Towards an embodied *in-vitro* electrophysiology: the NeuroBIT project

S. Martinoia[1], V. Sanguineti[2], L. Cozzi[2], L. Berdondini[3], J. van Pelt[4], J. Tomas[5], G. Le Masson[6], F. Davide[7]

[1]*Department of Biophysical and Electronic Engineering (DIBE), University of Genoa (ITALY)*
[2]*Department of Informatics, Systems and Telematics (DIST), University of Genoa (ITALY)*
[3]*Institute of Microtechnology (IMT), University of Neuchatel (SWITZERLAND)*
[4]*Netherlands Institute for Brain Research (NIBR), Amsterdam (THE NETHERLANDS)*
[5]*IXL Microelectronics Laboratory, CNRS-ENSEIRB-Université Bordeaux 1 (FRANCE)*
[6] *Institute INSERM E0358 François Magendie, Université Bordeaux 2 (FRANCE)*
[7] *Telecom Italia Learning Services (TILS),Telecom Italia SpA, (ITALY)*

Abstract

In-vitro cultured neurons form a bi-dimensional physical model of the brain. In spite of their simplified level of organization, they provide a useful framework to study information processing in the nervous system. NeuroBIT is an EU-funded project, aimed at developing algorithms and techniques that allow for establishing a bi-directional connection between cultured neurons and external devices, (e.g., robots). The main purpose is to enable 'embodied' in-vitro experiments, in which neural populations are provided with an actual physical body. Embodiment is likely to be crucial in studying the mechanisms of sensorimotor integration, control and adaptation in living systems. Here we present the general objectives of the project, and show the results of preliminary experiments and simulations.

Keywords: cultured neurons, micro-electrode arrays, neural interface

Introduction

Recently, Reger & al. [10] have proposed an innovative experimental paradigm, aimed at studying learning (in particular, sensorimotor adaptation) and, in general, synaptic plasticity in the nervous system. They connected a lamprey brain, isolated and kept alive *in-vitro*, bi-directionally to a mobile robot. Actuators were controlled by the recorded neural activity and sensors were used to drive neural stimulation, so that the robot played the role of an artificial body. Although the brain and the robot

were alien to each other, the resulting bio-artificial system was shown to be able of interacting with its environment (e.g., to follow or escape from a light source). Such capability can be analysed and manipulated experimentally: for instance, it is possible to simulate lesions and then to observe the resulting adaptation processes (if any), or to investigate on-line, closed-loop learning paradigms. In fact, embodiment has been suggested to be an essential condition for emergence of 'intelligent' behaviors. Similar experiments [3, 11] have been performed with populations of neurons, cultured on micro-electrode arrays (MEAs). Although the latter are extremely simplified models of the brain due to their inherently bi-dimensional structure and random connectivity, they allow chronic experiments and multi-site recording/stimulation. DeMarse & al. [3] interfaced a cultured neuronal network to a computer-simulated animal, moving inside a virtual world. Shahaf & Marom [11], through a simple conditioning paradigm, managed to induce a pre-determined, site-specific response. In both cases, very simple spatio-temporal stimulation patterns were used (isolated pulses or short bursts on few sites); however, in order to convey 'sensory' information, the patterns of stimulation that have to be delivered to the preparation should be structured in time, and distributed in space. The aim of the EU-funded NeuroBIT project is to develop the tools and the technologies for connecting portions of living nervous tissue bi-directionally with external devices (i.e., a robot), with the purpose of enabling 'embodied' in-vitro experiments on sensorimotor learning and memory. Here we describe the expected outcomes, discuss algorithms and techniques for interfacing the neural preparation with external devices, and show the results of experiments and simulations.

Materials and Methods

Neural preparation. Primary cultures of cortical neurons were selected as a suitable neurobiological system for chronic experiments[5]. Populations of *in-vitro* cultured neurons are spontaneously active and their pattern of activity can be modulated by means of simple training paradigms[6]. Neurons were extracted from rat embryos (17-18 days), and cultured on planar arrays of 60 TiN/SiN electrodes(Multichannel Systems). A mini-incubator, under development, consisting of a microelectrode array and a heating and temperature control system, will allow longer term experiments and to perform stimulation and/or recording during development.

