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Bioartificial Brains and Mobile Robots

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1. Introduction

The growth in neuroscience discoveries continues to be explosive, with new frontiers being reached every year in the understanding of new principles of the central and peripheral nervous system (CNS and PNS), in the interplay between structure and function at different scales (from molecules to behavior), and with the introduction of new technologies for direct transfer of information between natural neuronal systems and artificial devices.
Rapid advances in biomedical engineering and computer science are producing the methodologies required for predictive models of neural function that can interact with the brain in real time. The continuous achievements in microelectronics that allow ever-greater circuitry miniaturization together with increased speed and computational capacity are providing the next-generation hardware platforms for neuroprostheses and Brain Computer Interfaces (BCIs) or Brain Machine Interfaces (BMIs). On the other hand, in the last ten years, demonstrations of direct, real-time interfaces between living brain tissues and artificial devices, such as computer cursors, robots and mechanical prostheses, have opened new avenues for experimental and clinical investigations (Nicolelis and Lebedev, 2009). Interest in these BMIs has been kindled by the contribution that they may make to the treatment or rehabilitation of patients suffering from severe motor disabilities (Daly and Wolpaw, 2008; Hatsopoulos and Donoghue, 2009). When motor pathways fail, BMIs offer a physical bridge for movement intention to reach the external world (Donoghue, 2008). The first experimental demonstration that ensemble of cortical neurons could directly control a robotic manipulator was given in 1999 by the group of M. Nicolelis at the Duke University (USA) (Chapin et al., 1999). Since then, a continuous stream of research papers in the BMI field came out. Many groups (Nicolelis and Chapin, 2002; Taylor et al., 2002; Andersen et al., 2004; Schwartz, 2004; Hochberg et al., 2006; Velliste et al., 2008) have successfully shown the possibility of using cortical signals, recorded from different subjects, like rats, monkeys or humans, to move an artificial effector and, in these systems, the feedback was constituted by vision, tactile information and proprioception. BMIs have also been recently used as a tool for studying neural processing of information (Nicolelis and Lebedev, 2009). Nevertheless the limitations arising from the reduced possibility of a full control and observation of the system do not allow a systematic study on how information is processed and transmitted within and among cell-assemblies.

On the contrary, cultured cells or tissue allow for a proper control and observation of the phenomena taking place at cellular and cell-assembly levels but lack of an actual physiological situation in which the brain has a body and the body can interact with a specific environment. Since behavior emerges from complicate interactions of the nervous system with the body and the environment (Chiel and Beer, 1997), "embodiment" and "situatedness" are likely to be crucial in studying the mechanisms of sensory information processing, control and adaptation in living systems. For these reasons, closed-loop systems interfacing nerve cells to external devices have been used as an electrophysiological tool for studying adaptation and coding properties (Reger et al., 2000; DeMarse et al., 2001). More precisely, this framework allows to study interactions within cells and cell assemblies and to investigate the cellular and population mechanisms underlying learning and memory.

These mechanisms can be studied at different complexity levels. Networks of cultured neurons coupled to Micro Electrode Arrays (MEA) represent a kind of intermediate level where the control over the system is high and where it is possible to study universals of learning and memory, regardless of specific realizations (Marom and Shahaf, 2002). It should be noted that this neural preparation is an extremely simplified model of the brain: first of all it has a bi-dimensional structure, in spite of tri-dimensional brain organization; furthermore neural cells are dissociated and they re-organize autonomously even if they preserve functional synaptic interconnections. Nonetheless, the general morphological and physiological properties of the cell populations in culture correspond closely to the features of the original tissue (Shahaf and Marom, 2001).

Following the "embodied neurophysiology" approach, we interfaced a mobile robot with a population of neurons, extracted from rat embryos and cultured over a MEA (Novellino et al., 2007). The proposed paradigm represents an innovative, simplified and controllable bi-directional closed loop system where it is possible to investigate the dynamic and adaptive properties of a neural population interacting with an external environment by means of an artificial body (i.e., the mobile robot). Similar closed-loop approaches have been used to quantify the complexity of neural dynamics (Kositsky et al., 2009), to investigate the transition among different electrophysiological regimes (Wagenaar et al., 2005) and to investigate basic mechanisms of learning (Shahaf and Marom, 2001; le Feber et al., 2010). Closed-loop experiments are also relevant to the technology of neural interfaces (Mussa-Ivaldi and Miller, 2003; Nicolelis, 2003).

Along this line, Mussa-Ivaldi and coworkers (Reger et al., 2000) at Northwestern University proposed the first hybrid neuro-robotic system, an innovative experimental paradigm aimed at studying learning (in particular, sensorimotor adaptation) and synaptic plasticity in the nervous system (Fig. 1A). They bi-directionally connected the brain of a lamprey, maintained alive in vitro, to a small mobile robot. The robot had the function of an artificial body (physical embodiment) that provided sensory feedback to the brain and was controlled by the recorded electrophysiological signal converted in motor commands. In particular, the brain of a sea lamprey larvae was extracted and placed in a recording chamber where it was maintained at a constant temperature in a Ringer's solution. The activity was recorded from the visually identified neurons of the left and right Posterior Rhomb-encephalic Reticular Nuclei (PRRN) of the lamprey brain, and a simple interface decoder converted the spiking activities into driving signals for the corresponding wheels of the small robot. In parallel, an electrical stimulus, whose frequency was modulated by the light intensity perceived by the sensors housed in the robotic body, was delivered near the

region in which the axons of the Intermediate and Posterior Octavo-motor nuclei (nOMI and nOMP) cross.

