can also be found in Van Pelt et al. [18, 19].

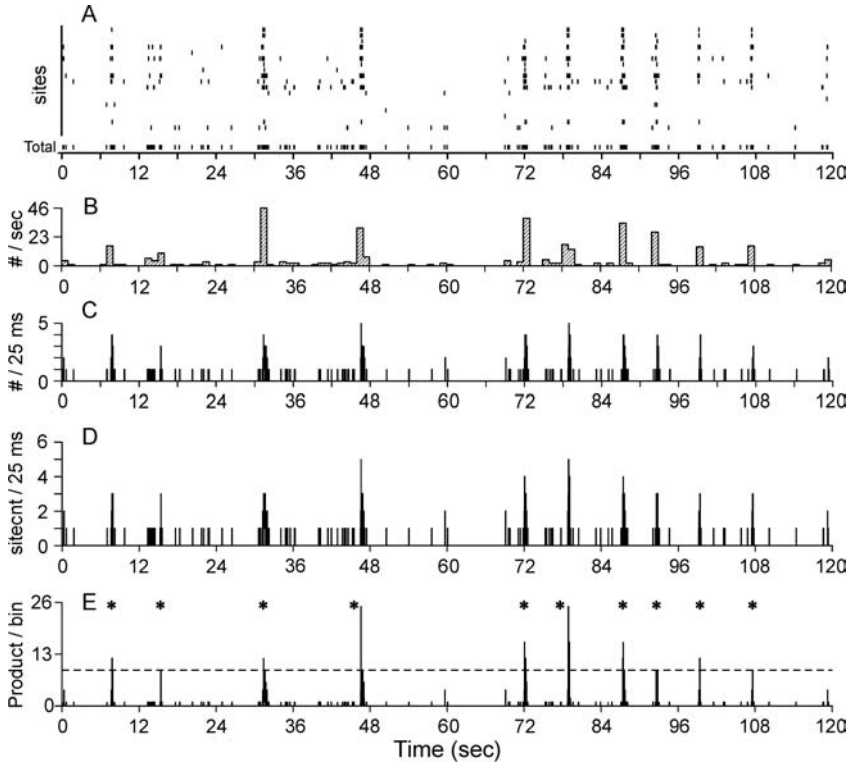

Fig. 2. Illustration of the network burst detection procedure. The different panels show (A) the time points of spikes at the different recording sites of a multi-electrode array, measured during a period of 120 sec, with the lowest trace showing the total spike train for all the sites, (B) a total network firing rate plot with time bins of 1 sec, (C) a total network firing rate plot with time bins of 25 msec, (D) a plot of the number of active sites with time bins of 25 msec, and (E) a plot of the product of total number of spikes times the number of active sites, calculated per 25 msec time bins. The dashed line in panel E denotes a criterion level of nine that the product at least has to reach for the detection of a network burst. This value will be obtained if within a 25 msec time bin a spike has been detected at least three different sites. Detected network bursts in panel E are indicated with a 'star'.

4. Experimental results

Fig. 3 reports the results obtained applying the single-channel burst detection technique explained in section 3.2 to long-term measurements from multi-electrode arrays.

Fig. 3A depicts the mean bursting rate shown by cortical cultures monitored for five weeks in vitro. The first stage of development (i.e. 7 DIV) is characterized by a total absence of organized activity. In some areas of the network, the only form of activity is represented by randomly generated single spikes (i.e. single supra-threshold signals recorded by a microelectrode), as reported by the graph bar in Fig. 3D. Fig. 3D represents the percentage between random spikes (i.e. spikes outside bursts) and the total number of spikes during the acquisition time (300 sec). At 14 DIV bursts start to be generated at quite low frequency, as shown by the low mean bursting rate in Fig. 3A and the high Inter Burst Interval in Fig. 3B, while the number of spikes within a burst reaches its

maximum (see Fig. 3C). In parallel, at 14 DIV we can assume that most spikes are generated within the burst and the percentage of random spiking reaches its minimum. At 21 DIV the highest bursting rate (Fig. 3 A) and, as a consequence, the minimum IBI (Fig. 3B) are found. Bursts are shorter at 21 DIV than at 14 DIV and the burst amplitudes start decreasing, with a good percentage of random spiking activity. At 28 and 35 DIV almost similar values for the considered parameters are extracted: it could mean that network has reached a stable regime, with a similar percentage of random and bursting spikes.

