Long-Term Bidirectional Neuron Interfaces for Robotic Control, and In Vitro Learning Studies

Steve M. Potter[1], Daniel A. Wagenaar[2], Radhika Madhavan[1], and Thomas B. DeMarse[3]

[1]Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University Medical School, Atlanta, GA, USA
[2]Department of Physics, California Institute of Technology, Pasadena, CA, USA
[3]Department of Biomedical Engineering, University of Florida, Gainsville, FL, USA
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Abstract—**There are two fundamentally different goals for neural interfacing. On the biology side, to interface living neurons to external electronics allows the observation and manipulation of neural circuits to elucidate their fundamental mechanisms. On the engineering side, neural interfaces in animals, people, or in cell culture have the potential to restore missing functionality, or someday, to enhance existing functionality. At the Laboratory for NeuroEngineering at Georgia Tech, we are developing new technologies to help make both goals attainable. We culture dissociated mammalian neurons on multi-electrode arrays, and use them as the brain of a 'Hybrot', or hybrid neural-robotic system. Distributed neural activity patterns are used to control mobile robots. We have created the hardware and software necessary to feed the robots' sensory inputs back to the cultures in real time, as electrical stimuli. By embodying cultured networks, we study learning and memory at the cellular and network level, using 2-photon laser-scanning microscopy to image plasticity while it happens. We have observed a very rich dynamical landscape of activity patterns in networks of only a few thousand cells. We can alter this landscape via electrical stimuli, and use the hybrot system to study the emergent properties of networks in vitro.**

Keywords—**MEA, rat, mouse, cortex. multi-electrode array; hybrot; animat; voltage-sensitive dye; 2-photon microscopy; cultured networks**

I. Introduction

Since the invention of the microelectrode, neuroscientists have been recording and stimulating individual neurons to study their role in the functioning of nervous systems. While this has been a fruitful approach in many respects, in some ways, it has mislead us into believing that individual neurons are important. At least in mammals, everything the brain does requires the combined activity of many, many interconnected neurons [1], not to mention the glia associated with them [2]. We are interested in studying the interactions in these ensembles of brain cells, and the emergent properties that result from them. We are especially interested in how neural circuits process and store information, and how this relates to learning and memory in animals and humans.

Commonly used in vitro systems for studying neural circuits have substantial limitations because of the fact that they are disembodied. This may seem like an obvious necessity for any in vitro system, but we re-examined this assumption and have created a system that may get around these limitations: embodied cultured networks.

II. Methodology

We grow cultures of dissociated brain cells (neurons and glia) on multi-electrode arrays (MEAs, MultiChannel Systems). Rat or mouse embryo cortex (E18) is dissociated by papain digestion followed by trituration, and plated onto MEAs coated with polyethylene imine and laminin at 50,000 cells per dish in a 10-50 µL drop, as previously described [3]. Cultures are maintained in dishes sealed with a gas-permeable membrane [4] that allows them to be grown in a non-humidified incubator and keeps them from getting infected upon repeated observations. We have used this technique to grow primary neuron cultures for up to two years. Because the incubator is not humidified, it is amenable to electronics, including continuous stimulation and recording via the MEA.

Our recording apparatus is the MEA60 from Multichannel Systems. Our stimulation apparatus is a custom made 64-channel neural stimulator (64-CNS, DeMarse, manuscript in preparation) that can dynamically switch between stimulation and recording on any channel. In combination with a new artifact subtraction algorithm [5], we can record neural responses as soon as one millisecond after stimulus pulses are delivered via the substrate electrodes. Stimuli are voltage-controlled, typically 300-700 mV, 100-500 µs biphasic pulses. Pulse parameters are adjusted to produce a submaximal, spatially-localized response (Wagenaar, manuscript in preparation).

Robots used are the Khepera (3 wheels, 8 cm) and Koala (6 wheels, 40 cm), by K-Team. These both have radio uplink/downlink for tetherless movement. Both have IR proximity sensors, and the Koala also has ultrasonic rangefinders.

III. Results and Discussion

A. Embodied cultured nets: A new research paradigm

The problem with in vitro preparations is that they have been removed from the animal. A cultured network of brains cells is much more accessible than an intact brain in an animal, and is simpler and easier to manipulate. But

because it lacks a body, it cannot express behavior, and it is cut off from all sensory input. We believe that this 'sensory deprivation' adversely influences network development in vitro, resulting in aberrant patterns of activity such as dish-wide bursts or 'barrages' [6]. These patterns resemble epileptic seizures and because they typically involve the entire network firing rhythmically in synchrony, they have little capacity for carrying useful information.

We have developed the software and hardware necessary to re-embody cultured networks [7]. This required creating a low-level data acquisition and processing system (implemented for Linux, available at http://www.its.caltech.edu /~pinelab/wagenaar/meabench.html). This software allows us to detect and extract spikes from the incoming 3MB/sec data stream, look for recurring patterns of neural firing, and use them to control the behavior of simulated animals, or 'animats' [8]. Sensory input to the animat is fed back to the cultured network as patterns of electrical stimulation, in less than 100 ms. By embodying the cultured network, its activity can now be expressed as behavior, and changes in that behavior with experience (sensory input) can be viewed as learning. This new paradigm of embodied cultured networks allows unprecedented access to a living, learning neuronal network, not feasible in vivo. The network is amenable to microscopic imaging at micron resolution, and millisecond time scale (Fig. 1).