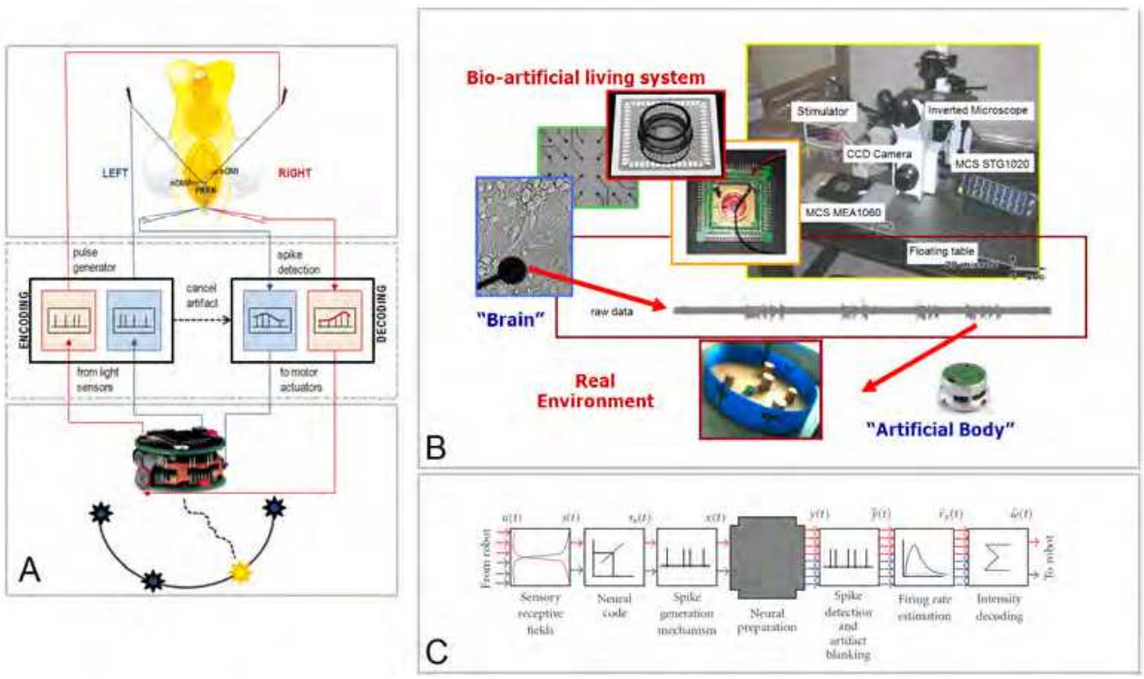

Fig. 1. A) Implementation of the first BMI realized at Northwestern University: a hybrid neuro-robotic system connecting a lamprey's brainstem to a small mobile robot. Signals from the optical sensors of the robot (bottom) are encoded by the communication interface into electrical stimuli, whose frequency depends linearly upon the light intensity. Stimuli are delivered by tungsten microelectrodes to the right and left vestibular pathways (top. nOMI and nOMP: intermediate and posterior octavomotor nuclei). Glass microelectrodes record extracellular responses to the stimuli from the posterior rhombencephalic reticular nuclei (PRRN). Recorded signals from right and left PRRNs are decoded by the interface, which generates the commands to the robot's wheels. These commands are set to be proportional to the average firing rate calculated from the corresponding side of the lamprey's brainstem. The robot is placed in a circular arena with light sources on the periphery. The neural system between stimulation and recording electrodes determines the motions in response to each light source. Modified from Mussa-Ivaldi et al., 2010, with permission. B) Implementation of the closed-loop system involving a bioartificial brain and a mobile robot. Dissociated cortical cells from a rat brain are grown onto a MEA. Once the in vitro neuronal network is formed, it is inspected under a microscope for visual compliance with internal standards. Furthermore, after three weeks of culturing, it is tested for activity, which should be synchronous in the whole network. If such criteria are met, the network is connected to the amplifier and its activity is recorded from selected electrodes to be decoded into motor commands for the artificial body, thus enabling interaction with the physical environment. C) General computational architecture of the closed-loop system: the signals coming from the infrared sensors (IR) of the robot are translated into patterns of stimuli that are delivered to the neural preparation through a set of selected stimulating electrodes. Then the activity recorded by two groups of electrodes is evaluated in terms of firing rate (i.e., mean number of detected spikes/s) and used as driving speed for each of the robot's wheel.

One of the main achievements provided by these experiments was that the alteration of the sensory input caused short- and long-term adaptive changes in the robot behaviors. This suggests that this system can be used as an experimental paradigm for investigating the properties of synaptic plasticity in the context of sensorimotor adaptation. However, one of the major limitations of this experimental set-up was the small number of electrodes used and the simple neuronal circuitry involved in such studies.

An alternative approach for investigating how neuronal information is processed was then proposed by Steve Potter's group at the GeorgiaTech where a new system, to interface an in vitro neuronal population to a computer-simulated environment, was developed. In particular, they employed Micro Electrode Arrays (MEA) dishes to record the neural activity from a population of neurons and use that activity to control a simulated animal, called 'Neurally Controlled Animat' (DeMarse et al., 2001). According to the developers, this approach makes possible a correlation between neural morphology, connectivity, and distributed activity, not feasible with in vivo neural interfaces (Nicolelis et al., 1998; Wessberg et al., 2000). In the preliminary experiment (DeMarse et al., 2001) they created a virtual environment where a simulated body was moved by the spatio-temporal patterns of activity across the living neuronal network coupled to a MEA. The motor commands for the Animat were computed from spatio-temporal patterns that were detected in real time from neural activity. A feedback signal was provided for each movement resulting in a collision with walls or barriers within the virtual environment, by means of electrical pulses. The main achievement of this work relies in the possibility to investigate a system where a living neuronal network communicates in real-time with an external agent which provides feedback to the network, thus generating electrophysiological patterns that can be associated with different behaviors. The concept was then extended to further hybrid devices that Potter and collaborators called "Hybrots", built by interfacing neuronal networks to other artificial devices (Bakkum et al., 2004; Bakkum et al., 2007). The preliminary results indicated that modifications in neural behaviors may occur as a consequence of the inner characteristics (timing) of the sensory feedback stimulation.

The proposed paradigm was not that far from having a simplified tool for studying "learning", seen as an "experience dependent" process (i.e. adaptive process) where the wiring process of the brain needs to be led by experience and by incoming sensory information.

With these premises, following what have been specifically proposed by our group, in the next sections we present the architecture and the developed closed-loop neuro-robotic system (Fig. 1B), the achieved results, limitations of the proposed paradigm and future prospects. Finally we introduce and discuss some open issues and future visions in the field of neuro-hybrid systems and BMIs.

2. A bioartificial brain bi-directionally connected to a mobile robot

Embodiment has been suggested to be an essential condition for the emergence of 'intelligent' behaviors (Chiel and Beer, 1997). This has been shown by pioneer publications (DeMarse et al., 2001; Shahaf and Marom, 2001; Martinoia et al., 2004), in which experiments have been performed with populations of neurons, micro-electrode arrays (MEAs) and then further extended to an actual neuro-robotic platform (Bakkum et al., 2007; Novellino et al., 2007) up to a neural inspired control in a biomimetic robot (von Twickel et al., 2011) and to a "ratcar" in which a vehicle is moved following electrophysiological signals extracted by

the cortical motor area of a rat (Fukayama et al., 2010). Among the possible approaches related to the choice of the robotic platform and of the control system (artificial, bioartificial or biological), there are common issues that can be explored by means of this experimental paradigm, namely: (i) how to convey information to the brain and (ii) how to extract information from the brain. In this framework, we designed an experimental paradigm in which the neuronal cortical culture coupled to a MEA is used as a central processing unit, i.e. the "bioartificial" brain, and it is then connected to an artificial body, i.e. the mobile robot (Fig. 1B). The proposed system acts as a BCI with the general purpose of enabling embodied in vitro experiments on sensorimotor adaptation and learning.

