Emerging Network Activity in Dissociated Cultures of Neocortex: Novel Electrophysiological Protocols and Mathematical Modeling

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Introduction

10.1 Outline

Since the early attempts at combining micro-fabricated transducers with in vitro neurobiological systems (Gross, 1979; Gross et al., 1985), cultures of neurons dissociated from the vertebrate nervous system have represented a convenient choice for several reasons (Stengler and McKenna, 1994). Neurons can be easily cultured over biocompatible substrates, grown in an incubator and maintained under healthy conditions for several weeks or more (Potter and DeMarse, 2001). This fulfills the requirements of the unconventional approach followed by MEA investigators: instead of invasively probing a single neuron by means of (e.g.) an intracellular glass-pipette electrode, let a population of neurons develop ex vivo and grow around multiple probes, for extended periods of time, under noninvasive conditions. On the other hand, the choice of cultured neurons is also related to the in vitro development of functional synaptic contacts (Nakanishi and Kukita, 1998) and to the emergence of spontaneous patterned electrical activity (Kamioka et al., 1996; Van den Pol et al., 1996). Thus, along a tradition of investigation that is common to physics (Amit, 1989), the possibility of accessing a reduced version of an active nervous system (Bulloch and Syed, 1992) constitutes a unique opportunity for the investigation of network electrophysiology. Indeed, such an approach makes it possible to dissect the interactions among individual neurons of a network and to look for collective mechanisms at the cellular and subcellular levels, through manipulation of the physicochemical conditions.

Under such perspectives, we review in this chapter electrophysiological data obtained from networks of neurons dissociated from the rat neocortex and cultured over arrays of substrate micro-electrodes (MEAs). In particular, we discuss a recent

This chapter is dedicated to the memory of the late Professor Massimo Grattarola, who pioneered the MEA technique in Italy.
experimental approach for the study of cortical network electrophysiology, and introduce a simple theory accounting for the emergence of the in vitro spontaneous collective activity. We conclude with some remarks on the perspectives of novel experimental protocols and mathematical modeling, as complementary tools to MEAs and traditional electrophysiological techniques.

10.1.1 Relevance of the Study of In Vitro Neocortical Networks

In the field of life sciences, we are assisting in an increase of interest for the function of biological networks of elements and for the complexity emerging from the interactions and combinations of such elements (e.g., the dynamics of motifs in neuronal/genetic/metabolic/biochemical networks) (Milo et al., 2002). The underlying inspirations of such a trend suggested interesting analogies with the physics of semiconductors and with the development of modern digital electronics (Grattarola and Massobrio, 1998). In fact, the design of semiconductor electronics proceeded first from very simple devices interconnected in complex manners (i.e., transistors and Boolean logic-gate networks), then evolved into a relatively simple interfacing of highly sophisticated units (e.g., cluster computing, parallel architectures, etc.).

In the case of the design of biological systems, evolution followed an opposite path. It started from the simple combination of complex (bio)molecular compounds (e.g., the assembly of a lipidic bilayer) and went on, assembling the nervous system of mammals, which functionally appears as a highly intricate map of networks, each composed of complex (sub)cellular elements. However, the most recent phylogenetic outcome of evolution, the neocortex, might reveal simpler principles (Douglas and Martin, 1990), irrespective of its anatomical complexity and heterogeneity. In addition, the very same basic electrophysiological processes might be carried out by each small region of the neocortex, by a kind of general-purpose canonical micro-circuitry. Actually, although there are several differences from layer to layer, with regard to projections, cell density, morphology, and size, a stereotypical organization seems indeed to dominate. For instance, Douglas and Martin (1990) focused their proposal on a canonical building block, underlining and emphasizing the tremendous recurrent excitation, estimated as 90% of the total afferent excitation.

In such a context, and under the perspective of ultimately understanding how the synaptic organization of the neocortex produces the complexity of cortical functions, the convergence of a mathematical theory and experimental results is imperative. Such an approach might be also devised to determine how many of the details underlying single channels, dendrites, neurons, and synapses, play a role at higher levels, and whether these details must be fully retained or largely simplified, at the level of large-scale cortical processing description.

One possibility to challenge existing theories and to develop new ones, is represented by the study of in vitro preparations as reduced and highly simplified neurobiological systems, with regard to the network-level electrical activity.