HYBROTS: HYBRIDS OF LIVING NEURONS AND ROBOTS FOR STUDYING NEURAL COMPUTATION

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ABSTRACT

We are developing new tools to study the computational properties of living neuronal networks. We are especially interested in the collective, emergent properties at the mesoscopic scale (Freeman 2000) of thousands of brain cells working together to learn, process information, and to control behavior. We grow dissociated monolayer mammalian cortical cultures on multi-electrode arrays. We created the electronics and software necessary for a real-time feedback loop that allows the neurons to trigger their own stimulation. A key part of this loop is a system for re-embodying the in vitro network. We use the neural activity to control either simulated animals (animats) or robots. By using networks of a few thousand neurons and glia, we have tremendous access to the cells, not feasible in vivo. This allows physical and pharmacological manipulation, and continuous imaging at the millisecond and micron scales, to determine the cell- and network-level morphological correlates of learning and memory. We also model the cultured network in software; This helps direct our experiments, which then improves the model. By combining small networks of real brain cells, computer simulations, and robotics into new hybrid neural microsystems (which we call Hybrots), we hope to determine which neural properties are essential for the kinds of collective dynamics that might be used in artificially intelligent systems.

INTRODUCTION

What is a memory? What is a thought? How do we make up our minds what to do next? Cognitive scientists and philosophers have been debating such questions for ages. Unfortunately, few neurobiologists concern themselves with Big Picture questions. For computer scientists designing artificial intelligences they hope will remember, think, or make good decisions, most of the reductionistic findings of cellular neurobiologists are of little use. There exists a large chasm between the top-down and the bottom-up approaches to studying the brain. In the Laboratory for Neuroengineering at Georgia Tech,[1] we are developing new research tools to help bridge this chasm, to allow top-down behavior-based approaches to go down to the cell and molecular level, and to allow the bottom-up reductionism of cellular neurobiology to connect to the cognitive level. We aim to explore the terra incognita of network-level neuronal and glial dynamics, at a variety of temporal and spatial scales. In mammalian brains at least, no memory, thought, or decision involves only one neuron. Yet most electrophysiology in the past half-century has been carried out on individual neurons. We hope to broaden our perspective on how ensembles of neurons (and glia!) work together, by developing and improving tools for studying many cells simultaneously. These tools include long-term cultures on multi-electrode arrays (MEAs), optical recording of neural signals, and multi-photon time-lapse microscopy. We apply these tools to dissociated cultures of a few thousand rodent brain cells. To tie our cell- and network-level inquiries to behavior, we re-embody our cultured networks by connecting them to artificial animals, either simulated or robotic. If we and others are successful with this new approach, we will learn the cell- and network-level substrates of memory, thought, and behavioral control, and may then be able to develop more brain-like artificial intelligences.
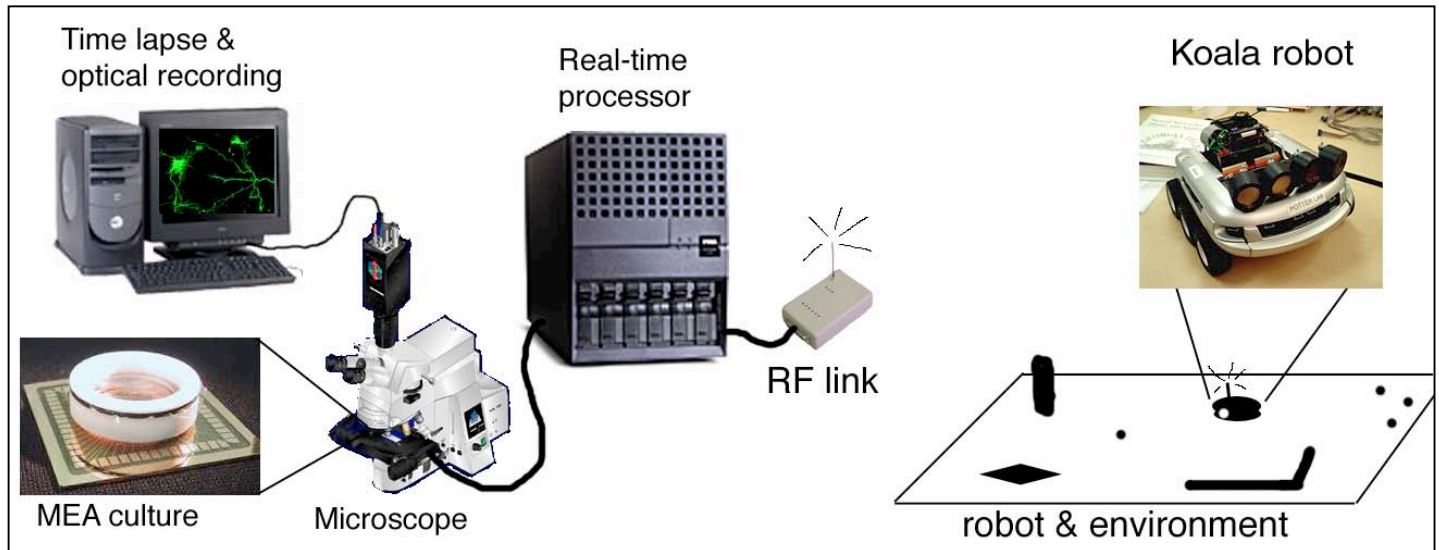
NOMENCLATURE