2.1 The bioartificial brain and the electrophysiology recording system

The brain model is an in vitro neuronal network of neurons and glia. In particular, the primary cultures of cortical neurons were prepared from fetal day 18 Wistar SPF rats according to previously described procedures (Chiappalone et al., 2006). Briefly, the cortex was dissociated through enzymatic and mechanical dissociation (0.125% Trypsin – DNAse I 0,025mg/ml – BSA 0.3% solution in HBSS without calcium and magnesium). The cells were seeded on standard and ad-hoc (Berdondini et al., 2005) 60-electrode TiN-SiN MEA chips with internal reference (Multi Channel Systems, Reutlingen, Germany) pre-coated with poly-D-lysine and laminin (0.1 mg/ml diluted in sterile MilliQ water) as 50 µl droplets (1500-2000 cells/mm^2), with subsequent addition of 1 ml of medium after the cells were attached (approximately 2 hours). Cultures were maintained in neurobasal medium (NB) supplemented with 2% B27 and 1% Glutamax-I, and half volume of the medium was exchanged once a week. Cells were maintained at 37^{o}C in a humidified atmosphere of 5% CO_2 until their use.

In these conditions, neuronal cells spontaneously grow by extending their dendritic and axonal arborisations re-establishing synapses. Within a few days of in vitro culture, the neurons already form a functionally connected network reaching the mature state in three weeks (Novellino and Zaldivar, 2010; Hogberg et al., 2011). The neuronal networks coupled to MEA forms a long-term non-destructive two-way interface with the cultured neuronal tissue; and under healthy conditions it is possible to maintain such cultures for months (Potter and DeMarse, 2001).

Experiments were performed in the range 18–42 DIVs, when the neuronal network reaches its "mature" state (Fig. 2), consisting of synchronized clustered activity with minute-to-minute fluctuations in the probability of firing (Marom and Shahaf, 2002).

If a network is electrically stimulated, the neuronal dynamics can deeply change (Novellino et al., 2003); however when weak stimuli are applied, the network shows dynamics whose spatio-temporal features are reproducible to a good degree (Jimbo et al., 2000; Vajda et al., 2008). For this reason, a very critical issue in neuroscience is the definition of a procedure able to induce pathway-specific synaptic changes at the network level either in the direction of potentiation or depression. In literature there are only a few examples of plasticity on cultured networks of cortical neurons (Jimbo et al., 1999; Tateno and Jimbo, 1999; Shahaf and Marom, 2001). More recently, our group demonstrated that a 'network potentiation' could be achieved by using a novel experimental protocol of electrical stimulation (Chiappalone et al., 2008). A high frequency sequence of bursts (internal frequency 20Hz) called 'tetanus' is delivered from one site of the MEA, coupled to a low frequency stimulation, either in-phase or out phase with respect of the tetanic burst, delivered from another electrode called 'co-activation' site.

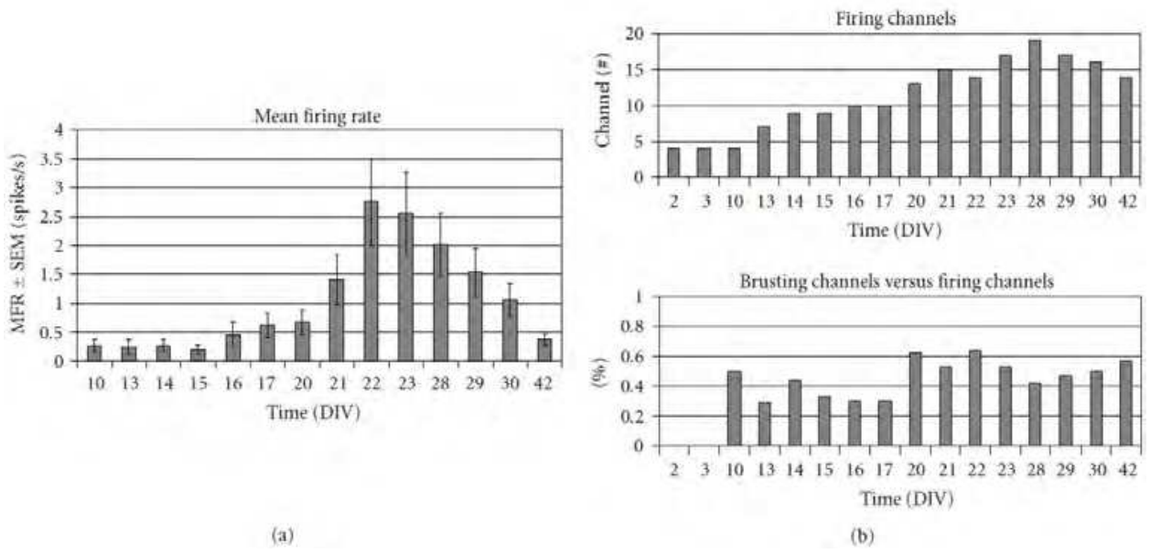

Fig. 2. The mean firing rate (MFR) reports the average spike number during the investigation period (i.e., 10 minutes) and it is an indicator of the spontaneous neuronal activity. Random spiking activity can be recorded at a very early stage of the network development, that is, DIV 2 or 3. The network is still immature and spiking is slow, sporadic, and sparse (i.e., active channels are likely to be far and unsynchronized). After one week of in vitro culture (e.g., DIV10) it is possible to record more frequent activity. The network reaches its maturity at the third week in vitro when activity reaches its peak, and the maximum number of active channels is recorded (top right), and then it starts decreasing. Burst behavior appears during the second week of culture (bottom right). We monitored the network behaviour up to DIV 42.

Experiments regarding the neuronal network electrical stimulation provide a lot of information about the possible functional states in which the network can fall in response to a well-defined stimulation. To mimic this natural morphological condition a specifically designed chip for studying in vitro neuronal network dynamic in interconnected sub-populations of neurons has been used. In particular the device presents physical barriers that induce a minimal constraint of the network in sub-populations and these clusters are interconnected via integrated microchannels (Berdondini et al., 2005). The described devices were demonstrated to guarantee a spontaneous synchronized global behavior and at the same time the sub-population of the stimulated cluster presents a higher probability of evoked response without preventing the stimulus propagation and activation of other sub-populations (Berdondini et al., 2005).

