

Chapter 8.12

Explorative Data Analysis of *In-Vitro* Neuronal Network Behavior Based on an Unsupervised Learning Approach

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ABSTRACT

In the present chapter authors want to expose new insights in the field of Computational Neuroscience at regard to the study of neuronal networks grown in vitro. Such kind of analyses can exploit the availability of a huge amount of data thanks to the use of Multi Electrode Arrays (MEA), a multi-channel technology which allows capturing the activity of several different neuronal cells for long time recordings. Given the possibility of simultaneous targeting of various sites, neuroscientists are so applying such recent technology for various researches. The chapter begins by giving a brief presentation of MEA technology and of the data

produced in output, punctuating some of the pros and cons of MEA recordings. Then we present an overview of the analytical techniques applied in order to extrapolate the hidden information from available data. Then we shall explain the approach we developed and applied on MEAs prepared in our cell culture laboratory, consisting of statistical methods capturing the main features of the spiking, in particular bursting, activity of various neuron, and performing data dimensionality reduction and clustering, in order to classify neurons according to their spiking properties having showed correlated features. Finally the chapter wants to furnish to neuroscientists an overview about the quantitative analysis of in-vitro spiking activity data recorded via MEA technology and to give an example of

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explorative analysis applied on MEA data. Such study is based on methods from Statistics and Machine Learning or Computer Science but at the same time strictly related to neurophysiological interpretations of the putative pharmacological manipulation of synaptic connections and mode of firing, with the final aim to extract new information and knowledge about neuronal networks behavior and organization.

INTRODUCTION

The last decade the Neuroscience field of research has taken great advantage by knowledge and methods from electronics, robotics and informatics, especially in order to develop new micro- or nano-technology instrumentations in neurobiology and molecular biology. Such very effective and profitable exchange of know-how is giving birth to a sort of new scientific expertise, also known under the denomination of “Neuroengineering” and “Neuroinformatics”. Apart the denomination chosen, the roles of mathematics and engineering science has become very important for neuroscientists, covering from the development of the instrumentation used to a number of various approaches in order to analyze neuronal activity. The last one it is the argument we will discuss in the present Chapter, by introducing the specific case of in-vitro neuronal cultured networks, the data analysis issues of the related recordings and some of the statistical and mathematical methods used to elaborate them, with the aim to extract meaningful and undiscovered models and notions about the neurobiological phenomena involved in such living networks.

At this point it is necessary to introduce the instrumentation used for recording from cultured networks, which is the Multi Electrode Array (MEA) (Maher, 1999; Potter, 2001; Segev, 2004; Baruchi, 2007; Shahaf, 2008; Eckmann, 2008; Shein, 2008), a technology able to complement classical methods in electrophysiology and to

solve the considerable limitations given by the single neuron recordings, as from the patch-clamp techniques, given the fact that it is very helpful to retrieve all the information coming from many synaptically connected neurons, with a good resolution both in time and in space, as explained in the following paragraph. The networks of cortical neurons grown on *in-vitro* cultures on MEAs have probably inter-neurons connections much simpler than in vivo population, but despite that they continue to show a behavior showing many of the original features untouched. Neurons on MEA can be of course stimulated in an electrical way or via the application of drugs, or more generally chemical stimuli, as for our Case Studies.

To this aim, a method and a related software tool, able to evaluate Neuronal Network (NN) features during different environment conditions and stimuli is necessary. Given that MEA data can be studied via signal processing, statistics, pattern recognition or Machine Learning methods, we considered techniques from each one, with special regards to the analysis of the neuronal network activity based on a so-called Unsupervised Learning approach. Related methods and algorithms were implemented in a software framework in Python programming language.

BACKGROUND

Brief Overview of Micro Electrode Array Technology and Data Features

MEA instrumentation comes in various different commercial variants (see <http://www.multichannelsystems.com/products-mea/microelectrode-arrays.html>), but the standard MEA presents usually 64 electrodes which are spatially displaced in an 8x8 square layout grid, and in particular the four electrodes at the vertices of such square matrix do not record neurons activity. Electrodes have a very small diameter of about 30 μm and each one is 200 μm far away from each other,

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