Animat: a simulated animal; Hybrot: robot controlled by living neurons; MEA: multi-electrode array.

Multi-electrode arrays and long-term culturing

Multi-electrode arrays for recording and stimulation of cultured neuronal networks were developed over two decades ago, independently by Pine (Pine 1980) and Gross (Gross 1979). These consist of culture dishes with ~60 cell-sized electrodes embedded in the substrate upon which dissociated brain tissue is grown. Extracellular electrodes are not harmful

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[1] http://neuro.gatech.edu/

Time lapse & optical recording — Real-time processor — Koala robot — RF link — MEA culture — Microscope — robot & environment

to the cells, and thus allow continuous recording and stimulation for as long as the culture is maintained. Only in the past 5 years or so has computer power been sufficient to deal with the data produced by such an array, and that is when MEA setups became commercially available. We use the Multichannel Systems MEA60 (Reutlingen, Germany). Noticing that the primary cause of death of neural cultures is either infection or changes in osmolarity, we developed a system for keeping cultures alive for over two years, by sealing them in a gas-permeable MEA culture chamber that keeps the bugs out and the water in (Potter & DeMarse 2001). This enables much longer-term experiments to be conducted than before, allowing us to go past the 'developmental' phase (which lasts about 90 days for these cultures (Kamioka et al 1996)) and well into maturity (and perhaps, senility?).

The recording technology is further along than stimulation technology. Although it is possible to buy systems for recording from 60 or even more channels, none are available for switching between stimulation and recording on that number of electrodes. Therefore, we developed two such systems. One, developed by DeMarse, has an onboard microprocessor that is programmed with stimulation parameters, for optimum flexibility. Another, developed by Wagenaar, uses real-time Linux running on a low-end PC to control a bank of switches that can be easily added to commercially available preamplifiers (Wagenaar & Potter 2004). DeMarse and Wagenaar also created the real-time software necessary to close the loop between recording and stimulation and to carry out a number of common and specialized data processing tasks on multi-unit data (Wagenaar et al 2001).[2]

Neurally-controlled animats: a new research paradigm

Why did we bother to create the hardware and software necessary to enable a 15-ms loop time between recording and stimulation, simultaneously on 60 electrodes? We feel that, because neural systems evolved to control a body and thereby interact with the world (Clark 1997), it may be more fruitful to study cultured networks that can likewise control a body and interact with the world, as opposed to the standard, disembodied in vitro approach. We have re-embodied our dissociated neuronal networks by allowing patterns in the neural activity to control the behavior of simulated animals or *animats* (Meyer & Wilson 1991). These include animats that exist on the computer screen, interacting with a virtual world, as well as robots moving about in the real world (see diagram). The hybrid robots or **hybrots** have sensory systems of our own choosing, and sense data is translated rapidly by our real-time software into distributed spatio-temporal patterns of electrical stimuli (DeMarse et al 2001). By closing the loop, from neurons firing action potentials, to detection of network activity patterns, to controlling behavior, to getting new sense data, and then to stimulating new action potentials, we approach a more naturalistic way of studying a living neural system. This is contrasted to much neurobiology research in animals in which the animal is restrained and anesthetized, unable to do much interacting with the world, and presented only with rarified stimuli of the experimenter's choosing. Unlike these lab animals, most of humans' and wild animals' inputs are the consequences of their recent actions. The same is true for the Neurally-controlled Animats.