In order to record activity, the neuronal network has been coupled to the MEA60 system supplied by Multi Channel Systems (MCS, Reutlingen, Germany). Briefly the experimental setup is constituted by the following elements (Figure 1.B): a neuronal preparation cultured over an MEA, a mounting support with a 60-channel amplifier (gain 1200x), a homemade 8-channel stimulus generator, to deliver both current and voltage desired stimulating signals, an inverted optical microscope, connected to a CCD camera (Hamamatsu, Japan), to visually monitor the cells during the experiment, an antivibration table and a Faraday cage (for further details see Novellino et al. 2007).

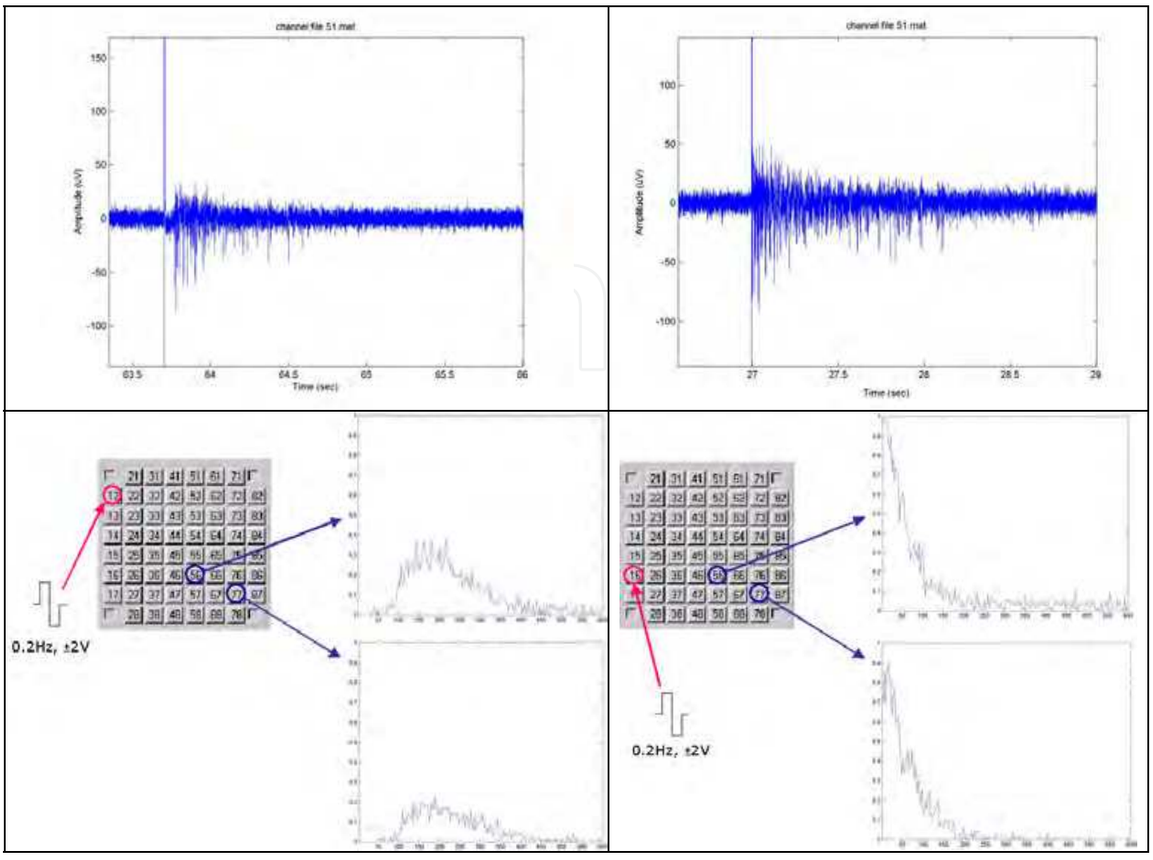

Fig. 3. Example of the neuronal activity recorded while applying an electrical stimulation to cell preparation. Top left, Zoom of the response after a stimulus: a "delayed" burst on channel 51 is evoked by the stimulus from channel 12. Top right, An early burst on channel 51 evoked by the stimulus from channel 72. Bottom left, Comparison between the PSTHs built by means of the activity arising from channels 56 and 77 after stimulation from electrode 12. The shape of the PSTH is the same on the two considered channels ("delayed response"). Bottom right, Comparison between the PSTHs built by means of the activity arising from channels 56 and 77 after stimulation from electrode 16. The shape of the PSTH is the same on the two considered channels ("early response").

The artificial body is a small mobile robot (Khepera II, K-team, http://www.k-team.com) equipped with eight infrared (IR) proximity sensors that provide information about the distance of the robot from obstacles and two wheels whose speeds are determined by neurons activity. These allow movements of the robot inside a circular arena (80 cm diameter), and interaction with several cylindrical obstacles.

2.2 Computational architecture, coding and decoding of information

To specifically implement a demonstration and to carry out experiments in the direction of adaptation in a closed-loop scenario, we developed an experimental set-up in which a bioartificial brain (the neuronal network) and its body (the mobile robot) were challenged to learn by experience a simple 'intelligent' adaptive behavior: to move in an unknown environment avoiding obstacles.

A model of adaptive behavior is represented by an agent who is motivated in trying to survive in a defined environment, without any external (i.e., human) help. The agent may generate its actions exclusively from the available sensory information, or may use some kind of previous "experience." The former type of agent is generally referred as "reactive" (Maes, 1994; Brooks, 2002).

To establish a bidirectional communication between the neuronal preparation and a mobile robot, the electrophysiological signals need to be translated into motor commands for the robot (decoding of neural activity), and at the same time the sensory signal from the robot need to be translated into a pattern of electrical stimulation (coding of sensory information). Figure 1C presents the general computational architecture of the proposed closed-loop system that can be summarized in the following three main logical blocks (i.e., from left to right in Figure 1.C).

1. Coding (i.e. the representation of the external sensory input in terms of electrical stimulation from the robot to the neural network): while the robot freely moves into the playground, its IR sensors see whether or not an obstacle is in the proximity and where it is (left or right side). The IR signals are weighted according to a sensory receptive field law and the two resulting stimulation signals, relative to the right and left eye of the robot, are then coded into a feedback stimulation according to a "binary" information coding (i.e. 0 or 1 Hz). The electrical stimulus is delivered when the sensory feedback overcomes a threshold, corresponding approximately to the presence of an obstacle at 5 cm distance.
2. Processing of electrophysiological signals: the spontaneous or evoked electrophysiological activity is sampled at 10 kHz and processed by means of an event detection algorithm (i.e. spike detection, see further), which also removes stimulation artifacts.
3. Decoding (from the neural preparation to the robot): the processed electrophysiological signals are translated into motor commands for the robot. In particular the instantaneous firing rate is low pass filtered to compute the "neural activity" that is then decoded into motor commands (wheels speed) according to a winner-takes-all (WTA) algorithm (see below).