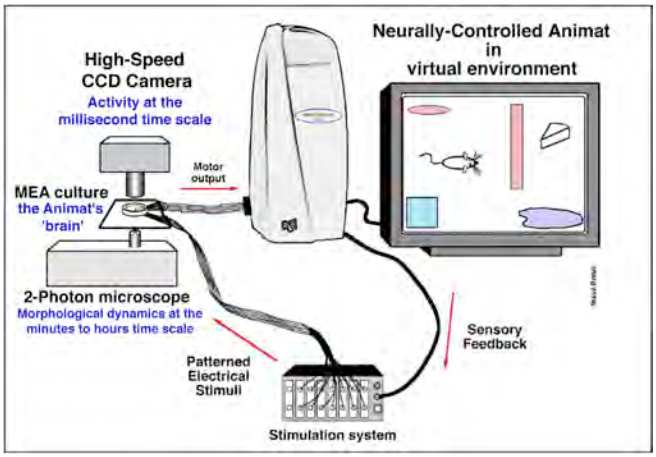

Fig. 1: Neurally Controlled Animats: Embodied cultured networks.

We have developed a system for quieting the pathological bursting that spontaneously develops in cultured networks [9]. Stimuli are delivered continuously at an aggregate rate of about 10 Hz to ten electrodes (1 Hz per electrode), mimicking the tonic level of input from the senses of the animat that provide the 'context' for salient stimuli. We implement this, for example, as efference copy or proprioceptive feedback, so that the network can make associations between what it just did, and the resulting behavior. We deliver salient stimuli as stronger trains of pulses on top of this background of quieting stimuli. These trains are tailored to induce distributed patterns of potentiation and depression across the network [10]. By first quieting the network with background stimuli, it is more responsive to the plasticity-inducing stimuli.

B. Hybrots: Hybrid neural-robotic animats

One reason for embodying a cultured network with a bidirectional neural interface and an animat situated in a simulated environment, is to bring it to a more natural activity state by supplying inputs continuously. But perhaps a more important reason is to allow the network's activity patterns to be expressed as behavior. Humans evolved skills for watching the behaviors of animals and each other. Even if the animat only serves as a visualization tool, it is much more effective than an oscilloscope trace at presenting network activity to the investigator. To make the behavior more detailed and realistic requires increasingly more complicated simulations. An excellent way to get all the complexity of the real world 'for free' is to use robots instead of detailed simulations [11].

We use the Khepera wheeled mobile robot (K-Team, Fig. 2) under control by neural signals from the MEA culture [12]. The robot is placed within a 1-m round playpen with several IR LEDs which are used as landmarks by the robot.

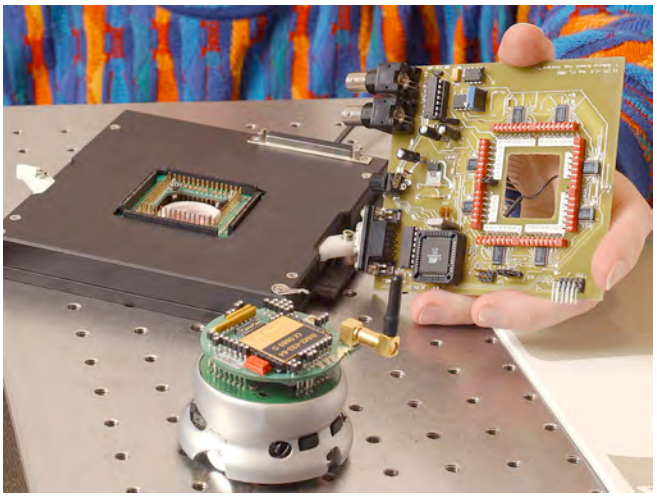

Fig. 2: Hybrot's main parts: Cultured neuronal network in sealed MEA, within the MultiChannel Systems preamplifier (upper left), 64-Channel Neural Stimulator (in hand) which plugs into the preamp, and Khepera mobile robot (K-Team). Not shown is a desktop computer that contains the A/D and robot interface cards, and runs MeaBench software.

Our hybrot can express phototaxis [12] as did the lamprey brain-slice controlled Khepera of Fleming et al. [13], but we have yet to observe a clear example of learning, i.e., improving at a set task. We expect that by using quieting stimuli and the correct sensory-motor mappings, the synaptic plasticity we have observed in these cortical nets can be expressed as hybrot learning.

C. *Watching learning while it happens*

If we do manage to observe a lasting change in behavior in the hybrot, as a result of its experience, we will be able to monitor the network with optical microscopy to see what changes in network connectivity and cell morphology underlie the learning process.