Imaging neural structure and function

Using re-embodied cultured networks has some unique advantages when compared to in vivo research. It is a living neuronal network, with much of the anatomical complexity and dynamics of real brain circuits (Dichter 1978), but with a manageable size of only a few thousand neurons and glial cells. We chose this number to provide complex network-level dynamics and still allow every cell in the network to be studied in detail. Unlike with real animals, the brain can remain very still on the microscope stage while the body is behaving. (As mentioned below, the body can even be halfway around the globe from the brain!)
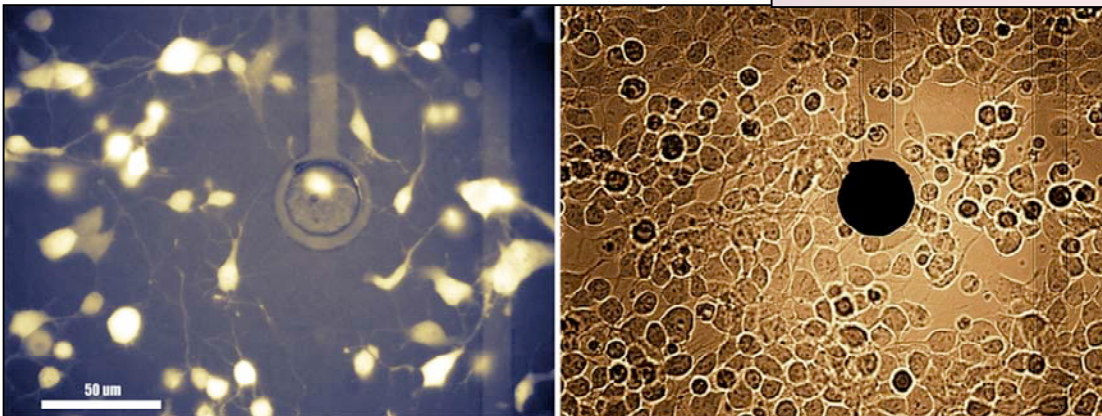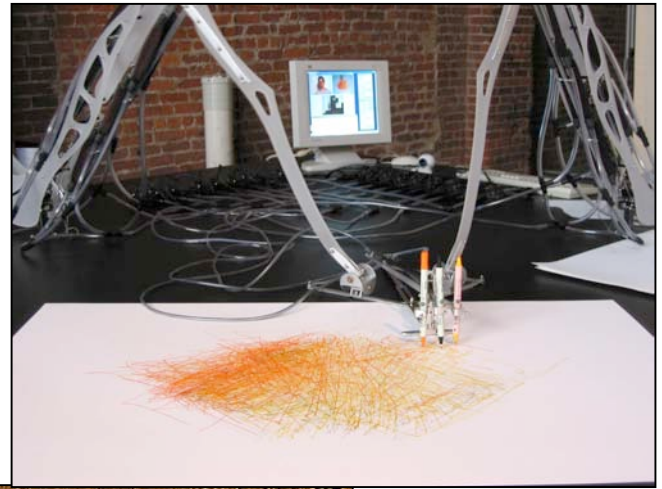
To image the morphological correlates of learning *while it happens*, we are building our own custom multiphoton microscope, based on the design of Tsai et al. (Tsai et al 2001).

[2] http://www.its.caltech.edu/~pinelab/wagenaar/meabench.html

It is optimized for keeping MEA cultures alive for weeks or months while they are being imaged and while they are controlling animats and receiving sensory inputs. In the Fraser lab at Caltech, Potter built one of the first 2-photon microscopes (Potter et al 1996b), and has administered the international multiphoton mailing list MPLSM-Users,[3] since 1994. 2-photon imaging is less harmful to living specimens than other microscopic techniques (Potter 1996, Potter 2000, Potter et al 1996a). We create cultures from transgenic mice in which some or all of the neurons are labeled with fluorescent proteins (Feng et al 2000) (see micrograph). This allows us to follow morphological dynamics of neurons and glia at the micron level. or changes in their connectivity at the network level.

We are also pushing the technology of high-speed imaging of neural activity. By labeling neurons with voltage-sensitive membrane dyes, one can monitor their electrical signals optically (Davila et al 1973), in more detail than possible using 60 extracellular electrodes. Pine and Potter built a 1000-frames-per-second CCD camera with the unique ability to only digitize pixels of interest, for maximum speed (US Pat. No. 6,633,331 (Potter et al 1997)). It allowed imaging action

potentials in cultured mammalian neurons in a single trial (Pine & Potter 1997), which is important for studying non-repeating neural patterns. We now use an even faster commercially available CCD camera (2kf/s, Redshirt Imaging). Phototoxicity and photobleaching of the dyes are still major problems with optical recording, and the solution to this problem is likely to come with the development of voltage-sensitive fluorescent proteins (Ataka & Pieribone 2002, Friedrich et al 1999, Siegel & Isacoff 1997). We expect that by combining optical recording with electrical recording and stimulation of many neurons simultaneously, new windows into emergent neuronal network dynamics will be opened.

