Available online at www.sciencedirect.com
ScienceDirect
Progress in Natural Science 19 (2009) 229–235
Progress in Natural Science
www.elsevier.com/locate/pnsc

# Detection of bursts in neuronal spike trains by the mean inter-spike interval method

Lin Chen [a,b], Yong Deng [c], Weihua Luo [a], Zhen Wang [a], Shaoqun Zeng [a,*]

[a] *Hubei Bioinformatics and Molecular Imaging Key Laboratory, Huazhong University of Science and Technology, Wuhan 430074, China*
[b] *College of Mechanical Engineering, Guangxi University, Nanning, 530004, China*
[c] *College of Optoelectronic Science of Engineering, Huazhong University of Science and Technology, Wuhan 430074, China*

Received 18 March 2008; received in revised form 28 April 2008; accepted 9 May 2008

**Abstract**

Bursts are electrical spikes firing with a high frequency, which are the most important property in synaptic plasticity and information processing in the central nervous system. However, bursts are difficult to identify because bursting activities or patterns vary with physiological conditions or external stimuli. In this paper, a simple method automatically to detect bursts in spike trains is described. This method auto-adaptively sets a parameter (mean inter-spike interval) according to intrinsic properties of the detected burst spike trains, without any arbitrary choices or any operator judgment. When the mean value of several successive inter-spike intervals is not larger than the parameter, a burst is identified. By this method, bursts can be automatically extracted from different bursting patterns of cultured neurons on multi-electrode arrays, as accurately as by visual inspection. Furthermore, significant changes of burst variables caused by electrical stimulus have been found in spontaneous activity of neuronal network. These suggest that the mean inter-spike interval method is robust for detecting changes in burst patterns and characteristics induced by environmental alterations.


*Keywords:* Inter-spike interval; Bursts; Spike trains; Multi-electrode arrays

## 1. Introduction

Many neurons fire action potentials in brief bursts of high-frequency discharge. Bursts are the particular electrical activity pattern of single neurons (in which the intrinsic properties of neuronal membrane might be involved), which represent a particular mode of neuronal signaling and have a distinct function in sensory information transmission [1]. The functional importance of generating bursts is to increase the reliability of communication between neurons and to avoid synaptic transmission failure. Bursts of action potentials might afford more precise information than action potentials that arrive singly, and they also provide effective mechanisms for selective communication between neurons [2], and produce long-term synaptic plasticity and information processing [3,4]. Furthermore, bursting behavior is the most important property for analyzing the dynamics of electrical activity during the development of neuronal networks [5–7], and for investigating the modification of neuronal networks which are induced by alteration of the physiological environment [8,9] (such as chemical exposure and electrical stimuli). The analysis of the inter-spike interval (ISI) within a burst is of important biological significance for understanding the characteristics of temporal coding in spike trains of neuronal networks [10–12].

Because of the complexity of the background spiking, detecting bursts is a long-standing challenge in investigating the dynamics of burst activity. Legendy and Salcman [13] assumed that the frequency of spike trains had a Poisson dis-

* Corresponding author. Tel.: +86 27 87792033; fax: +86 27 87792034.
*E-mail address:* sqzeng@mail.hust.edu.cn (S. Zeng).

1002-0071/$ - see front matter 
doi:10.1016/j.pnsc.2008.05.027

tribution and defined bursts of spikes by the value of the 'Poisson surprise' parameter. Cocatre-Zilgien and Delcomyn [14] used an inter-spike interval (ISI) histogram to detect critical interval values in the distribution represented by the break between short intervals within a burst and the longer intervals between bursts. Both of them defined a burst on statistical arguments of ISIs of a spike train. Kaneoke and Vitek [15] adopted a higher number of discharge density (the number of spikes in a short interval) to define a burst. Turnbull et al. [16] applied only two parameters, a minimum number of spikes per burst and a maximum ISI, to define a burst as a string of spikes, and this method has the virtue of simplicity and intuitiveness. Some researchers defined a burst as a number of inter-spike intervals all shorter than a given value [4,17]. However, all these methods depend on the parameters assumed for burst detection.

