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EFFECTS OF NMDA AND NON-NMDA RECEPTORS ANTAGONISTS ON THE BEHAVIOR OF CULTURED CORTICAL NETWORKS

Laura Bonzano, NBT, Neuroengineering and BionanoTechnologies Group, DIBE, University of Genoa,
Via Opera Pia 11A, 16145 – Genoa, Italy
laura.bonzano@dibe.unige.it

Marco Bove, Department of Experimental Medicine, Section of Human Physiology, University of Genoa,
Viale Benedetto XV 4, 16132 – Genoa, Italy
bove@dibe.unige.it
Sergio Martinoia, NBT, Neuroengineering and BionanoTechnologies Group, DIBE, University of Genoa,
Via Opera Pia 11A, 16145 – Genoa, Italy
martinoia@dibe.unige.it

ABSTRACT

Nowadays several research groups in neuroscience are interested in understanding the basic mechanisms of information processing within the Central Nervous System. Thanks to the application of silicon technology in the field of cellular biology, non-conventional electrophysiological techniques allow to screen the activity of brain tissue or nervous cell cultures in spontaneous and stimulus-evoked conditions.

Activity patterns in cultured networks of cortical neurons from rat embryos (E18) were investigated at different Days In Vitro (DIV), by using a multisite recording technique based on planar Micro Electrode Arrays (MEAs). Transitions in neuronal dynamics of cortical networks were induced administering antagonists of NMDA and non-NMDA receptors. Neurochemical effects on synaptic transmission were evaluated analyzing the whole network activity by means of ad-hoc developed algorithms. A decrease in excitatory synaptic transmission corresponded to a decrease in both spiking and bursting activity with a loss of network synchronization.

This experimental study constitutes a potential approach to quantify how structural modifications in a neuronal network can induce changes in the computational properties of the network itself.

Keywords: Neuronal networks, Micro Electrode Array, Excitatory synapses, Chemical stimulation

INTRODUCTION

A major aim of neuroengineering is to understand the basic mechanisms of information transmission in the Central Nervous System. Towards this goal neuroscientists choose an innovative experimental approach, studying simplified brain models: acute or organotypic slices, or primary neuronal cultures, coupled with Micro Electrode Arrays (MEAs) [1, 10, 12].

Exploiting this neuro-electronic system, it is possible to analyze the in-vitro spontaneous electrical activity of neuronal networks and the changes in activity patterns induced by electrical or chemical stimuli delivered to the network itself [3-5, 7, 8, 11, 20].

In this work cultures of dissociated cortical neurons of embryonic rats coupled with planar MEAs were considered [2, 19]. Particular attention was paid on the role of NMDA and non-NMDA receptors on cortical network behavior. This was done by adding antagonists of these receptors to the experimental solution at different concentrations [2, 9].

Network electrophysiological activity patterns were analyzed for the different chemicals stimulation conditions at both burst and spike level using custom developed algorithms [21]. The possibility to drive cortical network dynamics from a stable state towards another by modifying its excitatory synaptic transmission was explored.

NOMENCLATURE

MEA = Micro Electrode Array
DIV = Day In Vitro
NMDA receptor = N-metyl-D-aspartate receptor
non-NMDA receptor = K (kainate) or AMPA (α-amino-3-hydroxy-5-methylisoxazolepropionate) receptor.
APV = D-2-amino-5- phosphonopentanoic acid, an NMDA receptor antagonist
CNQX = 6-cyano-7-nitroquinoxaline-2, 3-dione, a non-NMDA receptor antagonist

MATERIALS AND METHODS

CELL CULTURE

The devices were sterilized and pre-treated with adhesion factors (Poly-L/D-lysine and laminin) in order to improve the coupling between neurons and electrodes.

Cortical hemispheres were isolated from rat embryos (Wistar rats, gestation day 18), dissociated, suspended in Neurobasal medium and placed on the MEAs (Figure 1). Neurons coupled with the MEAs were then kept in a humidified incubator (5% CO2, 37°C) until the performance of experiments.