Characterization of spontaneous and evoked behaviors. Spontaneous firing activity was measured continuously over a period of almost five weeks in vitro. To this end, the culture chamber was firmly sealed to prevent any evaporation of medium. Firing activity was measured by recording the times of occurrence of the spontaneous action potentials with a time resolution of 0.1 msec. For these long-term recordings, we used MEAs with different electrode diameters of 10, 20 and 30 μm, respectively. The spontaneous firing activity was analyzed at several time scales [12]. An overall impression of developmental changes was obtained by calculating the firing rate in number of spikes per hour for each recording site in the MEA over the whole 33 DIV period of recording. At a time scale of minutes the spontaneous firing activity showed an ongoing alternation of periods of low level firing rates and short periods of highly synchronized firing at many recording sites (network bursts). These network bursts have been analyzed in detail focusing on the spatial and temporal patterns of firing within these bursts at a time scale of msec. Network bursts were automatically detected by means of an algorithm based on the product of total

network firing rate and number of active sites. The time point at which this product was maximal was taken as the center of a network burst. Statistical estimates for the patterns of firing within network bursts were obtained by averaging the firing activity of all network bursts detected within periods of 4 hours, aligned according to their time centers.

Additional methods to characterize spontaneous behavior, particularly bursting, included the Inter-Spike Interval (ISI), the Inter-Burst Interval (IBI), and the mean duration of individual bursts [9]. A Wavelet-based denoising algorithm [8], and one for burst detection based on the Hurst parameter were also developed. More specifically, we used a modified version of an algorithm that was originally proposed[1] to monitor fractal-like behaviour of communications traffic.

In studies of the evoked behavior and in closed-loop experiments, we started to record the neural activity of the preparation after 17 Days In-Vitro (DIV) to allow for the formation of mature connectivity. To describe the spatial pattern of firing that is evoked by single stimuli in selected sites, we used post-stimulus time histograms (PSTH) in addition to the techniques developed to study spontaneous behavior.

Bi-directional neural interface. For each experiment, we identified two sets of MEA channels to be, respectively, the recording (Output) and stimulation (Input) sites, i.e. the 'motor' and 'sensory' areas of our model brain. A personal computer running a real-time operating system kernel is responsible for (i) neural recording from the Output sites, and generation of the control signals for the external device; and (ii) recording of the 'sensory' signals from the external device, and generation of the corresponding neural stimulation patterns on the Input sites. A second computer provides an experiment front-end to control system configuration and experimental

parameters. A specifically developed programmable stimulator generates continuously varying spatio-temporal patterns of stimuli. Stimulation channels can be configured individually, by direct programming, or as part of (future) training protocols.

Coding and decoding techniques. Sensory information from the external device has to be translated into a spatio-temporal pattern of stimulation. We assumed that the firing rate of neurons in the 'sensory' portion of the brain is largest when the sensor input has a specific, preferred value, and decreases otherwise. This corresponds to the notion of 'receptive field'. If the sensors are arranged topographically, so that each is characterized by a specific position, d_j, to account for partial receptive field superposition we assume that for each stimulation site, $i = 1,\ldots,M$, there is a 'preferred' stimulus position d_i, so that stimulus intensity, i.e. $s_i(t)$, $i = 1,\ldots,M$, is computed as:

$$s_i(t) = \sum_{j=1}^{6} G\left(\left\| d_j - d_i \right\|\right) \cdot u_j(t) = \sum_{j=1}^{6} G_{ij} \cdot u_j(t)$$

The time-varying activity of the simulated receptive fields was used to modulate the instantaneous frequency of the trains of stimuli delivered to the Input sites. We adopted a rate coding scheme, and treated stimulus intensity as the instantaneous rate of stimulation. We experimented two alternative methods for generation of the corresponding trains of stimuli: Poisson (i.e., rate specifies the mean of an inhomogeneous Poisson-distributed process), or perfect integrate-and-fire (i.e., rate directly modulates the frequency of a pulse train). The two methods correspond, respectively, to gamma distributions of, respectively, order 1 and order ∞ [4].

The raw signal from the Output sites is sampled at 10 kHz, and individual spikes are detected on-line by means of a threshold algorithm that is based on a 3 ms moving window. The threshold is established, for each experiment, during an early characterization phase on each individual channel. Blanking of stimulus artifact is based on the algorithm of Wagenaar & Potter [13]. For each recording site, the instantaneous firing rate $U_i(t)$, i = 1,…,N is estimated through a first order low-pass filter, with τ = 100 ms. The spatio-temporal pattern of neural activity is then translated ('decoded') into a lower dimensional set of motor commands. Any 'decoding' strategy is clearly arbitrary; one possibility (a 'spatial' decoding strategy) is to assume that the motor command is coded into the average activity of each Output region:

$$x(t) = \frac{x_{\max}}{U_{\max}} \frac{1}{N} \sum_{j=1}^{N} U_j(t)$$

Population coding is another simple and biologically 'plausible' rule, based on the idea that each recording site is assigned (a-priori) a 'preferred' control command (in the present case, an angular speed), and the control signal is computed as a weighted sum:

$$x(t) = \frac{\sum_{i=1}^{N} x_i \cdot U_i(t)}{\sum_{j=1}^{N} U_j(t)}$$

In the latter case, normalization guarantees insensitivity of the generated control signals on the baseline spontaneous activity (including network bursts).