Due to the enormous variety of burst structures and burst patterns associated with neuronal plasticity, behavior and the involvement of external stimulation, it is difficult to predetermine appropriate parameters for experimenters when they use the parametric methods to detect bursts from spike trains. It is important to use a self-adaptive algorithm for burst detection and objective analysis, so Tam [18] presented an auto-adaptive method in which a burst was defined by inter- and intra-burst intervals. However, this method is only valid for typical burst patterns in neuronal spike trains. Here, we describe an automatic method using 'mean inter-spike interval' (MISI) for burst detection, which identifies bursts through an auto-adaptive procedure. This method is based on characteristics of ISI sequences and identifies bursts as 'many' spikes with a 'small' interval (spike cluster). Although it is very simple, and can rapidly and accurately extract relevant features of the burst patterns to elucidate the dynamical changes in electrophysiological activity of neuronal networks.

## 2. Experimental methods

### 2.1. Cell culture

Animal care was in accordance with guidelines approved by the Animal Use Review Committee of Huazhong University of Science and Technology. Hippocampal cells were isolated from 18-day-old embryonic rats and were cultured on a multi-electrode array (MEA) dish (Fig. 1a) according to the method previously reported [7]. Cultures were maintained in an incubator at 37 °C with 5% $CO_2$, and a part of the medium was replaced by fresh medium every three days. Bursts activity usually appeared when networks formed after being cultured for 2–3 weeks.

### 2.2. Recording system and stimulation

Electrical activities were recorded with a square array of 60 titanium nitride electrodes (Multi Channel Systems, Reutlingen, Germany) in contact with the base of the culture, each was 30 μm in diameter, with 200 μm spacing

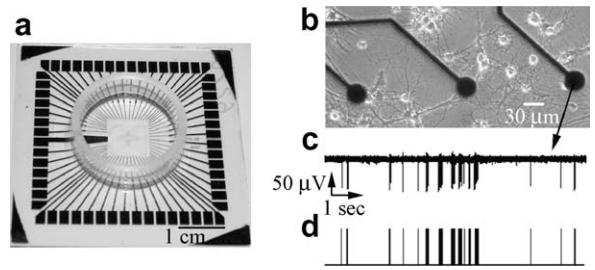


Fig. 1. Multi-electrode arrays. (a) Circular plate on top of microelectrodes. (b) Cultured hippocampal neurons (18 days *in vitro*) on a multi-electrode array plate. The solid black circles are microelectrodes with a diameter of 30 μm; the distance between adjacent electrodes is 200 μm. The neurons grew on the plate and developed a network. (c) Spontaneous electrophysiological activity from one recording electrode. (d) Raster plot showing the spikes detected. Spikes are represented by thin vertical lines.

between them (Fig. 1a and 1b). Stimuli were generated using a four channel stimulator (Multi Channel Systems, Reutlingen, Germany). After 1200× amplification, signals of electrophysiological activity were collected at a frequency of 50 kHz and were simultaneously recorded in 60 channels for 30–300 s using a multi-channel data acquisition card. The whole process was controlled through MC-Rack software. Experiments were performed when neuronal networks formed 2–6 weeks later.

The 0.2 Hz low-frequency electrical stimulus was applied to the neuronal network through a pair of active electrodes (42 and 44). The voltage stimulation mode with biphasic rectangular voltage pulses was used (positive phase first), and the strength was 500 mV and the pulse length was 200 μs. After a series of 300 stimuli, the spontaneous electrophysiological activity was recorded.

### 2.3. Inter-spike interval sequences

A prerequisite to any analysis method is the extraction of the spike times from the recorded electrophysiological activity by means of a standard spike detection algorithm. This transforms the continuously recorded signals into a discrete series of spikes. Each spike train can then be expressed as a series of $\delta$ functions

$$a(t) = \sum_{n=1}^{N} \delta(t - t_n), \tag{1}$$

with $t_1, \ldots, t_N$ denoting the series of spike times, and $N$ being the number of spikes.

In this study, the spike detection algorithm was used to extract the spike times from the recorded signals, with the threshold for acceptance of a signal set at $5\sigma$ to $7\sigma$ (where $\sigma$ is the standard deviation of the quiescent signal during 500 ms at the beginning of each measurement). A single electrode on a multi-electrode array may pick up electrophysiological signals from one or several neurons (Fig. 1c). Hence, the multiple neuronal spikes can be sorted by amplitude and waveforms as discriminators in a hierarchical clustering algorithm or principal component algorithm

[19]. To provide an example of spike distribution, a raster plot is used for describing the electrical activity of neurons, in which each line denotes a spike (Fig. 1d).