The motor commands ω(t), that is, the angular speeds of the wheels, are obtained by implementing the following winner-takes-all (WTA) mechanism:

$$\begin{cases} \omega_L(t) = \begin{cases} \left(\omega_0 - \sum_{i=1}^{N} C_i * \left[\hat{r}_i(t) \right]_R \right) & \omega_L \geq \omega_R \\ -\omega_b & \omega_L < \omega_R \end{cases} \\ \omega_R(t) = \begin{cases} \left(\omega_0 - \sum_{i=1}^{N} C_i * \left[\hat{r}_i(t) \right]_L \right) & \omega_R \geq \omega_L \\ -\omega_b & \omega_R < \omega_L \end{cases} \end{cases}$$

where ω_b is a constant angular speed (up to 2 rad/s), ω_0 is the maximum angular speed (i.e., 5 rad/s); $\hat{r}_i(t)$ is the instantaneous firing rate of the recording site i, C_i is a normalization coefficient and represent the inverse of the estimation of the maximum value that can be reached by the instantaneous firing rate on each group (left versus right) of electrodes. L and R indicate signals pertaining respectively to the left and the right wheel.

The control law implements inhibitory crossed connections between inputs and outputs: in absence of neural activity, the robot moves according to the maximum angular speed of both the wheels, whereas an increase in the activity results in a fixed decrease of the speed of the opposite wheel. WTA helps the robot turning away from the obstacle by increasing its rotational speed, on equal evoked response. This allows to better achieve a reactive control task, overcoming the problems caused by the short range of the IR sensors of the robot.

2.3 Data processing and statistics

Spike detection. The electrophysiological signals $y(t)$ acquired from MEA electrodes must be pre-processed in order to remove the stimulus artifact and to isolate spikes from noise. The spike detection algorithm uses a differential peak-to-peak threshold to follow the variability of the signal (Bove et al., 1997). A time window, sized to contain at most one single spike (4ms), is shifted along the signal, sampled at the frequency of 10 kHz. Within the window, when the difference between the maximum and the minimum exceeds the threshold, a spike is found and its time stamp is saved. In this way, the resulting spike train signal is sampled at 250 Hz. The threshold is proportional to the noise standard deviation (SD) and is calculated separately for each individual channel (typically as 7 × SD) before the beginning of the actual experiment.

Blanking of stimulus artifact. Stimulus artifacts are detected when the recorded signal exceeds a second, higher threshold. The artifact is then suppressed by cancelling the first sample in the spike train occurring immediately after it, corresponding to a signal blanking of 4ms after stimulus delivery.

Post-stimulus Time Histogram. To investigate the neural activity evoked by stimulation, we computed the post-stimulus time histogram (i.e., PSTH), which represents the impulse response of each site of the neural preparation to electrical stimulation (see Fig. 3). The PSTHs were calculated by taking 400 ms time windows from the recordings that follow each stimulus. We then counted the number of spikes occurring in a 2-4 ms bin and divided this measure by the number of stimuli (Rieke et al., 1997). For our cultures, typical PSTHs show an "early" (less than 50 ms) and a "late" (50–250 milliseconds) component (Marom and Shahaf, 2002; Cozzi et al., 2006).

Functional Connectivity Index (FCI). The amplitude of the early response to stimuli can be taken as an indicator of the functional connectivity between stimulation and recording site (Cozzi et al., 2006). The PSTH area over 100ms from stimulus averages the early evoked network response and thus it represents the network Functional Connectivity Index (CFI) and helps in differentiating strong and weak functional input/output connection.

Stimulus-Triggered Speed. The stimulus-triggered speed (STS) is constructed by averaging the speed waveform due to each stimulus. The STS indicated the average wheel speed (that is proportional to the neuronal activity of the "brain" region connected to that wheel) in response to each stimulus (sensory feedback). The 4 STS curves (i.e., the variations of the speed of the left and the right wheels in response to the left stimulus are the first two curves and constitute the first STS, and the variation of the speed of the left and the right wheels in response to the right stimulus constitute the second STS with the latter two curves) are a representation of the sensory-motor pathways and also indicate

the robot behavior performance and side-selectivity relationship between stimuli and speeds.

The behavior of the neurorobotic system can be evaluated by means of the *stimulus-triggered speed (STS)*, that is, the average linear speed elicited by a single electrical stimulus, and, as already showed (Novellino et al., 2007) this is the best parameters to catch the relationship between electrophysiology and robot performance and its evolution.

2.4 Experimental procedure

The experimental protocol consisted of the following steps:

1. baseline:

a. spontaneous activity recording (5 minutes);

b. pre-processing: test stimulus from 8 channels (serial stimulation). To test the response to stimulation from different sites in different areas of the neuronal network, trains of 50 electrical stimuli are delivered (±750mV amplitude, 500μs duration, and duty cycle 50%).

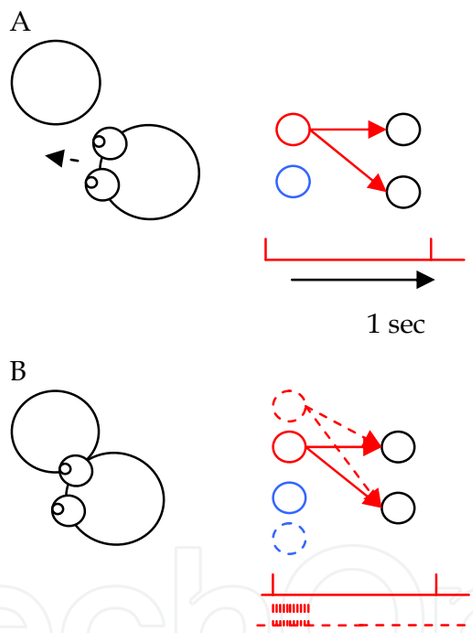

Fig. 4. Schematic representation of the sensory feedback stimulation as well as the conditioning stimulation protocol. A. Let's assume the robot is approaching the obstacle. When the robot is close enough to the obstacle, the robot's eyes detect the obstacle and the sensory feedback (1Hz) is delivered from the input neurons (red circle) to evoke activity in the output neurons (black neurons). If the robot bumps into the obstacle we can have two different stimulations: if the system is running the "non-conditioning phase" of phase 3.a and 3.c, the stimulation is only delivered from the input neuron, as shown in this panel. B. If the system is running the conditioning phase (i.e. the experimental phase is 3.b), and the robot bumps into the obstacle the co-activation neurons (i.e. red dashed circle) are enabled to deliver the "in-phase tetanic stimulation" in parallel with the normal feedback stimulation (bottom) to enhance the robot reactive behavior performance for obstacle avoidance.

