Journal of Neuroscience Methods 259 (2016) 143–155

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Computational Neuroscience

Joint analysis of extracellular spike waveforms and neuronal network bursts

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HIGHLIGHTS

- Spike and burst statistics give limited information on changes in networks.
- Here, spike sorting combined with burst detection.
- Spike waveform type participation in bursts revealed.
- Spike type compositions of bursts change under network modifications.
- New kind of information obtained on the changes in bursting networks.

ARTICLE INFO

Article history:
Received 2 June 2015
Received in revised form
23 November 2015
Accepted 24 November 2015
Available online 7 December 2015

Keywords:
Neuronal network
Microelectrode array
Burst detection
Spike waveform
Spike sorting
Electrophysiological signal analysis

ABSTRACT

Background: Neuronal networks are routinely assessed based on extracellular electrophysiological microelectrode array (MEA) measurements by spike sorting, and spike and burst statistics. We propose to jointly analyze sorted spikes and detected bursts, and hypothesize that the obtained spike type compositions of the bursts can provide new information on the functional networks.
New method: Spikes are detected and sorted to obtain spike types and bursts are detected. In the proposed joint analysis, each burst spike is associated with a spike type, and the spike type compositions of the bursts are assessed.
Results: The proposed method was tested with simulations and MEA measurements of *in vitro* human stem cell derived neuronal networks under different pharmacological treatments. The results show that the treatments altered the spike type compositions of the bursts. For example, 6-cyano-7-nitroquinoxaline-2,3-dione almost completely abolished two types of spikes which had composed the bursts in the baseline, while bursts of spikes of two other types appeared more frequently. This phenomenon was not observable by spike sorting or burst analysis alone, but was revealed by the proposed joint analysis.
Comparison with existing methods: The existing methods do not provide the information obtainable with the proposed method: for the first time, the spike type compositions of bursts are analyzed.
Conclusions: We showed that the proposed method provides useful and novel information, including the possible changes in the spike type compositions of the bursts due to external factors. Our method can be employed on any data exhibiting sortable action potential waveforms and detectable bursts.

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http://dx.doi.org/10.1016/j.jneumeth.2015.11.022
0165-0270/

1. Introduction

Regardless of decades of research, neuronal networks, and their development and functioning, are still not fully understood. The analysis of electrophysiological data is one of the methodologies for advancing our knowledge. Developing new methods to derive more information from the available measurement data is highly desirable. Here, we propose a new joint spike and burst analysis method for analyzing extracellular network electrophysiology data. We illustrate the method with simulated signals, and as the test bench we use human stem cell derived neuronal networks cultured on microelectrode arrays (MEAs). Such networks have been shown to develop spontaneous electrical activity and show histiotypic behavior (Bużańska et al., 2005, 2006; Heikkilä et al., 2009).

MEAs are commonly employed in the assessment of the electrical activity of neuronal networks both *in vitro* and *in vivo*. MEA recordings carry information on the electrical activity in tissues and cell cultures at network and cell levels (Gross et al., 1977; Thomas et al., 1972; Pine, 1980; Egert et al., 1998), *e.g.*, from neurons in the vicinity of the MEA electrodes. Physically, MEAs record extracellular field potentials as voltage signals, which can exhibit contributions from both action potentials and lower frequency neuronal activity, in addition to noise. Here, we consider that an action potential is synonymous with a voltage spike over any area of neuronal cell membrane recorded via a MEA electrode. In the recordings, spikes may occur as individual spikes, or as trains or bursts manifesting network activity (Kandel and Spencer, 1961; Connors et al., 1982; Gray and McCormick, 1996).

To use neuronal networks on MEAs as biosensors was proposed by Gross and Rhoades (1995), who described the effects of several pharmacological agents on bursting, and also mentioned the possibility to measure average spike waveforms. Several studies have suggested various spike and burst related metrics to quantify neuronal network behavior (Bal-Price et al., 2008; Johnstone et al., 2010; Defranchi et al., 2011; Hogberg et al., 2011; Novellino et al., 2011; Alloisio et al., 2015). In previous studies, parameters such as spike count, the number of bursts, mean spike rate, mean burst rate, the number of spikes in bursts, burst duration, interburst interval, and the percentage of spikes in bursts have been commonly used (Johnstone et al., 2010; Novellino et al., 2011; Uchida et al., 2012). Furthermore, patterns and spatial distributions of activity are inherent and crucial aspects in network electrophysiology (Banerjee and Ellender, 2009; Uhlhaas et al., 2009; Crumiller et al., 2011).