The inter-spike interval sequences ($\mathrm{ISI}_n$) are defined as the history of time intervals between consecutive spikes in the spike train. Let $t_n$ be the occurrence time of the $n$th spike in a set of $N$ spikes. Then the $\mathrm{ISI}_n$ is a variable:

$$\mathrm{ISI}_n = t_{n+1} - t_n, \quad n = 1, 2, \ldots, N-1. \tag{2}$$

## 3. Burst definition method

In this section, we introduce a quantitative method for burst definition. Before describing this method, the features of different neuronal spike firing patterns need to be illustrated by plots of ISI distributions. Four neuronal firing patterns are commonly observed in the recorded data, including typical bursts, alternate single spikes and bursts, tonic spikes, and sporadic spikes. Raster plots of these four typical spike trains recorded over 100 s are shown in Fig. 2a (each vertical line denotes the occurrence of an identified spike). Clearly, the neuronal spike trains are organized in very dissimilar ways in these four cases. In addition, the ISI histogram [20,21] was used to characterize the ISI distribution for these four cases. The ISI histograms of the tonic firing and the sporadic firing indicated that no bursts existed in these two cases, as shown in Fig. 2b3 and b4. In contrast, the ISI histograms of the alternate single spikes and bursts firing and the typical bursts firing showed strong peaks at 7–20 ms and 1–10 ms, respectively (Fig. 2b1 and b2), and the profiles of ISI histogram conformed to an exponential distribution. These features reveal that spikes are highly organized in bursts. We propose a new burst detection method according to intrinsic properties of the detected burst spike trains, and the scope of application of this method is burst firing pattern (such as Fig. 2a1 and a2).

In an inter-spike interval sequence ($\mathrm{ISI}_n$), the mean inter-spike interval is given by

$$\mathrm{Mean} = \sum_{n=1}^{N-1} \mathrm{ISI}_n \Big/ (N-1). \tag{3}$$

Because bursts are high-frequency spike episodes within a spike train, it follows that the inter-spike intervals within a burst will be small, less than the mean value of the $\mathrm{ISI}_n$. Here, the new method to detect bursts consists of the following steps:

1. Determine the mean value of the $\mathrm{ISI}_n$ from formula (3).
2. Construct a new ISI sequence, $L(n)$, which can be extracted from the original $\mathrm{ISI}_n$ sequence. If the $\mathrm{ISI}_n$ < mean, then put it into the sequence $L(n)$. So each element of the $L(n)$ is less than the mean. The elements of $L(n)$ are mainly ISIs within bursts but some are ISIs between two spikes.
3. Calculate the mean of $L(n)$ from formula (3) and term this value ML. The value of ML is used as a parameter to define bursts. ML is an auto-adaptive parameter derived from measured spike trains.
4. Define bursts as two or more successive ISIs in the original $\mathrm{ISI}_n$ sequence with a mean value smaller than ML (Eq. (4)).

