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 rat Central Nervous System and put in culture show, after a few days, spontaneous activity with a typical electrophysiological pattern ranging from stochastic spiking to synchronized 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 algorithms for detection and analysis of bursts in single channel spike trains and of synchronized network bursts in multi-channel spike trains, significant changes have been revealed in the firing dynamics at different stages of the developmental process.

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

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1. Introduction

Experimental investigation of the electrophysiological activity in 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 studied and basic physiological mechanisms can be quantitatively characterized [9, 13].

Taking advantage of the substrate-embedded micro electrode-array technology [1, 2, 9], experiments on cortical preparations over arrays of 60 planar microelectrodes were performed in order to monitor the electrophysiological activity of the neuronal network from both spatial and temporal point of view during the network maturation process [6, 10].

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, 14, 15]. For these reason, we are convinced that burst analysis is an important tool to characterize properties of the spontaneous firing dynamics in neuronal networks.

Two burst detection algorithms, for both single-electrode as well as for multi-electrode spike trains, with complementary goals, are here presented and applied for analyzing an actual experimental session, in order to identify the major changes in the network dynamics during the in-vitro development. Investigation on the neuronal spontaneous electrophysiological activity during development is one of the key issues for understanding the functional neuronal circuits formation and their implication in network plasticity and adaptability. We think that the application of complementary methods aimed at extracting relevant features of the burst patterns can be useful to elucidate fundamental mechanisms arising in a developing neuronal network.

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 Micro Electrode Arrays (MEAs) with 60 electrodes of 30µm diameter, arranged on a 8x8 square array, and with mutual distances of 200µm (MultichannelSystems, Reutlingen, Germany), pre-coated with adhesion promoting molecules (Poly-D-Lysine and Laminin), at the final density of $6\text{-}8 \cdot 10^4$ cells/device and maintained in Neurobasal medium (Gibco) 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.

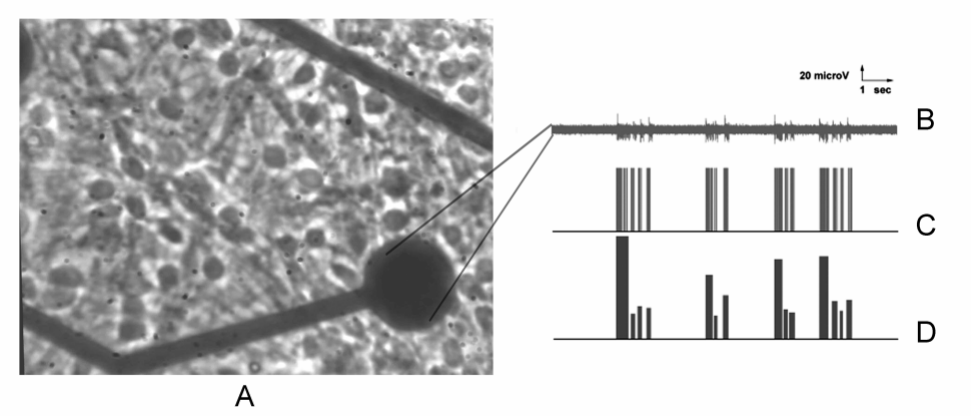

Fig. 1. (A) Dissociated cortical neurons on MEA surrounding a planar microelectrode (30 μm diameter). (B) Electrophysiological activity from one recording electrode; (C) spike detection result; (D) single-channel burst detection.

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 [3, 11]. Briefly, a sliding window, sized to contain at most one single spike (i.e. 3-4 msec), is shifted over the signal and, when the difference between the maximum and the minimum within the window 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 [3, 8], is separately defined for each recording channel.

Given the size of the electrodes (30μm) and the averaged size of neuronal cell bodies (5-10μm), electrical activity is often picked up from more than 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.

3. Burst Detection Algorithms

In order to analyze what is referred to as rhythmic and clustered electrophysiological activity of neurons, several approaches have been proposed in literature, with quantitative analysis already been performed since the '60, when Rodieck and colleagues [12] studied non rhythmic bursts characterizing the distribution of the Inter Spike Interval. Also Cocatre-Zilgien and Delcomy (1992) used the interspike interval distribution to detect the critical interval value that represents the break between short intervals within a burst and the longer intervals between bursts [4], the so called Inter Burst Interval (IBI). This method has further been generalized by Corner et al. (2002) by constructing graphs of burst properties for any given value of the burst criterion [5].

Taking inspiration from [5], we decided to implement a simple methodology for burst definition and detection, but we wanted that the result of the automatic procedure was as closer as possible to what the experimenter interprets during electrophysiological signals inspection. At the same time, having 60 channels to record from, it was important to define an additional method to extract important information regarding the burst synchronization and the level of activation of the recording channels during acquisition.