We have developed a number of techniques for continuously imaging neural cultures and brain slices, using multi-photon microscopy [14]. 2-Photon microscopy is much less harmful to living specimens than other types of fluorescence microscopy [15], allowing extended time-lapse movies of the structural dynamics of labeled cells (Fig. 3). We have observed changes in dendritic spines at the submicron level at time scales from seconds to days with this technique.

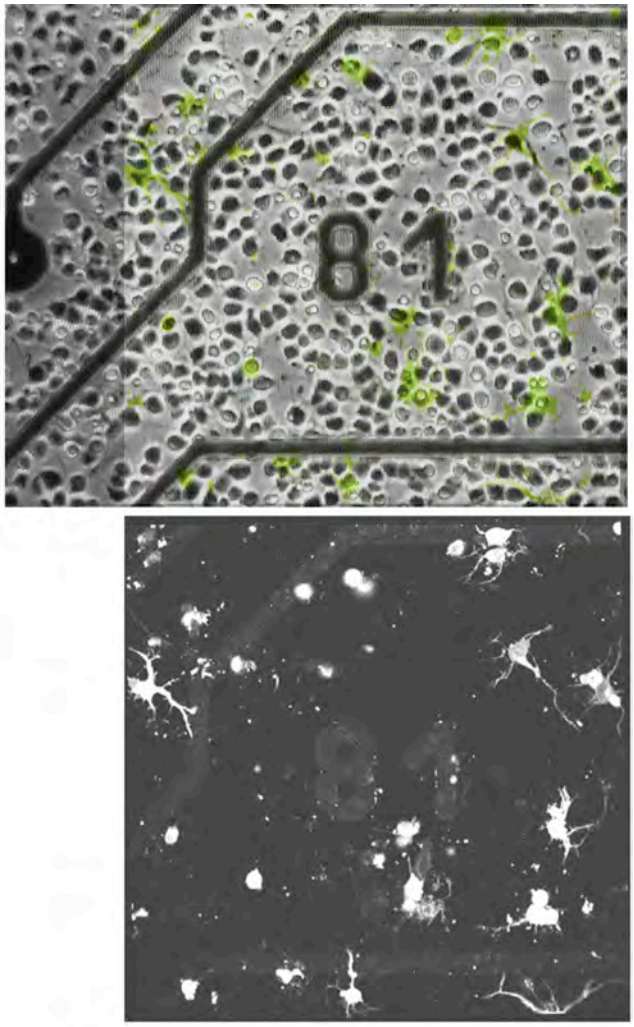

Fig. 3: Multi-photon fluorescence microscopy of cultured cortical neurons and glia on an MEA. A subset of the cells (top) is labeled either with lipophilic dyes (DiO, in this case) or fluorescent proteins, and repeatedly scanned by the 2-photon microscope (bottom) in a warmed microscope chamber, while conducting multi-site recording and stimulation. Cell bodies and MEA leads are 10 μm across.

The precise timing between action potentials is thought to carry information in neural circuits [16], and conversely, neural circuits are tuned to detect subtle changes in the timing of their inputs, as expressed in long-term potentiation or depression of synaptic strengths [17]. Each of our electrodes picks up action potentials (spikes) from 2-10 neurons, and we have not been very successful at assigning spikes to certain neurons, or spike sorting. This is partly due to the noisy signals one gets with extracellular electrodes, and partly due to the fact that it is very hard to resolve overlapping spikes.

To help with the spike sorting problem, and to observe network dynamics in more detail than possible using 60 electrodes, we will employ high-speed imaging with voltage-sensitive dyes [18]. By optically recording the membrane potential at the same time as it is recorded with electrodes, one can obtain the 'ground truth' of which cell fires when, and then optimize spike-sorting algorithms to give that result. We built a high-speed CCD camera [19] for optical recording that can image action potentials in single mammalian neurons without averaging [20]. Frames rates faster than 1000 per second are possible by selecting only the pixels of interest. Presently, the major limitation with this technique is dye phototoxicity. The stained neurons die from light exposure within a few seconds of continuous imaging, or a few minutes of periodic imaging. Unfortunately, the scanning mechanism of 2-photon microscopy is too slow for optical recording of transmembrane potential, so we must use wide-field illumination. We look forward to improvements in potentiometric probes, such as bigger signals and less phototoxicity, and to the development of fast-scanning multiphoton setups for optical recording, possibly using acousto-optic deflectors [21]. One could also use 2-photon uncaging to stimulate neurons [22], instead of electrical pulses.

IV. Conclusion

We have described a number of technologies that enable the study of distributed processing, and eventually learning, in cultured networks. Optical neural interfaces have the potential to supplant electrical ones, eventually. Until then, a combination of optical and electrical interfacing should yield new information about the emergent dynamics of neuronal networks. We hope that the information gained using embodied cultured nets will provide the basis for a deeper understanding of vital brain functions such as learning, memory, perception, and motor control. Such an understanding will point the way toward cures and treatments for patients in which these processes have gone awry.

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