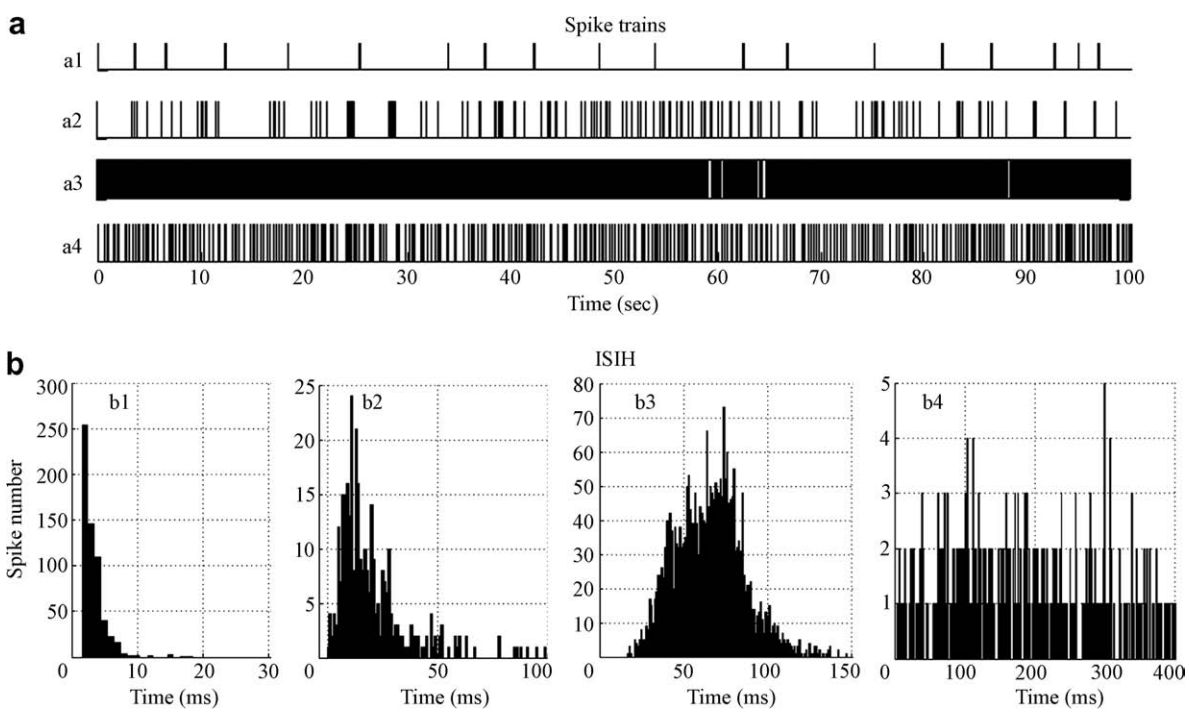


Fig. 2. The ISI histograms for different firing patterns. (a) Raster plot of spike trains for four different firing patterns: a1 – typical bursts, a2 – alternate single spikes and bursts, a3 – tonic spikes, and a4 – sporadic spikes. (b) ISI histograms of the four firing patterns (b1–b4) recorded over 100 s, bin = 1 ms.

$$\frac{1}{k}\sum_{n=i}^{i+k} \mathrm{ISI}_n \leqslant \mathrm{ML}, \quad k = 2, 3, \ldots \tag{4}$$

5. Confirm the beginning and the end of each burst period from the original spike train, the burst duration as the sum of ISIs within the burst, and the number of spikes per burst as $k + 1$.
6. Construct a graph which illustrates the time of burst emergence, burst duration and number of spikes per burst.

## 4. Results

### 4.1. Identifying bursts within two different firing patterns

The 'mean inter-spike interval' method was applied to the extraction of bursts from two of the spontaneous electrophysiological activities of neurons (typical bursts firing pattern – a1, and alternate single spikes and bursts firing pattern – a2, as shown in Fig. 1). Bursts were identified following the steps in Section 3. The values of 'ML' were 4 ms and 39 ms for a1 and a2, respectively, shown as dashed lines in Fig. 3a and b. The plots of joint inter-spike interval histograms, in which the ordinate and abscissa are on a logarithmic scale, are helpful to demonstrate the ISI distribution within a spike train. In Fig. 3, the initial spikes in bursts are shown at bottom right; the final spikes in bursts are on top left, spikes within bursts are at bottom left, and sporadic spikes are at top right. It is clear that most spikes within bursts are located near the mean value (dashed line, ML).

Using the above-mentioned values of ML, bursts have been automatically identified. Parts of the identified bursts are shown in Fig. 4a and b (a period of 20 s). Fig. 4a and b shows burst variables including burst duration, inter-burst intervals, the number of bursts and the number of spikes per burst. In a typical burst, as shown in Fig. 4a, the maximum number of spikes is 36, but it is only 11 in the bursts alternating with single spikes (Fig. 4b). These figures show the average frequency of spikes within a burst, and they also display the position and distribution of bursts within spike trains. Fig. 4c and d shows the cumulative spike number versus the time at which the spike occurred. Many groups of spikes are arranged nearly vertically as described by Turnbull et al. [13] who labeled them as 'strings' and defined them as bursts. It is obvious that the bursts identified by 'mean inter-spike interval' method correspond perfectly to these 'strings' which are easily picked out by visual inspection.