Control of a mobile robot. The above coding and decoding methods are relatively general, and can be applied to interface the neural preparation with different kinds of

external devices. To prove the feasibility of closed-loop, embodied experiments, we connected the neuron culture to a Khepera II miniature mobile robot, with two wheels and eight infra-red (IR) proximity sensors, which can move inside a circular playground, containing a number of obstacles. We used two separate sets of recording sites to control the left and right wheels of the robot, and two stimulation sites to code the activity of left and right sensors.

Results

Neuron culture characterization. Cultures can be maintained healthy and spontaneously active over many weeks *in-vitro*. Starting with isolated cells after plating, neurons soon grow out by forming dendritic and axonal arborizations. At the end of the first week in vitro, sufficient synaptic connectivity has been formed to allow the network to generate spontaneously action potentials and synchronized network bursts. During further development the networks bursts showed a significant broadening in the 3rd week in vitro, and a subsequent shortening of the leading edge in the 4th week in vitro. Neurons appeared to fire within network bursts with significant temporal relationships, while these temporal patterns remained highly stable over long periods of recording. Many neurons maintained their phase relations over the full period of recording. Analysis of the mean firing rates in relation to the size of the electrodes revealed that the mean firing rate recorded at electrodes with diameters of 10 and 20 μm was not different, but was doubled at the 30 μm diameter electrode, which implies that the latter has detected action potentials from more than one neuron (multi-unit), whereas the smaller electrodes have recorded activity from single units.

Burst on Hurst Detection (BOHD) Algorithm. To characterize burst activity, we developed an innovative method based on the self-similarity or fractal property of the recorded neural signal. The basic assumption is that self-similarity of the signal increases during bursts. By using the Hurst parameter [7] as an estimator of fractal behavior, we can identify intervals of bursting activity; see Figure 1 for an example.

Figure 1 near here

A distinctive property of this approach is that no previous spike detection is necessary as this approach rejects automatically the noise through aggregation. Analysis is quite robust to the presence of noise, as noise typically has short-term dependences. **Analysis of evoked behaviors**. An underlying requirement for the assumption of functional specialization is that different patterns of stimulation are able to induce different spatial distributions of activity. To assess whether this was the case, we systematically stimulated the preparation on a number of different sites (half the total) with bipolar pulses, delivered every 5 s, and analysed the resulting neural activity at population level. Analysis of IBI, burst duration and PSTH shows that population activity can indeed be modulated; there is a clear dependence of population dynamics on the sites of stimulation, and distinct patterns of activation can be induced.

We developed a simple procedure for selection of the most suitable Input and Output sites (i.e. the sensory and motor areas) of our brain model. We selected as Outputs those sites that were significantly responsive to electrical stimulation. Input sites were selected based on their capability to evoke different spatial distributions of activity on the Output sites. To drive the development of cultured neurons toward functional specialization, we also developed micro-electrode arrays with a compartmentalized structure. We plan to use them in alternative to the matrix-shaped MEAs.

Robot control through neural activity. We initially focused on a simple obstacle avoidance task, i.e. a 'Braitenberg vehicle' [2] as the target behavior. Decoding and encoding schemes were tested in controlling the movements of the robot. Figure 2 shows a portion of a closed-loop experiments. We used 16 sites as outputs (eight per wheel). The speeds of the robot wheels were computed through a 'spatial' decoding scheme. Sampling of sensory inputs and updating of speed commands was performed at 10 Hz. As the robot approaches an obstacle by its left/right side, the increased signal recorded by at least one of the sensors on that part is reflected on the increasing of the stimulation frequency of the corresponding channel; see Figure 2.

Figure 2 near here

Discussion

Network bursts constitute a prominent mode of spontaneous firing in dissociated rat cortical cultures. The observed temporal relationships of firing of individual neurons within these network bursts may reflect the pattern of synaptic connectivity and the flow of spontaneous firing through the network. Therefore, stability of these temporal relationships suggests a stable connectivity, even during periods of developmental changes, so that the spatio-temporal pattern within network bursts can be considered as a dynamic blueprint of the neuronal network. Ongoing research will investigate whether these dynamic blueprints are related to input-output relationships, and whether changes in the network induced by strong stimulation also alter these dynamic blueprints. Experiments on evoked behavior focused on the effects of spatio-temporal stimulation, and confirmed previous studies (e.g. [11]), suggesting that naïve preparations are relatively unstructured, with high connectivity and 'weak' synapses. Spatial selectivity of evoked responses supports the choice of using

specialized regions for input and output. As for closed-loop experiments, a further step will be to experiment stimulation protocols that are driven by robot performance and are capable of inducing changes in selected synapses, thus leading to specific behaviors.