2. input-output mapping:

Reactive behavior means the robot is able to immediately react to the presence of an obstacle where immediately means in a time shorter than 100ms, that is, we need input-output pathways characterized by a relatively early (up to 50 ms) and sustained response meaning a "high strength" in the functional connectivity (Novellino 2007). Practically at least 2 channels for input (sensors), 2 channels for conditioning input (sensors), and 2 channels for output (motors) were chosen according the stimuli induced activity and "selectivity maps".

3. closed–loop experiment: Robot running (5 + 5 + 5minutes):
a. free running;
b. obstacle avoidance with the application of the in-phase tetanic protocol (when the robot hits an obstacle, a conditioning stimulus is delivered from the collision side), according to our previous studies (Chiappalone et al., 2008).
c. free running.
4. Input/output mapping checking:
a. spontaneous activity recording (5minutes);
b. test stimulus from the chosen stimulating channels.

To avoid manual removal of the robot and possible damage due to wheels' motors heating in case of a collision against an obstacle, a step-back mechanism has been enabled. Figure 4 schematizes the 3rd step.

3. Results and discussion

3.1 Spontaneous activity requirements

We applied a quite restrictive selection of the experimental chips. In particular we only selected mature cells (i.e. after the third week in vitro) which presented synchronized native bursting activity (minimal bursting rate 0.2Hz) all over the network. I/O pathways (and co-activation sites) selection consisted in serial delivery of 50 stimuli from at least 7 different sites (i.e. 350 stimuli).

3.2 Identification of input-output sites

In order to obtain a reactive behavior, we need the network to promptly respond after the feedback stimulation, that is, we need input-output pathways characterized by a relatively early (up to 50 ms) and sustained response meaning a "high strength" in the functional connectivity. If the network reacts to the sensory feedback and the evoked electrophysiological response is characterized by a relatively long activation phase (up to 200–300 ms), the robot would not be able to react to the presence of an obstacle in 100 ms (i.e., the delay among successive serial communications between the system and the robot). This is one of the reasons why we need to accurately select the input-output pathways. We need the stimulus-evoked response to be fast, prolonged, reliable, and therefore effective for the entire duration of the experiments (i.e., all day long). As already said, the general aim is to have a robot that follows a specific task on the basis of the spontaneous/stimulated electrophysiological activity shown by the neuronal culture. To this end, it is a fundamental prerequisite to characterize the collective activity of the network that will be connected to the robot (i.e., analysis of both spontaneous and stimulus evoked neuronal activity).

The post-stimulus time histogram (PSTH) (i.e., the average number of spikes obtained in response to a stimulus, at different latencies) is then used for quantifying the strength of connections between a specific stimulating sites and all the other recording sites (Figure 4).

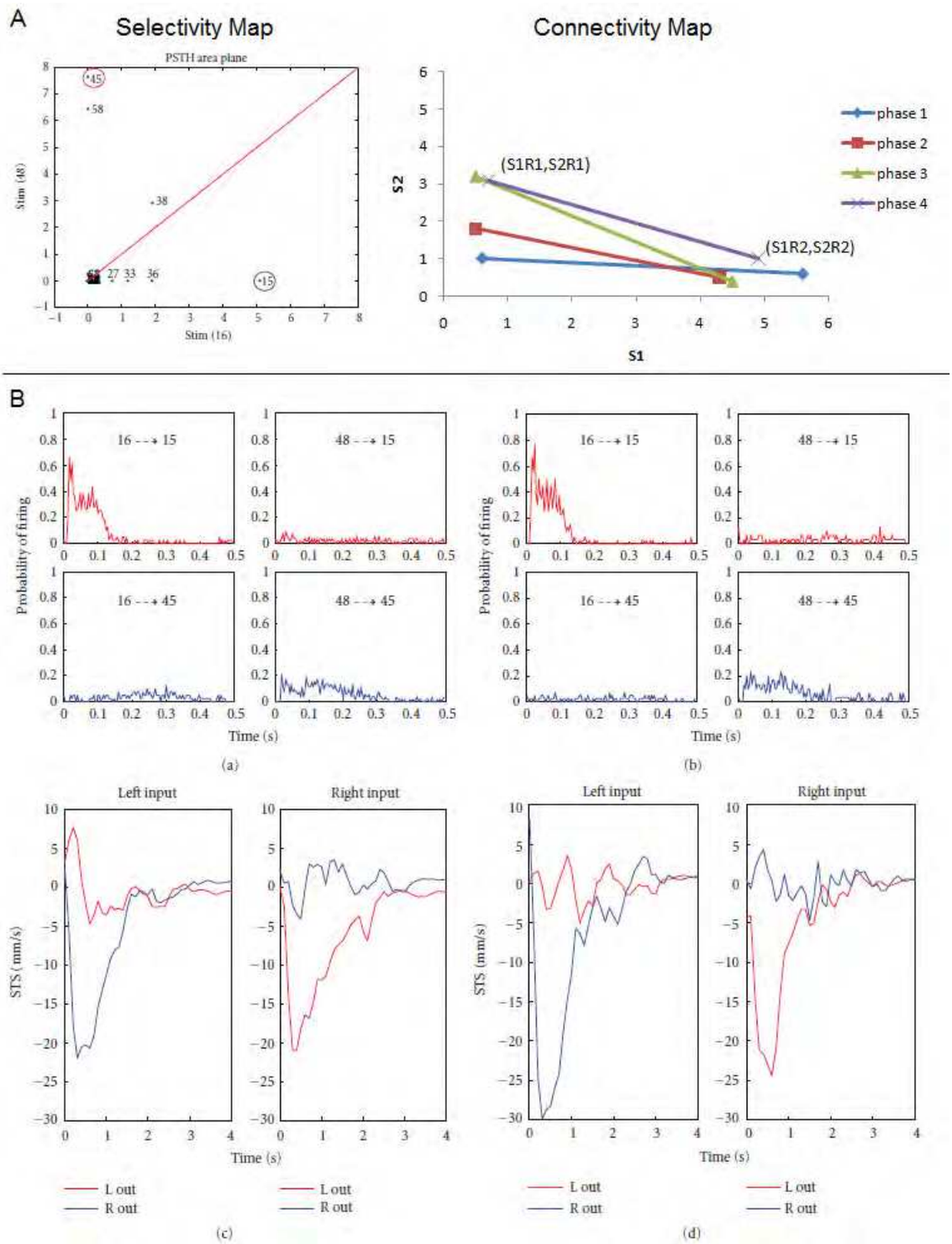

Fig. 5. **A)** (left) Selectivity Map for a representative experiment: each point represents the evoked activity in a channel from the two stimulation channels, i.e. x-coordinate is the PSTH area in response to electrical stimulation delivered from Stim 16 (S1), and y-coordinate is is the PSTH area in response to electrical stimulation delivered from Stim 48 (S2). The circled