Two embodiments for cultured networks

What is the basis of creativity? Does something have to be alive to be artistic? In collaboration with Guy Ben-Ary and Phil Gamblen at SymbioticA, the art-science lab at the University of Western Australia, we created a 'semi-living artist' called MEART. This is a hybrot consisting of a dish of cultured neurons in our lab in Atlanta controlling a robotic drawing arm in Perth. We process the neural activity in real time, creating a 'population vector' (Georgopoulos 1994) that is sent across the internet in a third of a second to command the arm's next movement. A video camera watches the drawing

process, comparing the work in progress to an image of a person to be drawn. The difference is used to generate a feedback signal which triggers the multi-site stimulator. An advantage of the hybrot approach is that we can create sensory-motor mappings of our own choosing, and try out many of these to gain insight into the nature of brain-body-world interactions. We have discovered that most of the reasons why MEART is still at the toddler stage of artistic ability stem from poor control of actuators, and too-sparse feedback (see photo).

The body of another hybrot in our lab is the Koala wheeled robot (K-Team). We discovered that every cultured network shows a robust network phenomenon of short-term potentiation and refractory period: the net's response to the second of two stimuli is boosted when the stimuli are less than 30 ms apart, while the response is depressed if they are 100-500 ms apart. Shkolnik mapped this curve to a control algorithm for robot following (Shkolnik 2003). While a smaller robot (K-Team Khepera) is randomly driven around by the computer, the neurally-controlled Koala approaches and tracks it at a certain distance. The distance of the target is mapped onto the timing between the two stimuli (on the sensory side), and the magnitude of the network's response determines the distance traveled toward the goal in one sensory-motor loop. By using real robots, we can save the trouble of simulating complex physics of the real world, such as friction, noise, inertia, etc. (Holland & McFarland 2001).

Simulating network dynamics

Cultured networks express barrages of action potentials that last ~100 ms, and recur every few seconds. We believe that these may be erasing our attempts to encode memories into these networks. Our working hypothesis is that this is a pathological form of activity, like epilepsy, that results from the culture being cut off from sensory input (except when it is being used in a closed-loop animat experiment). With our multi-site stimulator, we are bringing the cultures back to a more naturalistic mode of behavior, in which dish-wide barrages are reduced by a continuous application of background stimuli (Madhavan et al 2003). We are still working out the ideal parameters of such stimuli (Wagenaar et al 2004), and to help us test ideas out, Chao created a model network that also exhibits these dish-wide barrages. This is a fairly simple network of 1000 integrate-and-fire neurons, about 30% of them inhibitory, as in our living networks. The simulated net, like the living one, seems to be cured of its seizures by sprinkling in stimuli across several electrodes. Through an iterative process in which the network properties of the modeled network inform our experiments with hybrots, and the results of the hybrot experiments allow refinement of the model net, we will make faster progress toward discovering which network dynamics are important in learning and behavior.

CONCLUSION

This overview of the present state of the Hybrot Approach we have developed over the past 5 years is conspicuously lacking in a demonstration of any of the 'cognitive' traits mentioned at the beginning. We have developed a lot of technology, but the exciting results that bridge the chasm between top-down and bottom-up approaches lie in the future. We expect that they will take the form of new network dynamics—that the favored fundamental units of brain-like computation will no longer be neurons or synapses, but dynamic attractors, properties of networks that have been missed by single-unit techniques. Perhaps new types of computational approaches will spring from structural and functional studies of mesoscale neuronal network dynamics.