To apply the string method, a parameter $t_s$ (the maximum inter-spike interval between adjacent spikes in a burst) was selected, and on this basis a burst was identified. We also analyzed our data using the string method and compared the result with those obtained from our mean inter-spike interval method. When $t_s$ was set at 34 ms or 104 ms for the two firing patterns, the burst characteristics obtained from the string method were similar to those obtained from the 'mean inter-spike interval' method. Four characteristics of bursts are compared in Tables 1 and 2, which demonstrate that the values of these characteristics obtained by the two methods are approximately the same, the difference between the values being not more than 5% (Tables 1 and 2). However, our method has the virtue that the values of parameters used in the analysis (ML, etc.) are obtained by an objective process.

### 4.2. Changes of spontaneous burst activity by electrical stimulus

Electrical stimulus may influence spontaneous activity of the neuronal network. The 'mean inter-spike interval' method was used to detect bursts to see whether there

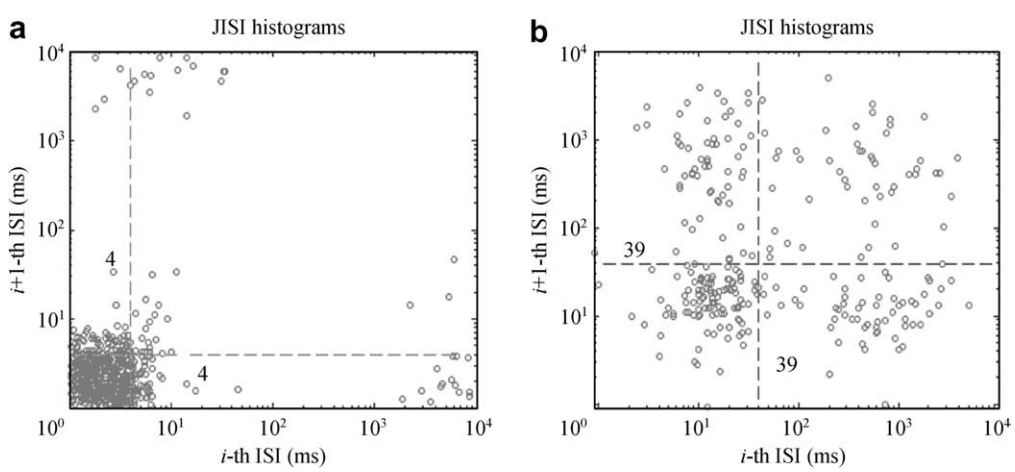


Fig. 3. The joint inter-spike interval histograms of two burst firing patterns (a1, typical bursts; a2, alternate single spikes and bursts in Fig. 1). Each plot shows the inter-spike interval following a spike (ISI$i + 1$) as a function of the preceding one (ISI$i$), with the ISI on a logarithmic scale on the ordinate and abscissa. Each '○' denotes a spike. The values of ML are shown as dashed lines.