3.1. Theoretical definition of the spike burst

In this paragraph we will give a mathematical outline of the spike burst definition based on the interspike interval criterion method. Let $ST(t)$ be the spike train recorded from a single electrode [11, 14, 15]:

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

where *N* is the total number of spikes, t_n is the occurrence time of the *n*-th spike and $d(t)$ 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:

$$ISI_n = t_n - t_{n-1} \qquad (2)$$

We can simply define the bursts as sequences of densely packed spikes, with a duration equal to the sum of the inter spike intervals within the burst and separated by an interval, called Inter Burst Interval (IBI), relatively long compared to the burst duration [14]. In this way, the burst train $BT(t)$, with a total number of M bursts, can be described as:

$$BT(t) = \sum_{m=1}^{M} \left(A_m \Pi\left(\frac{t - t_m}{t_m} \right) \right) \qquad (3)$$

where t_m denotes the starting time of the *m*-th burst in the $BT(t)$, $\Pi\left(\frac{t}{t}\right)$ is the rectangular function denoting the occurrence of a burst at time $t = t_m$ and lasting t and A_m is the burst amplitude. The generic burst amplitude A_i can be calculated as:

$$A_i = \frac{1}{t_i} \int_{t_i} \sum_{n=1}^{N_{t_i}} d(t - t_n)\, dt = \frac{N_{t_i}}{t_i} \qquad (7)$$

where t_i is the burst time duration, N_{t_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, or, equivalently, by two specific parameters such as the number of spikes per burst and the burst duration.

3.2. Burst detection in single-channel spike trains

For burst detection, the spike train is analyzed using 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 is defined as the maximum Inter Spike Interval for spikes within a burst (maxISI, set at the value of 100msec); the second one is defined as the minimum number of consecutive spikes belonging to a burst (minSpikes, set at the value of 10 spikes). The values of 100msec and 10spikes for maxISI and minSpikes, respectively, have been set after a series of comparisons between the results of the automated burst detection and the visual inspection of three experimenters (data not shown). Even if 100msec can appear a high value, it has been shown to give the better performance results, since each burst, especially in the early developmental stages, present a long tail with lower frequency components, which must be included as part of the burst itself.

In summary, spike bursts are defined as sequences of spikes with interspike intervals, each one smaller than the maxISI, and containing at least a number of spikes equal to minSpikes. Spike bursts are represented as rectangular functions from the first up to the last spike in the bursts, and with amplitude equal to the spike rate within the burst, as defined by the equations (6) and (7) and reported in Fig. 1D.

3.3. Network Burst detection in multi-channel spike trains

Network bursts are short episodes of synchronized firing among many recording sites and may be 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 defining an 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. But, 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 time point at which the product within a network burst attains its maximal value is used to

define a center-of-mass based time center of the network burst [16]. The chosen value for the bin width of 25 msec turned out to give good results. Although the method is not critical on the precise choice of the time bin, higher values for the bin width will smear out the clustering and synchronization effect too much, while smaller values will not capture anymore the synchronization effect. The approach is illustrated in Fig. 2. The network burst center time points have further been used for time alignment in averaging the firing rates in network bursts in order to obtain an averaged estimate of the firing rate profiles within networks bursts, both for the total network activity and for the individual sites. Applications of this method can be found in Van Pelt et al. [16, 17].