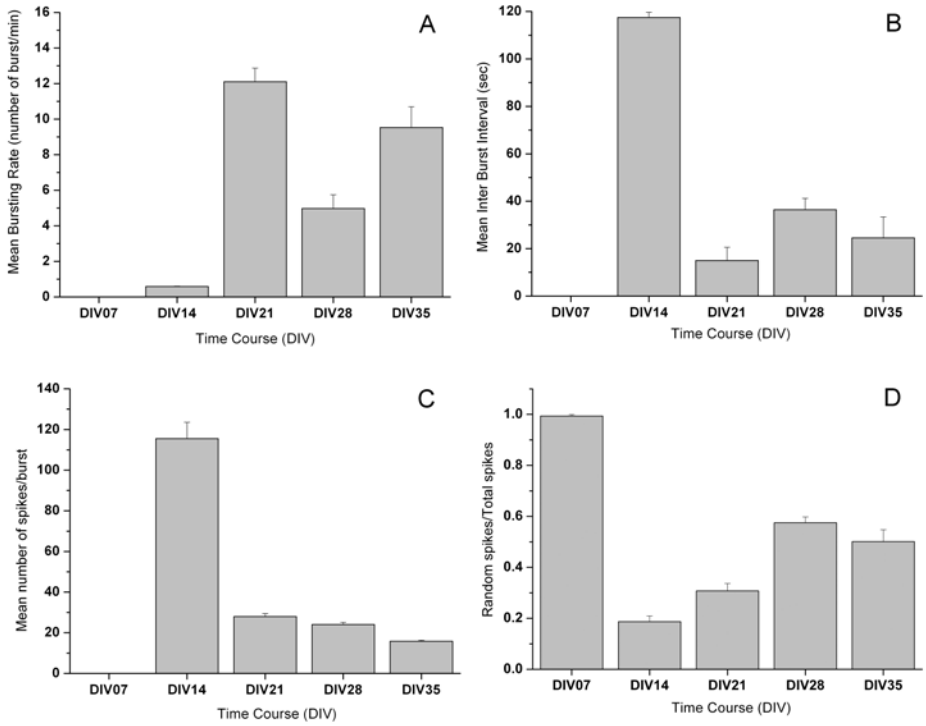

Fig. 3. Single-channel Burst results. (A) Burst rate (number of burst per minute) at different developmental stages: 7, 14, 21, 28 and 35 DIV. (B) Inter Burst Interval amplitude (sec). (C) Mean number of spikes within a burst: note that the maximum is reached at 14 DIV. (D) Percentage of random spikes (i.e. outside the bursts) during development: this parameter reaches stable values after 28DIV. Data are presented as mean±se.

Parameters such as burst shape and duration can be easily extracted looking at the network burst results presented in Fig. 4. Network burst analysis can also confirm results obtained employing the single-channel burst detection.

Network bursts have been detected from 300-sec traces of spontaneous firing at the different developmental stages according to the procedure explained in Fig. 2. Additionally, the most intense network burst was subsequently searched within a time window of 4 sec around a detected network burst for further analysis. By summing these selected bursts, time-aligned according to their time centers, profiles for the mean firing rates

during network bursts have been obtained, as illustrated in Fig. 4. The four panels illustrate the developmental changes in the firing rate profiles, showing broad network bursts at 14 DIV, but increasingly prompt onsets and shorter trailing phases during subsequent weeks in vitro. Burst shape and duration at 21, 28 and 35 DIV look quite similar, but at 21 DIV burst activity is much higher than at 28 and 35 DIV, as can be deduced looking at the firing rate profiles.

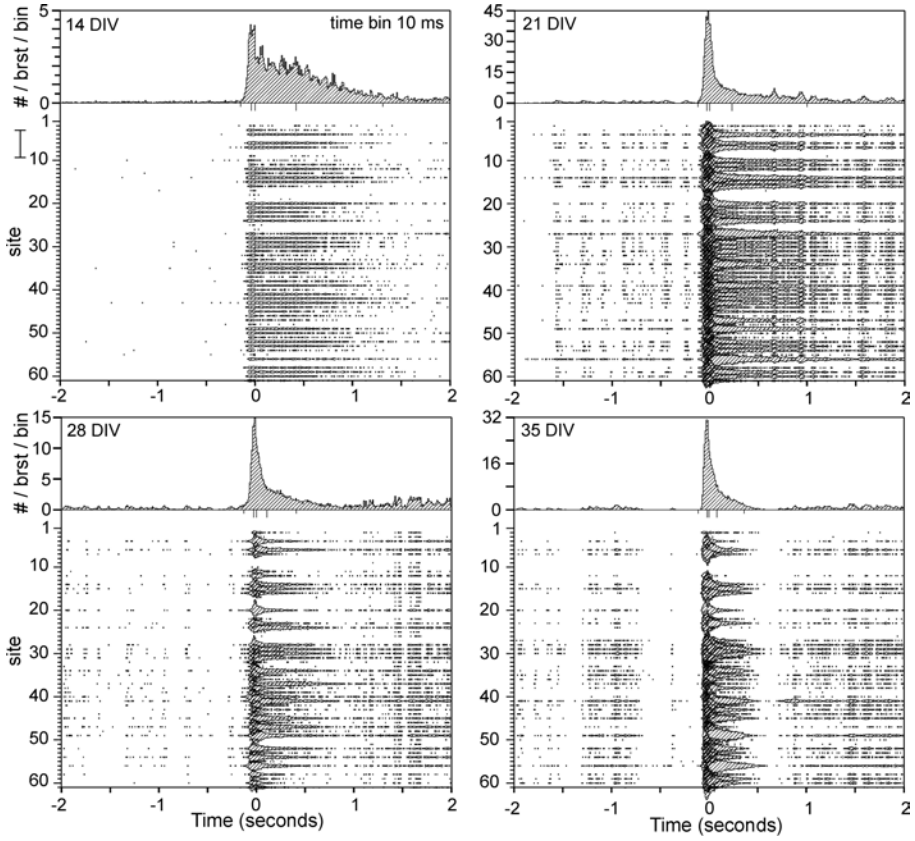

Fig. 4. Averaged firing rate profiles within network bursts at four different developmental stages. The network bursts have been detected from 6 minute traces of spontaneous firing activity according to the algorithm explained in Fig. 2. For the averaging, the individual bursts are time-aligned according to their time centers, while firing rates are calculated for 10 msec time bins. The scale bar denotes 1 spike per time bin per burst.

5. Discussion and conclusions

It is well known that neuronal networks store information in the spike timing or in the changes of the delay between two consecutive spikes, but not only. Also the collective behavior, the so called burst carries information [7, 17] and it is often encountered in neural patterns of activity. Moreover, most spike train statistics lack in methods to characterize bursting behavior. The two presented burst detection algorithms provides a

robust and complementary way to analyze burst activity and the reported results point out that we can extract useful information on the evolution of the dynamics of *in-vitro* neuronal systems by analyzing such a burst behavior both at single channel and at network level.

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