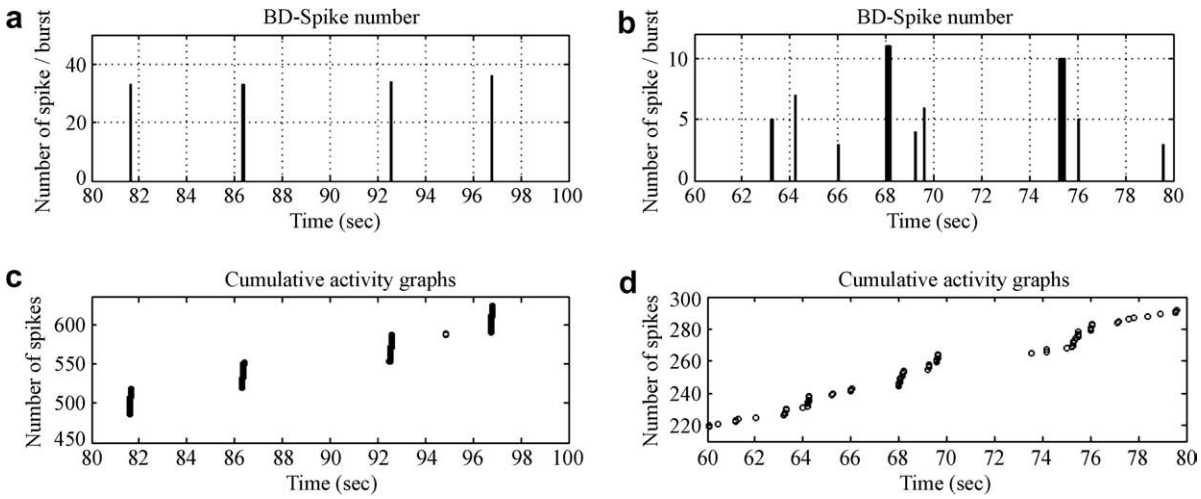


Fig. 4. Bursts identified in the typical burst firing pattern and in the alternate single spikes and bursts firing pattern (recorded over 100 s). (a) and (b) Duration and number of spikes in bursts. The width of a bar denotes the burst duration and its height represents the number of spikes per burst. (c) and (d) Cumulative activities of the two firing patterns, in which a vertical string of spikes are defined as a burst based on Ref. [16].

Table 1
Results of bursts detection by mean inter-spike interval method for two burst firing patterns.

| Parameters | ML (ms) | Number of bursts | Mean burst duration (ms) | Spikes in bursts/total spikes | Mean ISI in bursts (ms) |
|---|---|---|---|---|---|
| Typical bursts | 4 | 17 | 103 | 621/625 | 2.89 |
| Alternate single spikes and bursts | 39 | 42 | 91 | 238/329 | 19.6 |

Table 2
Results of bursts detection by string method for two burst firing patterns.

| Parameters | $t_s$ (ms) | Number of bursts | Mean burst duration (ms) | Spikes in bursts/total spikes | Mean ISI in bursts (ms) |
|---|---|---|---|---|---|
| Typical bursts | 34 | 17 | 104 | 622/625 | 2.91 |
| Alternate single spikes and bursts | 104 | 44 | 91 | 245/329 | 20.0 |

was a change in spontaneous burst activity evoked by electrical stimulus.

Fig. 5a shows spontaneous spike trains from channels 14, 27, 68 and 77 in the multi-electrode arrays, which were recorded for 40 s before and 80 s after the electrical stimulation. It can be seen that the distribution and density of spikes before stimulation are obviously different from those after stimulation. The average spontaneous spike rate of these four channels changed from $9.9 \pm 1.2$ to $10.9 \pm 1.0$ spikes/s (mean $\pm$ SD) by the stimulation, and the mean burst rate (bursts/s) increased by 71.6%. This indicates that the burst rate has a wider dynamic range than the spike rate.

The two histograms in Fig. 5 show the changes in burst parameters before and after electrical stimulation. Burst duration and the number of spikes per burst are markedly decreased ($^{**}p < 0.01$) for channels 14, 27 and 77, and so as for channel 68 ($^{*}p < 0.05$). After stimulation, the average burst duration and number of spikes per burst for the four channels decreased by 74% and 62%, respectively. These changes show that the characteristics of bursts have altered after electrical stimulation, that is, long bursts occurring with low frequency have changed into short bursts of high frequency (Fig. 5a).

## 5. Discussion

In this paper, we introduce a mathematical method to automatically identify bursts of spikes in neurons. This method is based on the characteristic of spike trains, that is, the nature of inter-spike intervals within bursts rather than the shape of ISI histograms or the discharge density of ISI [14,15]. In this method, the distribution of inter-spike intervals does not have to be assumed to have a Poisson distribution and does not need to be characterized by fitting an equation to the experimental data [13]. The 'mean inter-spike intervals' method can auto-adaptively set a parameter (mean inter-spike interval) according to intrinsic properties of the detected burst spike trains, and can adaptively identify bursts from the spike trains without any prior assumptions about the parameters of identified bursts (the characteristic time scales of bursts or inter-burst