channels are the channels that answer with the best selectivity. (right) Connectivity Map for a representative experiment: displacements of the right and left recording channels during the four acquisition phases in closed-loop on the basis of their PSTH areas with respect to the right and left stimulating channels. Phase 1 indicates the first 'unconditioned' phase. **B)** PSTH and STS in a neurorobotic experiment. (a) PSTHs for two electrodes (chosen as recording—motor electrodes) with respect to two stimulating sites. Electrode 15 responds well to stimulation from electrode 16 and poorly to stimulation from electrode 48; on the contrary electrode 45 responds well to 48 and poorly to 16. This tendency is maintained and even improved after the robotic experiment (b). The STS graphs before (c) and after (d) the robotic experiment proves again the selectivity of the chosen electrodes and the improvement in the performances (increased STS area).

It is the impulse response (in terms of instantaneous firing rate) to a single test stimulus. The algorithm for the selection of the output (motor) and input (sensory) sites supplies the I/O pairs corresponding to maximum selectivity and it is based on network effective functional interconnectivity, i.e. Selectivity Map, then it is used to check the evolution of the selected I/O pairs, i.e. Connectivity Map (Figure 5A).
The ideal case is described in the following: given two (or more) stimulating channels (e.g., S1 and S2) and two groups of recording sites (e.g., R1 and R2), the strength of the connectivity S1-R1 and S2-R2 is "high" and simultaneously, the strength of the connectivity S1-R2, and S2-R1 is "low" (i.e., good selectivity in input-output pairs). The described scheme guarantees, somehow, that the responses in the two (groups of) recording sites are different on the basis of the stimulating electrodes. Of course the above is an ideal situation and, since the mean connectivity of the network is high, also due to the high density of plated cells, it is hard to get highly specific responses in the input-output pathways.
The methodology that we developed to make a selection of the pathways is the "selectivity map". Each dot represents the PSTH area at a specific recording site given that there was a stimulation from a couple of stimulating sites (Fig. 5A). All the possible input-output combinations are explored and only the pathways producing responses lying more distant from the diagonal (i.e., closer to the axis) are selected. Those specific pathways (of sensory-motor activations) can be then conveniently utilized for driving the robot and for implementing simple reactive behaviors (e.g., obstacle avoidance), as presented in previous sections.

3.3 The robot behavior

Figure 6 shows an example of the robot behavior during three phases of the experiment. The mobile robot starting position was indicated by a green circle, robot position was sampled at 0.5Hz. The robot was moving inside its playground and occasionally it hit either obstacles or playground walls (red dots). During the learning phase when the mobile robot bumped into any obstacle the punishing-conditioning feedback stimulation (11pulses at 20Hz) from the co-activation site was delivered. The robot performed its explorative-obstacle avoidance reactive behavior into the playground for 10 minutes for any phase (see Methods).
Robot behavior performance parameters, such as number of hits, covered space etc, do not allow quantifying any relationship between the motor response and the sensory information, but, considering different phases, if the robot covered the same area and the trajectories are in the same order of length, then the two phases are comparable. A reduction of the number of hits should indicate an improvement of the robot's behavior; anyhow any improvement in the robot's behavior must correspond to an improvement in the relationship

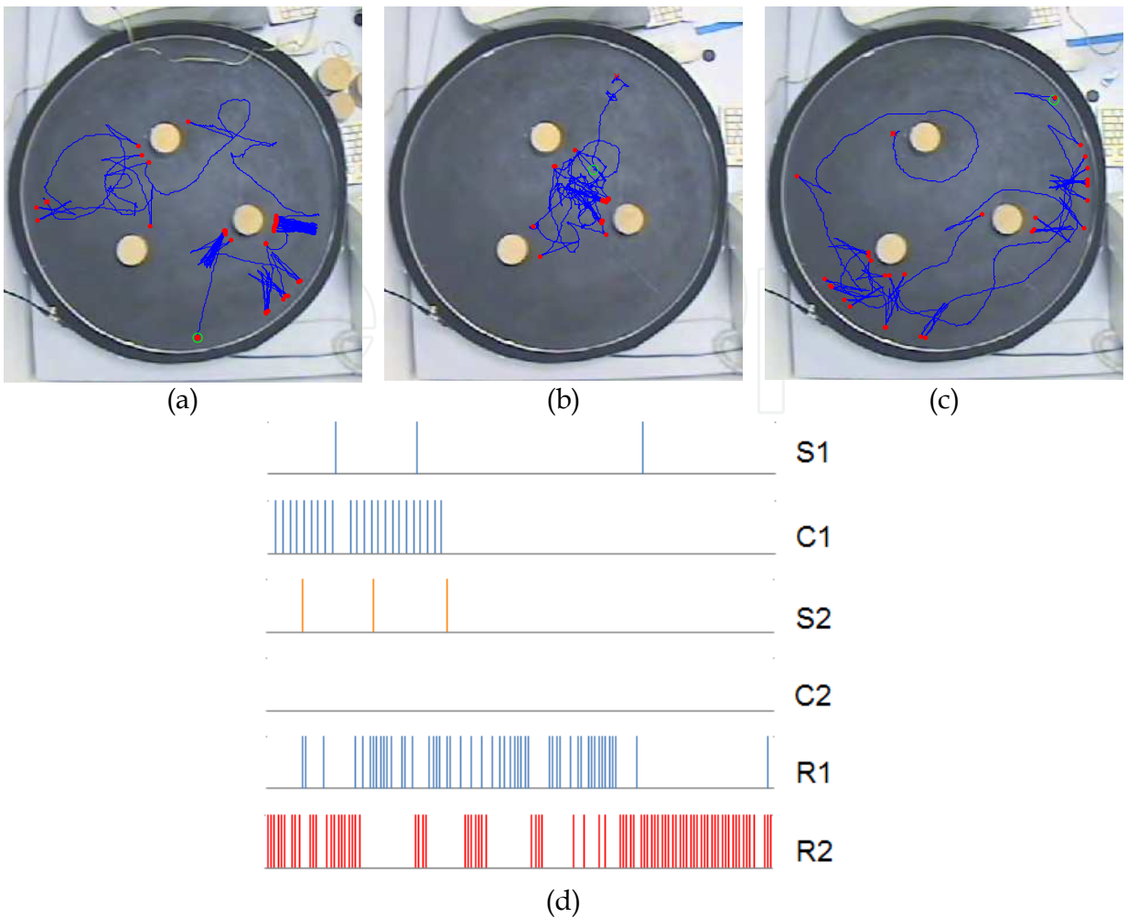

Fig. 6. Robot trajectories and performances in a neurorobotic experiment. (a) Robot trajectory during the first free running phase. (b) Robot trajectory during the learning phase. (c) Robot trajectory during the last free running phase. (d) From top: right feedback stimulus, right co-activation stimulus, left stimulus, left co-activation stimulus, two right output channels and two left channels. The window is 5 sec. It is possible to note that according to the WTA method (see Methods) only the right co-activation stimulus was delivered.