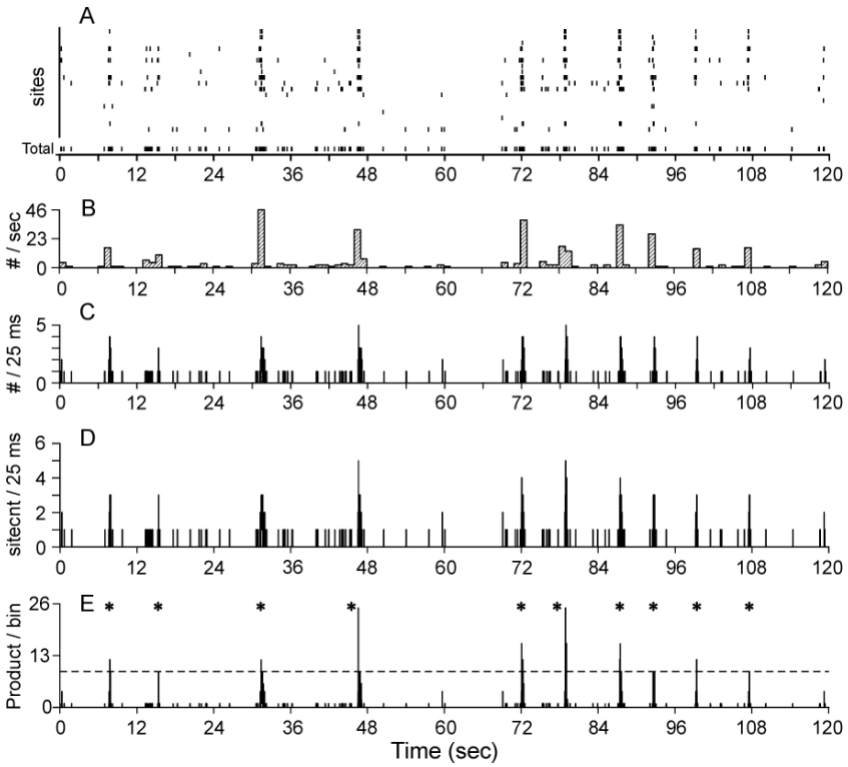

Fig. 2. Illustration of the network burst detection procedure. The different panels show (A) the time points of spikes at the different recording sites of a multi-electrode array, measured during a period of 120 sec, with the lowest trace showing the total spike train for all the sites, (B) a total network 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 at 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, and averaged over all the active channels (i.e. the

channels presenting at least two bursts, necessary to define the inter burst interval, in an acquisition session of 300 sec). The number of "active" bursting channels changes along with the development: at 7DIV a few sites show bursting behavior, proving the low level of network maturation in terms of synaptic connections. At 14 DIV about the 50% of the channels are characterized by collective activity and this trend is maintained also in the succeeding weeks (21 and 28 DIV), where this percentage improves till the 70%. AT 35 DIV the percentage of bursting channels decreases to the 35%, indicating that a small part of the network shows single-channel bursts. 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 spike bursts. 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.

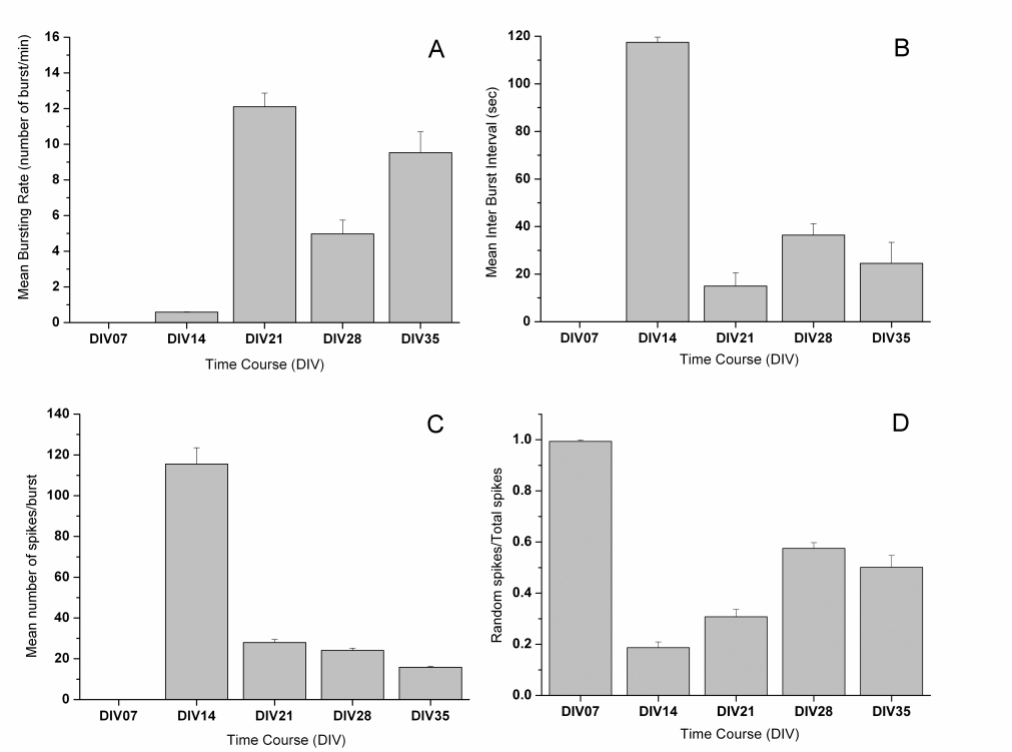

Fig. 3. Single-channel burst results, averaged over all the active channels. (A) Burst rate (number of burst per minute) at different developmental stages: 7, 14, 21, 28 and 35 DIV. (B) Inter Burst Interval amplitude (sec). (C) Mean number of spikes within a burst: note that the maximum is reached at 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 could also confirm results obtained employing the single-channel burst detection, even if electrode presenting single-channel bursts could not participate to the network burst or vice versa.