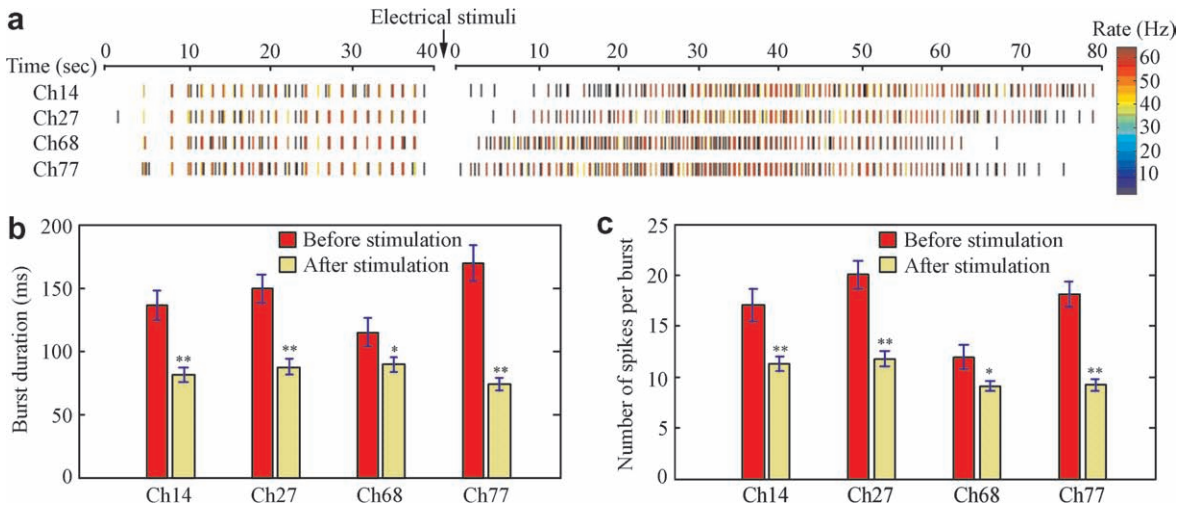


Fig. 5. The variety of spontaneous burst activities after electrical stimulation. (a) Raster plot from before and after a series of 300 electrical stimulations applied. Synchronized bursts occurred before stimulation and a lot of short bursts appeared after it (tissue was cultured for 20 days *in vitro*). (b) Effects of stimulation on burst duration. (c) Effects of stimulation on the number of spikes per burst (significance of differences between before and after stimulation were tested using Student *t*-tests $^{**}p < 0.01$; $^{*}p < 0.05$).

intervals [13–17]). When the mean value of several successive inter-spike intervals is not larger than the parameter, a burst can be identified. The 'mean inter-spike intervals' method presented in this paper performs a complete search of bursts within a spike train for different burst firing patterns. Moreover, this method performs in a robust way based on a very general definition of a burst. This method is easy to monitor statistical changes in burst variables, such as burst duration, number of spikes in a burst and burst period in real time.

This method also performs well in identifying bursts in datasets recorded from multi-electrode arrays, this will benefit to obtain different types of burst organization under different physiological conditions [4–7,11]. The characteristics of four different firing patterns shown in Fig. 2a, and the ISI histograms and the joint ISI histograms from others [13,14,20,21] demonstrate that bursts genuinely exist in these spike trains (Figs. 2b1, b2 and 3). Fig. 4a and b prove that the bursts detected by this method accurately reflect the detected physiological activity of neurons, and the results also agree with those from visual inspection (Fig. 4c and d) and are similar to the results obtained by the 'string' method (Tables 1 and 2).

Furthermore, this method is a sensitive technique for revealing the changes in spike and burst patterns induced by environmental alterations (pharmacological exposure or electrical stimulation). For example, we found that the spontaneous burst firing patterns changed significantly after a series of 300 0.2 Hz electrical stimulations (Fig. 5a). In the case that the assumed parameters [4,14,16,17] were used, it would be difficult for the experimenter to determine parameters that reflect the variety of burst activity. However, our new method can objectively adjust 'ML' values for identifying bursts from spike trains under different physiological conditions. When bursts have been identified, burst parameter values can be calculated to quantify these differences, such as the mean burst duration and the number of spikes per burst (Fig. 5b and c).

It has been shown that 'mean inter-spike intervals' method works well in identifying bursts with most spike trains. However, there are two limitations to this method. First, when it is applied to spike trains which contain a period of sporadic spikes with long ISIs, or numerous short ISIs (a burst firing pattern), it will lead to a large ML value and a long artificial 'burst'. Therefore we suggest that it would be better to select the periods in which bursts are obviously present before calculating ML to identify bursts. Secondly, if the first ISI of a burst is much larger than the following ISIs within the burst and the mean value of two successive ISIs is larger than ML, then this ISI may be not considered as a component of the burst.