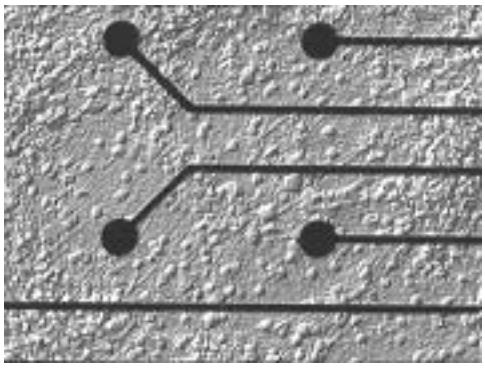

Figure 1 Part of 15 DIV culture of dissociated cortical neurons placed on MEA

EXPERIMENTAL SET-UP

Primary cultures of cortical neurons were plated over arrays of 60 planar microelectrodes (MEA 1060 by Multichannel System, MCS, Reutlingen, Germany) and kept alive in healthy conditions for several weeks.

The network electrophysiological activity was monitored and recorded with an experimental set-up based on the MEA60 System (Multichannel Systems), consisting of:

- The Micro Electrode Array (MEA 1060): a planar array of 60 TiN/SiN electrodes (30μm diameter, 200μm spaced)
- A mounting support with integrated 60 channels pre- and filter amplifier with a gain equal to 1200x
- A pc equipped with MC_Card, a PCI-based data acquisition card (a maximum of 128 recording channels, 12-bit resolution), for real time signal monitoring and long -term acquisition
- The software MCRack used for real time signal monitoring and long-term acquisition from the MC System
- An inverted microscope (connected to a TV-camera)
- An anti-vibration table and a Faraday cage

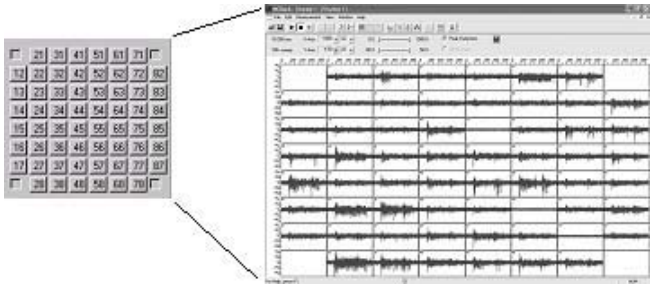

Figure 2 The layout of the MEA and the display of the software MCRack showing the corresponding signals in 1 sec time windows

Neuronal signals were recorded, through all the 60 electrodes, starting from the second week in-vitro in order to allow a certain level of synaptic maturation in the formed neuronal network [17].

Signals were monitored and recorded through the software MCRack, following their fluctuations on a display reproducing the exact layout of the array (an example is given in Figure 2).

EXPERIMENTAL PROTOCOLS

Characterization of the behavior of cortical neuronal networks was achieved performing chemical stimulation protocols in the second and in the third week in-vitro.

Attention was mainly focused on the study of the role of excitatory synapses in the neuronal dynamics.

Two experimental protocols were adopted, consisting of the administering of an NMDA antagonist (APV, D-2-amino-5-phosphonopentanoic acid) and a non-NMDA antagonist (CNQX, 6-cyano-7-nitroquinoxaline-2, 3-dione) to neuronal cultures. APV and CNQX were delivered on four different neuronal cultures each, two at 15 DIV and two at 22 DIV. Experiments were divided into phases corresponding to the delivery of three increasing concentrations of the same drug on the culture (25, 50, 100 μM for APV; 10, 50, 100 μM for CNQX). Experimental sessions started with a 20-min recording of the network spontaneous activity in physiological solution (0.7mM Mg^{++}, 1.3 mM Ca^{++}), defined as control condition. Then chemicals were added to the bath solution, in a not localized way so that the whole network was exposed to them. Recording for each chemical condition lasted 20 min. In both experimental protocols, the chemical treatment phases were preceded and followed by a recording in physiological solution in order to verify the recovery and the stability of the network activity.