between the motor response and the feedback sensory information (i.e., the STSs). Furthermore the shapes of the PSTHs must be similar to those of the PSTHs obtained during the characterization phase in order to ensure the stability of the response of the neuronal culture. If the area of the PSTHs drastically decreases at the end of the closed loop phases it means that the neuronal network has been fatigued by excessive repeated stimulations (Shahaf and Marom, 2001). The wellness and stability of the culture are "sine qua non" conditions to be verified before describing the neurorobotic behavior by means of the robot's performance indicators. Under these conditions, the performance of the neural preparation in controlling the robot can be represented by the STSs, depicted in Figures 13(c) and 13(d). The STS is the only parameter that permits to understand and demonstrate whether a different behavior of the robot actually corresponds to a different dynamics of the neuronal activity, and for this reason it can be considered the best indicator of the performance of the overall neurorobotic system. The comparison of the STSs and

connectivity maps obtained during each phase illustrates that a modification in the robot's behavior has occurred.

4. Conclusions and perspectives

The possibility to control actions from thoughts is becoming reality thanks to recent advances in Brain Machine Interfaces (Donoghue, 2008). However modern BMIs are mostly unidirectional (i.e. without providing sensory information to the subject) and aimed at restoring lost motor functions at the level of the Peripheral Nervous System. In a wider perspective, neural interfaces must be bi-directional devices that substitute motor, sensory or cognitive circuits within the brain, which might have been damaged as a result of an injury or a disease. In this context it becomes crucial to gain the necessary knowledge to be able to efficiently interact with the nervous system to realize new class of neuroprostheses and BMIs aimed at treating those diseases where a portion of CNS or PNS is damaged.

An in-vitro bi-directional closed-loop neuro-robotic interface, as the one we presented here, goes in the direction of elucidating and optimizing means to interact with the nervous system. Using simplified experiment models, the proposed approach aims at shedding some light into the mechanisms of information coding and of efficient bi-directional interaction with external artificial devices. At the same time, the proposed system has indeed some important biological limitations, such as: lacking of a specific structure-architecture (as in brain slices); lacking of any 3D structure (as the layers in a cortical brain region). One way to overcome this limit relies in the possibility to 'confine' the network in interacting cell sub-populations (Berdondini et al., 2005) that can behave as dynamically connected cell assemblies, as done by the cortical areas within the brain or to move to 3D neuronal culture (Pautot et al., 2008). Indeed, all that can be usefully tested and studied in such a simplified experimental framework then need to be translated to an in-vivo situation in which an external devices is connected to specific areas of the CNS. With specific reference to the results reported here, still there are things that need to be further addressed. The understanding of input/output mapping is fundamental and site selections is still representing a major factor of the overall system performance. Successful modification and improvement of robot behavior was recorded for 30% of performed recorded experiments. 90% of the unsuccessful experiments (i.e., 70%) did not even satisfy the preliminary requirements for input to output connection/correlation, meaning that a deeper understanding of how input-output mapping can be wired and re-wired has still to be fully understood.

In spite of the discussed limitations, the finalized experiments represent a fundamental step towards a better understanding of the biological principles of information processing that can be obtained by multi-site parallel recordings and multi-modal stimulation. Such information will be extremely valuable for developing our understanding of how real neuronal networks wire up, process information and change their connectivity (i.e. plasticity). More specifically, the use of a bi-directional neuro-robotic system allows to focus along three main research lines: (i-IN/OUT transformation, transfer function) investigate network dynamics and derive the input-output function of neuronal assemblies; (ii-CODING and DECODING) design new strategies for effectively sending appropriate information to cell assemblies, by developing stimulation protocols, and for efficiently extracting information from those assemblies; (iii-PROCESSING) derive the coding and information transmission properties of neuronal assemblies by designing computational

models which might substitute the dynamic behavior of neuronal population under observation (test).

In the field of BMI and neuroprosthesis, focused investigations by using a neuro-robotic paradigm are expected to give a relevant boost to the development of next generation of devices. Particularly, a significant improvement over current BMIs is expected from the newly gained capability to efficiently extract information from stimulus evoked activity, which will provide new algorithms and tools (i.e. optimized decoding scheme).

A second aspect relies directly in the bi-directional interface that plays a central role in the entire embodied system and from which the capability to optimally code information will constitute the basic of the new interfaces (i.e. optimized coding scheme) when missing sensory information has to be conveyed to the brain.

Finally, the paradigm of an artificially embodied network can provide a new investigation tool in the field of neuroscience where plasticity, dynamics and functional architecture can be studied in a context in which the brain tissue is not isolated but benefits of a direct interaction with the external environment. As a whole, this framework of study mainly targeting advanced BMI and neuroprosthetic applications is aimed at offering a future hope for potential therapy for the rehabilitation of severely impaired patients but also an useful platform to test various ideas on how population of neurons encode information in the brain.

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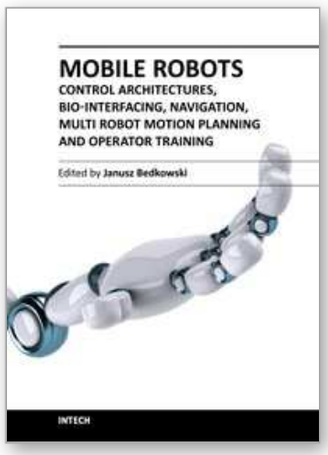

Mobile Robots - Control Architectures, Bio-Interfacing, Navigation, Multi Robot Motion Planning and Operator Training

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The objective of this book is to cover advances of mobile robotics and related technologies applied for multi robot systems' design and development. Design of control system is a complex issue, requiring the application of information technologies to link the robots into a single network. Human robot interface becomes a demanding task, especially when we try to use sophisticated methods for brain signal processing. Generated electrophysiological signals can be used to command different devices, such as cars, wheelchair or even video games. A number of developments in navigation and path planning, including parallel programming, can be observed. Cooperative path planning, formation control of multi robotic agents, communication and distance measurement between agents are shown. Training of the mobile robot operators is very difficult task also because of several factors related to different task execution. The presented improvement is related to environment model generation based on autonomous mobile robot observations.

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