This new method is a technique for automatically detecting bursts in neuronal activity and for quantitatively characterizing the dynamic changes of complex bursts in cultured neuronal networks. It displays robustness in identifying bursts under different physiological conditions. All these demonstrate that the mean inter-spike interval method should be useful to detect changes in burst patterns and characteristics under a wide variety of conditions.

## Acknowledgments

The authors are grateful to Prof. William John Cram for helpful discussion and revision of this manuscript. This work was supported by National Natural Science Foundation of China (Grant No. 30727002) and National High-Tech Research and Development Program of China (2006AA02Z343). The authors would like to acknowledge the MEA group of our laboratory for supplying experimental data.

## References


[1] Krahe R, Gabbiani F. Burst firing in sensory systems. Nat Rev Neurosci 2004;5(1):1323.

[2] Izhikevich EM, Desai NS, Walcott EC, et al. Bursts as a unit of neural information: selective communication via resonance. Trends Neurosci 2003;26(3):161–7.

[3] Lisman JE. Bursts as a unit of neural information: making unreliable synapses reliable. Trends Neurosci 1997;20(1):38–43.

[4] Harris KD, Hirase H, Leinekugel X, et al. Temporal interaction between single spikes and complex spike bursts in hippocampal pyramidal cells. Neuron 2001;32:141–9.

[5] Van Pelt J, Wolters PS, Corner MA, et al. Long-term characterization of firing dynamics of spontaneous bursts in cultured neural networks. IEEE Trans Biol Med Eng 2004;51(11):2051–62.

[6] Chen CP, Chen L, Lin YS, et al. The origin of spontaneous synchronized burst in cultured neuronal networks based on multi-electrode arrays. Biosystems 2006;85:137–43.

[7] Li X, Zhou W, Liu M, et al. Firing patterns of long-term cultured neuronal network on multi-electrode array. Prog Nat Sci 2006;16(12):1337–42.

[8] Chiappalone M, Vato A, Tedesco M, et al. Network of neurons coupled to microelectrode arrays: a neuronal sensory system for pharmacological applications. Biosens Bioelectron 2003;18:627–34.

[9] Li Y, Zhou W, Li X, et al. Dynamics of learning in cultured neuronal networks with antagonists of glutamate receptors. Biophys J 2007;93:4151–8.

[10] Richmond B. Information coding. Science 2001;278(5345):24933–4.

[11] Chen L, Yong D, Chen WJ, et al. Temporal coding of cultured neuronal spontaneous firing spike train on Multi-electrode array. Prog Nat Sci 2007;17(10):1445–60.

[12] Wu JF, Wang WT, Wei XF, et al. An analysis of neural information within electrogenic hippocampal epileptic network in rats. Acta Biophys Sin 2006;22(4):259–67.

[13] Legendy C, Salcman M. Bursts recurrences of bursts in the spike trains of spontaneously active striate cortex neurons. J Neurophysiol 1985;53(4):926–39.

[14] Cocatre-Zilgien JH, Delcomyn F. Identification of bursts in spike trains. J Neurosci Methods 1992;41(1):19–30.

[15] Kaneoke Y, Vitek J. Burst oscillation as disparate neuronal properties. J Neurosci Methods 1996;68:211–23.

[16] Turnbull L, Dian E, Gross G. The string method of burst identification in neuronal spike trains. J Neurosci Methods 2005;145:23–35.

[17] Chiappalone M, Novellino A, Vajda I, et al. Burst detection algorithms for the analysis of spatio-temporal patterns in cortical networks of neurons. Neurocomputing 2005;65/66:653–62.

[18] Tam DC. An alternate burst analysis for detecting intra-burst firings based on inter-burst periods. Neurocomputing 2002;44/46:1155–9.

[19] Lewicki MS. A review of methods for spike sorting: the detection and classification of neural action potentials. Network: Comput Neural Syst 1998;9:53–78.

[20] Rodieck RW, Kiang NYS, Gerstein GL. Some quantitative methods for the study of spontaneous activity of single neurons. Biophys J 1962;2:351–68.

[21] Dekhuijzen AJ, Bagust J. Analysis of neural bursting: nonrhythmic and rhythmic activity in isolated spinal cord. J Neurosci Methods 1996;67:141–7.