DATA ANALYSIS

Extracellularly recorded spikes are embedded in biological and thermal noise, ranging from 10 μV up to 40 μV peak-to-peak, and they can be detected using a spike detection algorithm based on a sliding window, sized to contain at most one single spike (i.e. 3-4 msec), and a peak-to-peak threshold set as a multiple of the standard deviation (7*SD) of the noise for each recording channel [18].

Mean Firing Rate (average number of spikes per second) was calculated on the whole 20min recording for the different antagonists concentrations (0 μM corresponds to the control condition) and normalized, for each experiment, to the mean of the control value.

To take into consideration even the typical bursting behavior showed by cortical neuronal networks [13, 22], an analogous parameter, the bursting rate, was extracted. An algorithm for burst detection was based on spikes timing and amplitude [21], by means of a threshold for the sum of detected peaks amplitudes in combination with an inspecting mobile window in order to ignore isolated spikes.

RESULTS

APV mainly reduced the global activity of the network as shown in Figure 3. Increasing in APV concentration drove the neuronal dynamics from a synchronized bursting activity (Figure 3a and Figure 3b) to an asynchronous behavior (Figure 3c) down to a state characterized by the occurrence of a few isolated spikes (Figure 3d).

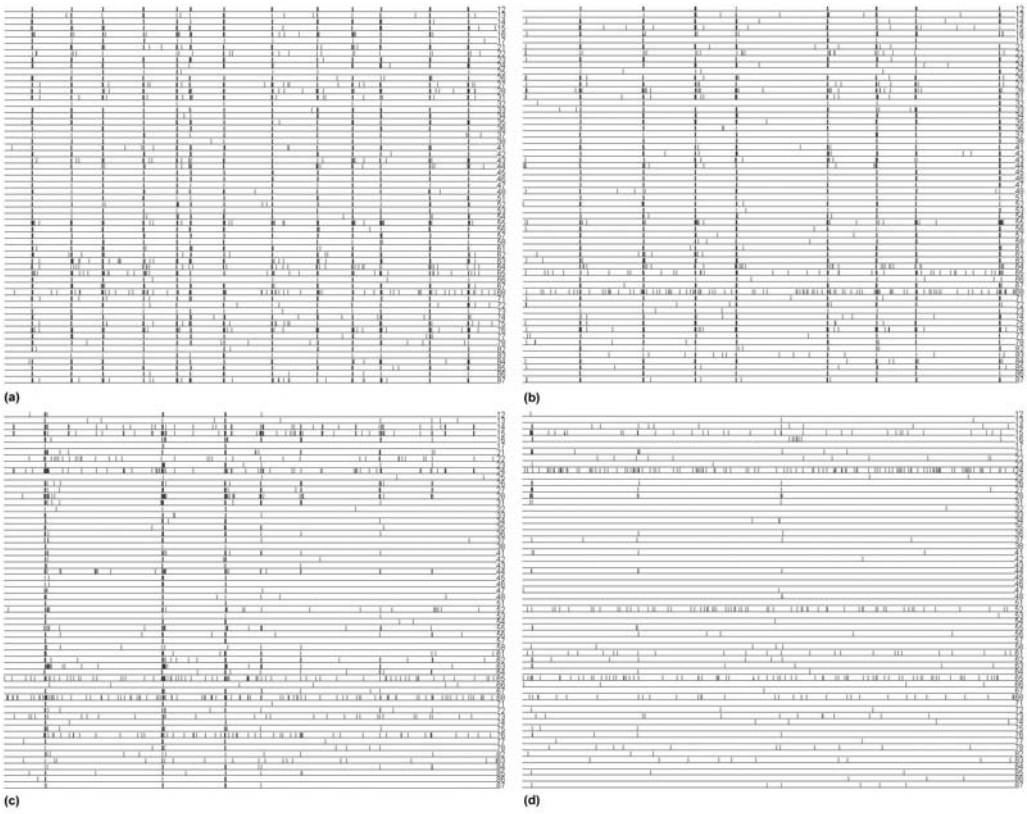

Figure 3. An example of raster plots of a representative experiment, showing detected spikes in 1 minute for each of the 60 recording channels: control condition (a), 25μM APV (b), 50μM APV (c), 100μM APV (d), respectively.

