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

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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 for connecting cultured neurons bi-directionally to external devices, in order to enable 'embodied' *in-vitro* experiments, in which neural populations are provided with an actual physical body. Embodiment may 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 experiments and simulations.

Introduction
Recently, Reger & al. (2000) 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 are alien to each other, the resulting bio-artificial system has been 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 (DeMarse & al., 2001, Shahaf & Marom 2001) were 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. (2001) interfaced a cultured neuronal network to a computer-simulated animal, moving inside a virtual world. Shahaf & Marom (2001), 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
Experimental set-up. Primary cultures of cortical neurons were selected as a suitable neurobiological system for chronic experiments (Jimbo & Kawana 1992). Populations of *in-vitro* cultured neurons are spontaneously active, and their pattern of activity can be modulated by means of simple training paradigms (Jimbo & al. 1999). Neurons were extracted from rat embryos (17-18 days), and cultured on planar arrays of 60 TiN/SiN electrodes.

A mini-incubator will allow more long-term experiments, and even to stimulate/record the preparation during development. It consists of a microelectrode array integrated within cluster chambers, and a heating and temperature control system. Both micro-fabrication and conventional mechanical machining are used to build the different parts, in order to facilitate assembly and final use.

The robotic body is a Khepera II miniature mobile robot, with two wheels and eight infra-red (IR) proximity sensors; the robot can move inside a circular playground, with a number of obstacles.

For each experiment, two sets of MEA channels are selected to be, respectively, the recording and stimulation sites, i.e. the 'motor' and 'sensory' areas of the model brain. A specifically developed stimulation device allows to generate continuously varying spatio-temporal patterns. Stimulation channels can be configured individually, by direct programming, or as part of training protocols. The activity at 'motor' sites is recorded by a target PC that implements a real-time controller, which takes care of (i) neural recording and generation of the robot control signals; and (ii) recording of the robot sensory signals and generation of neural stimulation patterns. In future versions, part of the computations will be performed by a DSP-based system. An experiment front-end is available on a host PC, to control system configuration and experiment parameters. An additional PC is used for storing recording and stimulation patterns in a data-base, for subsequent analysis and, eventually, for 'global' long-term modulation of closed-loop experiments.

On-line processing: decoding. Simple thresholding is used for spike detection and to distinguish between spikes and stimulus artifacts. The threshold is established, for each experiment, during an early characterization phase on each individual channel by estimating the noise standard deviation. The resulting spike trains are applied to leaky integrators, consisting of first-order low-pass filters with a 100 ms time constant. The spatio-temporal pattern of neural activity is then translated into a lower dimensional set of control commands. Any 'decoding' strategy is clearly arbitrary; we started investigating population coding as a simple and biologically 'plausible' rule: 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 the linear combination of the preferred commands, weighted by the normalized estimated activities. We used two separate sets of recording sites to control the left and right wheels of the robot. Due to normalization, control signals are insensitive of baseline spontaneous activity.

On-line processing: coding. Sensory information, provided by the IR sensors, has to be translated into neural activity, i.e. a spatio-temporal pattern of stimulation. As for decoding, any coding scheme is arbitrary. We adopted a coding scheme based on receptive fields, i.e. we assumed that the firing rate of neurons in the 'sensory' portion of the brain is largest when an obstacle has a specific, preferred orientation, and decreases otherwise. The time-varying activity of the simulated receptive fields was used to modulate the pulse trains delivered to the stimulation sites. In particular, activity modulated either the instantaneous rate of stimulation, or the mean of an inhomogeneous Poisson-distributed stochastic process (Perkel & al. 1967, Dayan & Abbott 2001).

Off-line analysis and modelling. To characterize population behaviors, we focused on analysis of Inter-Spike Interval (ISI), Inter-Burst Interval (IBI), mean duration of single bursts, and Post-Stimulus Time Histogram (PSTH). A wavelet-based denoising algorithm, and one for burst detection based on the Hurst parameter were also developed. The resulting information, together with simple classification procedures, will be used to define electrode clusters that can be identified as input (i.e., stimulating channels) and output (i.e., recording channels). The above parameters will also be monitored during the different phases of a training experiment to detect induced changes (Novellino & al. 2003).

Different coding, decoding and learning strategies were examined in simulated populations of interconnected neurons, based on either integrate-and-fire models and a Hebbian rule, or biologically realistic conductance-based neurons, using the NEURON simulation environment.

Results

Neuron culture characterization. We started to record the neural activity of the preparation after 17 Days In-Vitro (DIV) to allow for the formation of mature connectivity. Preliminary experiments were performed by using an electrical stimulation protocol consisting of single bipolar pulses, delivered every 5 s. Since an essential requirement for a population coding strategy being effective is that different patterns of stimulation can induce different spatial distributions of activity, we systematically stimulated the preparation on a number of different

sites (half the total) and analysed the resulting population neural activity. 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.

Robot control through neural activity. Decoding and encoding schemes were tested in controlling the movements of the robot. In particular, experiments were performed in which the robot moved in open-loop, driven by spontaneous neural activity. As expected, the observed trajectory was relatively smooth due to the normalizing effect of population coding. Closed-loop experiments are currently under way.

Discussion

Preliminary experiments focused on the effects of spatio-temporal stimulation, and confirmed previous studies (e.g. Shahaf & Marom 2001), suggesting that naïve preparations are relatively unstructured, with high connectivity and 'weak' synapses. Although bursting behavior dominates spontaneous activity, it can be altered by repeated stimulation (Van Pelt & al. 2001). 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.

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