Burst analysis is necessary in analyzing network activity and the network effects of different *in vitro* treatments (Johnstone et al., 2010). Previously, several different burst detection methods, mostly based on experimentally pre-defined parameters such as interspike interval (ISI) and the number of spikes in bursts (Chiappalone et al., 2005; Turnbull et al., 2005; Wagenaar et al., 2006; Pasquale et al., 2010), have been proposed, for example, to study rat cortical or hippocampal neuronal networks. Burst definitions which are more adaptive to the analyzed network have also been proposed (Pasquale et al., 2010; Kapucu et al., 2012). Such adaptability is called for in the analysis of maturing networks, such as human stem cell derived networks (Kapucu et al., 2012).

In spike analysis, spike waveform cut-outs are sorted, and the waveforms in each resulting class, or cluster, can be averaged to obtain the representative spike waveform types (Gibson et al., 2012). Despite its challenges, spike sorting is required for isolating or identifying single neuronal cell activities in a population firing in an orchestrated manner (Buzsáki, 2004), and different spike sorting algorithms have been utilized in various studies (Santhanam et al., 2006; Sun et al., 2010; Truccolo et al., 2011). Most related to our work, Illes et al. (2014) utilized raster plots of the sorted spikes.

In this paper, a novel joint analysis of sorted spike waveforms and detected bursts is proposed. The joint analysis provides information on the participation of the spike types in bursts for the particular data at hand. In other words, spike type compositions of the bursts, and their changes, *e.g.*, in time or due to external effects, can be assessed using the proposed framework. To our best knowledge, such a joint analysis has not been proposed previously.

A motivation for the development of the joint analysis has been an earlier study on the relationship between single spike features and network bursting in hippocampal pyramidal cells (Harris et al., 2001), which indicated that conditions that cause high firing rates do not necessarily produce high bursting in pyramidal cells. Moreover, the relation between firing rate and bursting may change differently for bursts with different intraburst ISIs (Harris et al., 2001). This may also be the case with our cells, or with any other neuronal network. If this is the case, increase in the activity of a spike type would not guarantee a higher probability of its participation in bursts. Thus, joint analysis would be necessary to assess the burst participations of different spike types.

The joint analysis is illustrated with simulated data containing spikes with different waveforms, organized as individual spikes and bursts, and demonstrated with real MEA data from *in vitro* human neuronal networks undergoing a pharmacological experiment to alter the networks. We show that the proposed framework yields information on the networks and on the changes therein, which is not obtainable by spike and burst analysis nor by spike sorting alone.

The methods presented in this paper were implemented in Matlab and run in a standard laptop PC. The Matlab code for the proposed joint framework is publicly freely available in the Matlab Central File Exchange (http://www.mathworks.com/matlabcentral/fileexchange/54277-joint-analysis-of-extracellular-spike-waveforms-and-neuronal-network-bursts).

2. Materials and methods

In this paper we demonstrate our proposed joint analysis together with the conventional methods. The methods are organized in three sections: Section 2.1 Cell preparations and the pharmacological experiment; Section 2.2 MEA measurements; and Section 2.3 MEA measurement analysis, describing the spike count statistics, spike sorting, burst detection, the proposed joint analysis (Fig. 1) illustrated with simulated data, and mathematical considerations on the proposed joint analysis.

2.1. Cell preparations and the pharmacological experiment

2.1.1. Cell culturing

Human stem cells were used as the starting material for neuronal cultures (Lappalainen et al., 2010). University of Tampere has ethical approval from Pirkanmaa Hospital District to derivate, culture, and differentiate human embryonic stem cells (Skottman, R05116), and the permission from the National Authority for Medicolegal Affairs (1426/32/300/05) to conduct human stem cell research. After differentiation and subsequent plating on MEAs (Heikkilä et al., 2009), the cultures were grown on the MEAs for seven weeks. Each MEA well was considered as one cell culture.

2.1.2. Pharmacological experiment

The proposed method is demonstrated by analyzing MEA data measured from 12 neuronal cell cultures which were pharmacologically manipulated for different effects on neuronal networks. The pharmacological experiment consisted of the following phases