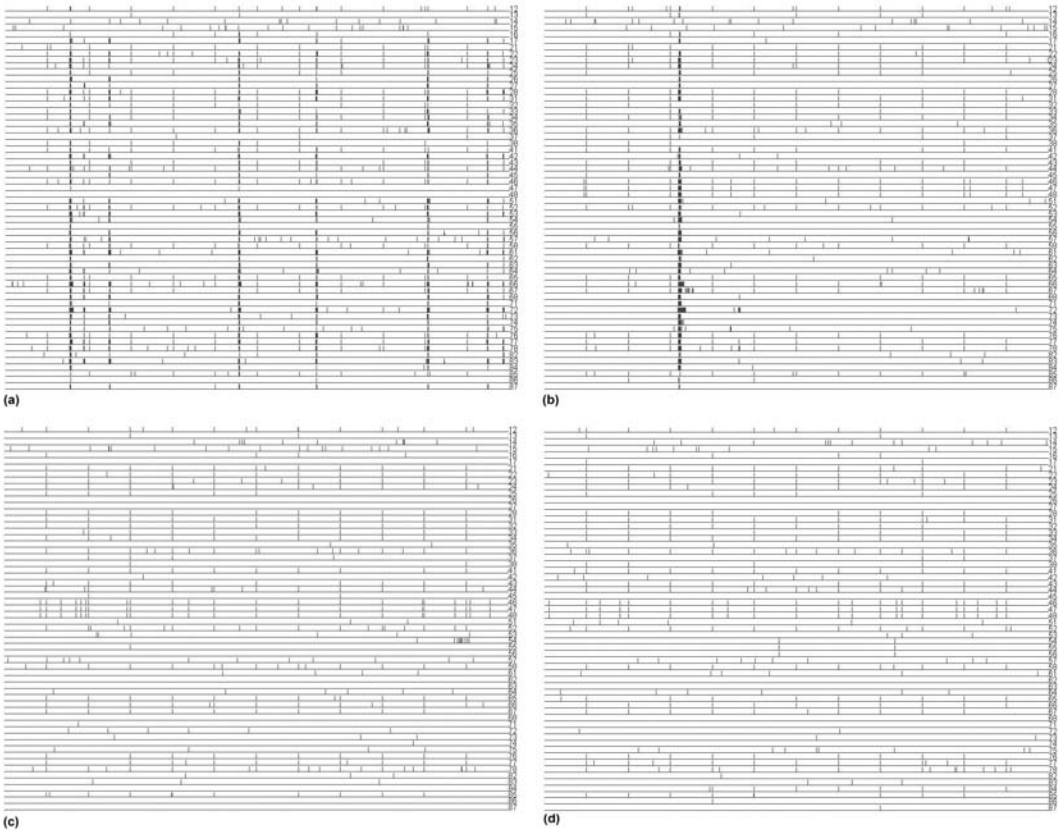

Figure 4. An example of raster plots of a representative experiment, showing detected spikes in 1 minute for each of the 60 recording channels: control condition (a), 10μM CNQX (b), 50μM CNQX (c), 100μM CNQX (d), respectively.

Inhibitory effects were also obtained with CNQX as shown in Figure 4. Increasing in CNQX concentration drastically reduced the neuronal bursting activity (Figure 4b) and at high concentrations a few isolated synchronized spikes were observed (Figure 4c and Figure 4d).

MFR decreased as a function of the APV concentration at both 15DIV and 22DIV (Figure 5a). ANOVA showed significantly different changes across concentration (df = 3,6; F = 163,71; P = 0,000004) and DIV (df = 1,2; F = 19,75; P = 0,047). APV effect was significant when the concentration was over 50μM (Newman-Keuls Post hoc test: for 50μM: P< 0.001; for 100μM: P< 0.001). Interestingly, a significant difference in MFR for an APV concentration of 50μM was observed between neuronal cultures at different stages of maturation (15DIV vs. 22DIV: P< 0.05).