Network bursts have been detected from the 300-sec traces of spontaneous firing at the different developmental stages and averaged according to the procedure explained in Fig. 2 and section 3.3. The four panels in Fig. 4 illustrate the developmental changes in the firing rate profiles (14, 21, 28 35 DIV): at 7DIV the network did not present any network burst. Fig. 4 shows broad network bursts at 14 DIV, but increasingly prompt onsets and shorter trailing phases during the subsequent weeks in vitro (21, 28 and 35 DIV). 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 (see the different scales in the panels of Fig. 4), as can be deduced also from the firing rate profiles. In addition, we can note that the number of recruited channels participating to the network burst reaches its maximum at 21DIV (i.e. maximum value of the network burst), denoting that at this age the network is highly active and synchronized.

This synchronization seems to be well maintained in the subsequent ages, probably indicating that the network has stabilized its behavior and reached a condition with properly formed connections.

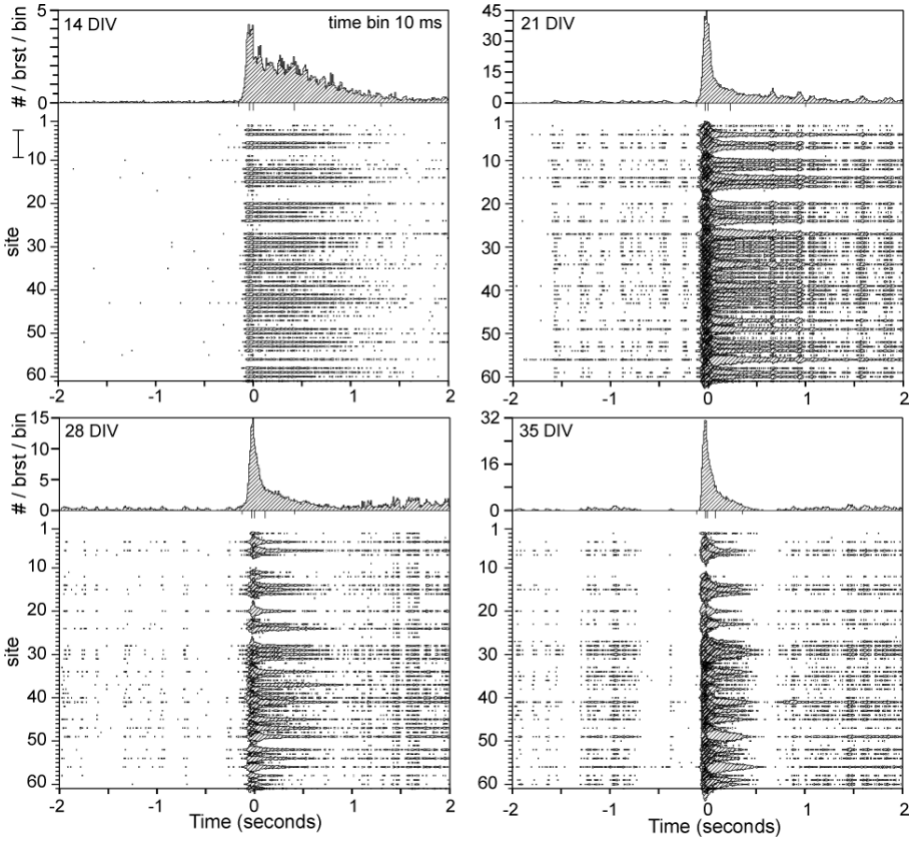

Fig. 4. Averaged firing rate profiles within network bursts at four different developmental stages. The net work bursts have been detected from 6 minute traces of spontaneous firing activity according to the algorithm explained in Fig. 2. For the averaging, the individual bursts are time-aligned according 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. Note the different scales among the four panels, in order to better appreciate the changes in the network burst shape and amplitude during the development.

5. Discussion and conclusions

Both the single channel burst analysis approach and the multichannel network burst approach have revealed significant developmental changes in the firing dynamics in cultured neuronal networks. Both methods capture complementary information of the bursting behavior in a network because a burst at the single channel level not necessarily needs to be part of a synchronized network burst and, alternatively, firing at a particular channel during a synchronized network burst not necessarily needs to be detected as a single channel spike burst. The threshold for network burst detection has purposely been set low such that synchronized firing within a small group of neurons at a minimum of three sites within 25 msec is already captured as a network burst event.

Further analysis is needed to quantify this complementarity. The reported changes in the firing dynamics may further stimulate investigations in developmental and plasticity mechanisms in network connectivity and excitability involved in the emergence of spontaneous network firing activity.

Acknowledgments

Novellino's Ph.D. fellowship is sponsored by eTT s.r.l. (http://www.ettsolutions.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".

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