Acknowledgement

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Figure 1. Evolution of the Hurst parameter in relation to the neural signal from one recording site

Figure 2 A closed-loop experiment. A. From top to bottom: neural activity in the output (motor) areas, estimated instantaneous firing rates and computed angular speeds of the robot wheels. B. From top to bottom, activity of the robot sensors, rates and corresponding patterns of stimulation

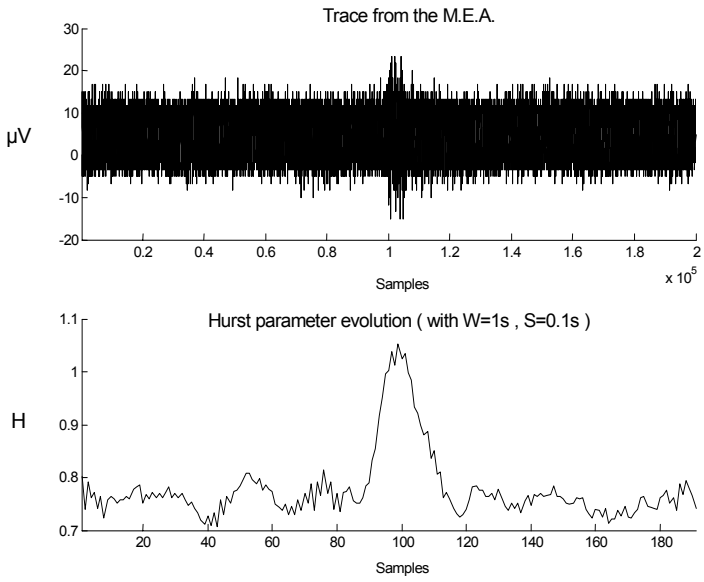
Trace from the M.E.A.
μV
30
20
10
0
-10
-20
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
x 10^5
Samples
Hurst parameter evolution (with W=1s , S=0.1s)
H
1.1
1
0.9
0.8
0.7
20
40
60
80
100
120
140
160
180
Samples

Figure 1

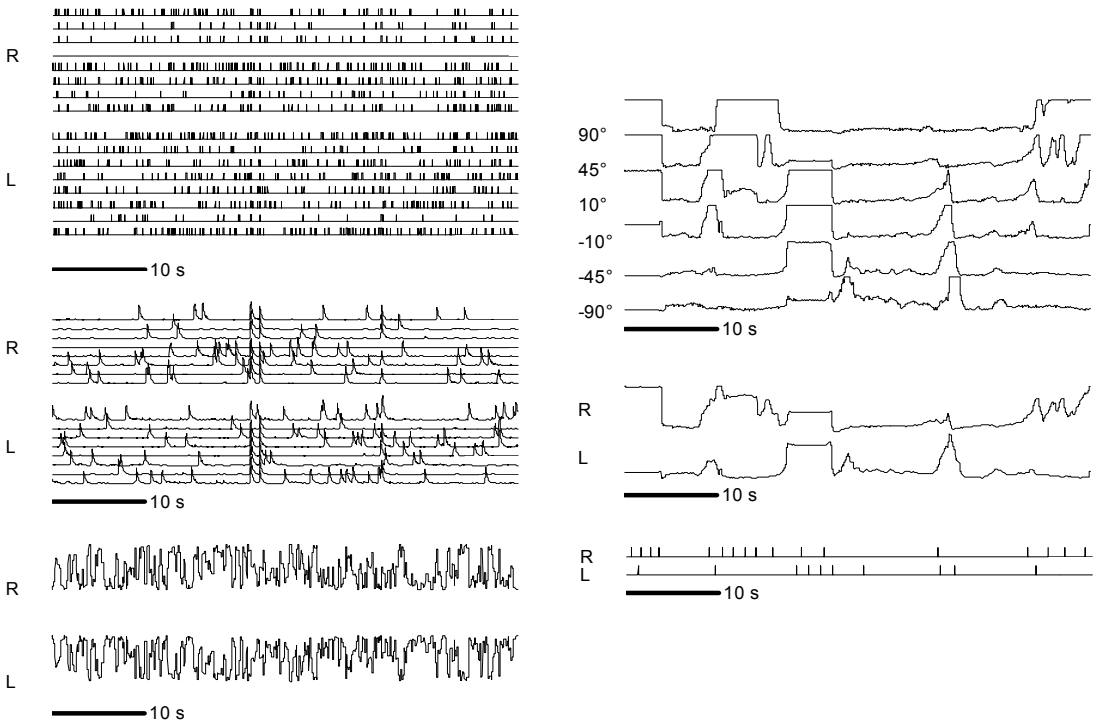

Figure 2