CNQX significantly changed MFR across concentrations (df = 3,6; F = 77,35; P = 0,000035) whilst only slightly across DIV (df = 1,2; F = 12,08636; P = 0,073707) (Figure 5b). CNQX concentrations greater than 10μM induced a significant decrease in MFR with respect to control condition (always P < 0.001). However, among these concentrations no significant difference in MFR was observed since MFR reached a steady state value. It should be noted that at 22 DIV a 10μM CNQX concentration only slightly reduce the MFR activity whilst a strong and significant effect was evident at 15 DIV.

Both APV and CNQX effectively suppressed bursting activity (Figure 6). The inhibitory effect was more evident when the spontaneous activity was better organized [16] and it was remarkable at the highest antagonist concentrations.

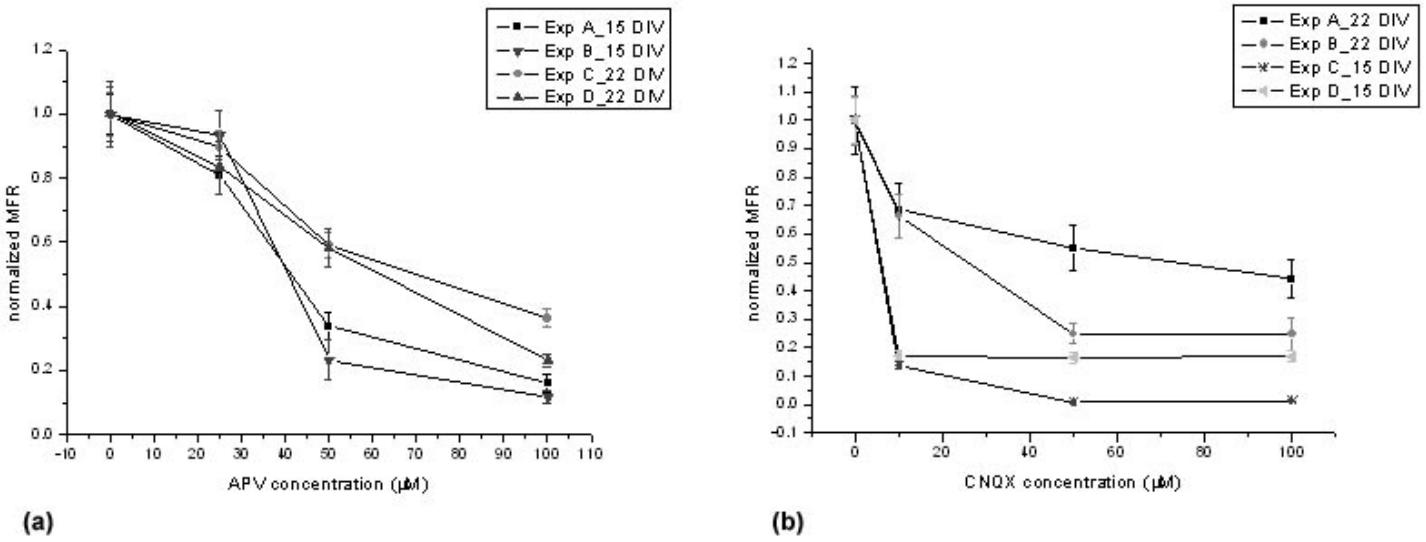

Figure 5. Dose-response curve for APV (a) and CNQX (b): MFR was calculated for experiments performed at 15 and 22 DIV.