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REFERENCES

Ataka K, Pieribone VA. 2002. A genetically targetable fluorescent probe of channel gating with rapid kinetics. *Biophysical Journal* 82: 509-16

Clark A. 1997. *Being There: Putting Brain, Body, and the World Together Again*. Cambridge: MIT Press

Davila HV, Salzberg BM, Cohen LB, Waggoner AS. 1973. A large change in axon fluorescence that provides a promising method for measuring membrane potential. *Nature* 241: 159-60

DeMarse TB, Wagenaar DA, Blau AW, Potter SM. 2001. The Neurally Controlled Animat: Biological Brains Acting with Simulated Bodies. *Autonomous Robots* 11: 305-10

Dichter MA. 1978. Rat cortical neurons in cell culture: Culture methods, cell morphology, electrophysiology, and synapse formation. *Brain Research* 149: 279-93

Feng GP, Mellor RH, Bernstein M, Keller-Peck C, Nguyen QT, et al. 2000. Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. *Neuron* 28: 41-51

Freeman WJ. 2000. Mesoscopic neurodynamics: From neuron to brain. *Journal of Physiology-Paris* 94: 303-22

Friedrich RW, Gonzalez JE, Potter S, Chien C-B, Tsien RY, et al. 1999. GFP-based optical recording from a C. elegans sensory neuron. *Soc. Neurosci. Abstr*. 25: 742

Georgopoulos AP. 1994. Population Activity in the Control of Movement. In *Selectionism and the Brain*, pp. 103-19. San Diego: Academic Press

Gross GW. 1979. Simultaneous single unit recording in vitro with a photoetched laser deinsulated gold multimicroelectrode surface. *IEEE Transactions on Biomedical Engineering* 26: 273-9

Holland O, McFarland D. 2001. *Artificial Ethology*. Oxford: Oxford University Press

Kamioka H, Maeda E, Jimbo Y, Robinson HPC, Kawana A. 1996. Spontaneous periodic synchronized bursting during formation of mature patterns of connections in cortical cultures. *Neuroscience Letters* 206: 109-12

Madhavan R, Wagenaar DA, Potter SM. 2003. Multi-site stimulation quiets bursts and enhances plasticity in cultured networks. *Society for Neuroscience Abstracts* 29: 808.14

Meyer JA, Wilson SW. 1991. *From Animals to Animats: Proceedings of the First International Conference on Simulation of Adaptive Behavior*. Cambridge: MIT Press

Pine J. 1980. Recording action potentials from cultured neurons with extracellular microcircuit electrodes. *Journal of Neuroscience Methods* 2: 19-31

Pine J, Potter SM. 1997. A high-speed CCD camera for optical recording of neural activity. *Soc. Neurosci. Abstr*. 23: 259.6

Potter SM. 1996. Vital imaging: Two photons are better than one. *Current Biology* 6: 1595-8

Potter SM. 2000. Two-Photon Microscopy for 4D Imaging of Living Neurons. In *Imaging Neurons: A Laboratory Manual*, ed. R Yuste, F Lanni, A Konnerth, pp. 20.1-.16. Cold Spring Harbor: CSHL Press

Potter SM, DeMarse TB. 2001. A new approach to neural cell culture for long-term studies. *J. Neurosci. Methods* 110: 17-24

Potter SM, Fraser SE, Pine J. 1996a. The greatly reduced photodamage of 2-photon microscopy enables extended 3-dimensional time-lapse imaging of living neurons. *Scanning* 18: 147

Potter SM, Mart AN, Pine J. 1997. High-speed CCD movie camera with random pixel selection, for neurobiology research. *SPIE Proceedings* 2869: 243-53

Potter SM, Wang CM, Garrity PA, Fraser SE. 1996b. Intravital imaging of green fluorescent protein using 2-photon laser-scanning microscopy. *Gene* 173: 25-31

Shkolnik AC. 2003. *Neurally Controlled Simulated Robot: Applying Cultured Neurons to Handle and Approach/Avoidance Task in Real Time, and a Framework for Studying Learning In Vitro*. Masters Thesis, Emory University, Atlanta

Siegel MS, Isacoff EY. 1997. A genetically encoded optical probe of membrane voltage. *Neuron* 19: 735-41

Tsai PS, Nishimura N, Yoder EJ, Dolnick EM, White GA, Kleinfeld D. 2001. Principles, design, and construction of a two photon laser scanning microscope for in vitro and in vivo brain imaging. In *Methods for In Vivo Optical Imaging*, ed. R Frostig: CRC Press

Wagenaar DA, DeMarse TB, Potter SM. 2001. *A toolset for realtime analysis of network dynamics in dense cultures of cortical neurons*. Presented at 7th Joint Symposium on Neural Computation Proceedings, La Jolla

Wagenaar DA, Pine J, Potter SM. 2004. Effective parameters for stimulation of dissociated cultures using multi-electrode arrays. *J. Neurosci. Methods* (in press)

Wagenaar DA, Potter SM. 2004. A versatile all-channel stimulator for electrode arrays, with real-time control. *J. Neural Engineering* 1:39-44

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