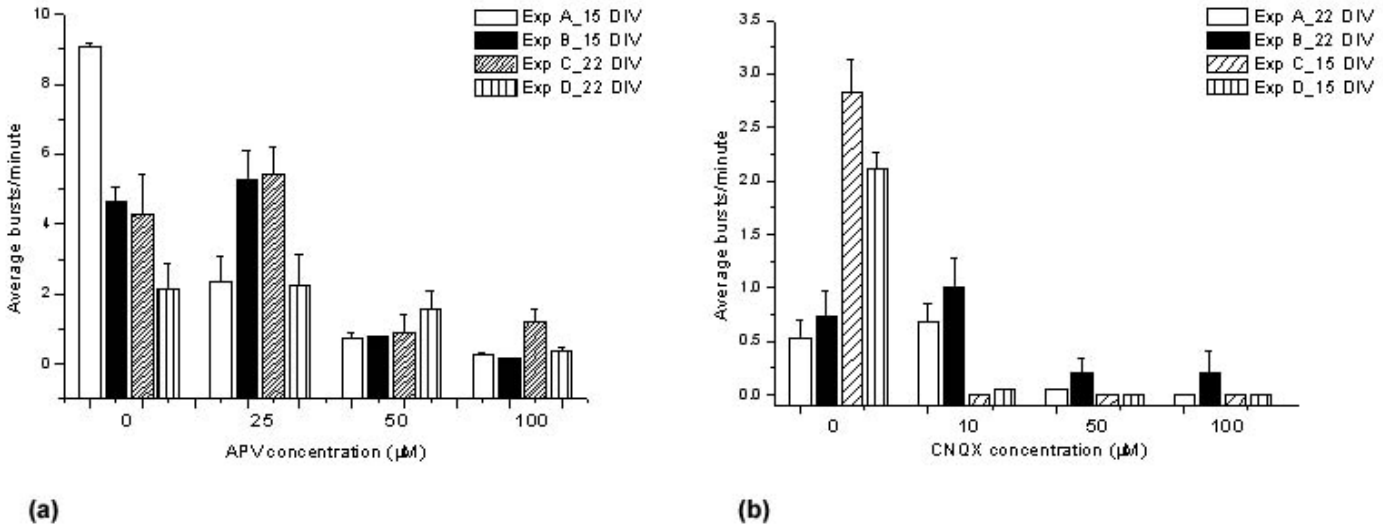

Figure 6. Average bursts/minute vs. APV concentration (a) and CNQX concentration (b).

DISCUSSION

NMDA receptors strongly affect the neuronal activity in cultured cortical networks and modifications in their functionality can alter the electrophysiological dynamics.

Experimental findings concerning the reduced effect of APV on 22 DIV cultures could indicate an increasing in NMDA synapses density [14] between the second and the third week in-vitro. These data are consistent with previous work [17] showing an increase in the development of excitatory synaptic connections in networks of cortical neurons during the same period.

NMDA receptors influence bursting activity as well. However, when the network spontaneous activity has a low bursting rate, APV has only a slight effect for high concentrations. This finding suggests that there could be a correspondence between the density of NMDA receptors and the network bursting rate, which is an indicator of the efficacy of information transmission in the neuronal network.

Even non-NMDA receptors are involved in the definition of the global cortical network activity since the presence of one of their antagonists can strongly reduce the firing rate. However, it seems that the inactivation of non-NMDA receptors does not suppress the whole network spiking activity. On the other side, bursting activity completely disappears with the delivery of CNQX. The maturation of non-NMDA receptors is dramatically important for the resulting neuronal dynamics as observed at low CNQX concentrations.

All these findings show that the spontaneous network activity is supported by neurons which fire only if activated by presynaptic cells and not by neurons showing intrinsically bursting activity [2, 6]. Therefore, the dynamical states of cortical neuronal networks are strongly dependent on the intrinsic properties of the network, especially on the elements which allow the information transmission among neurons [15].

On the basis of the presented results we could hypothesize that, in cultured cortical neuronal networks, the lack of NMDA receptors does not allow the formation of a network stable state in terms of neuronal dynamics (asynchronous spikes). On the other hand, in mature cortical cultures, their presence without the contribution of non-NMDA receptors could maintain the network in a state of synchronous spiking activity without burst occurrence. These phenomena could be explained by (i) a different mechanism of synaptic integration at the level of the two glutamatergic receptors, (ii) a different number and distribution of NMDA and non-NMDA receptors in cortical neuronal cultures during in-vitro development.

CONCLUSIONS

Such experimental approach represents a potential way to measure the relation between structural modifications in a neuronal network and the resulting changes in the computational properties of the network itself. Multisite recording with MEAs constitutes a really powerful technique to study neuronal circuitry, developmental evolution and synaptic potentiation and depression phenomena.

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