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Animat in a Petri Dish: Cultured Neural Networks for Studying Neural Computation

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Introduction

The accessibility of neural cell cultures makes them ideally suited for the study of emergent, network properties of living neural circuits. These cultures exhibit many of the properties of neural circuits in vivo, including the formation of rich synaptic connectivity, the development of complex patterns of spontaneous activity, and synaptic plasticity in response to electrical or pharmacological stimulation (Basarsky *et al.,* 1994). Traditionally, such cultures are studied using one or two microelectrodes. We believe that such approaches are unable to capture the spatio-temporal distribution of activity and may therefore miss many interesting collective properties of biological neural networks.

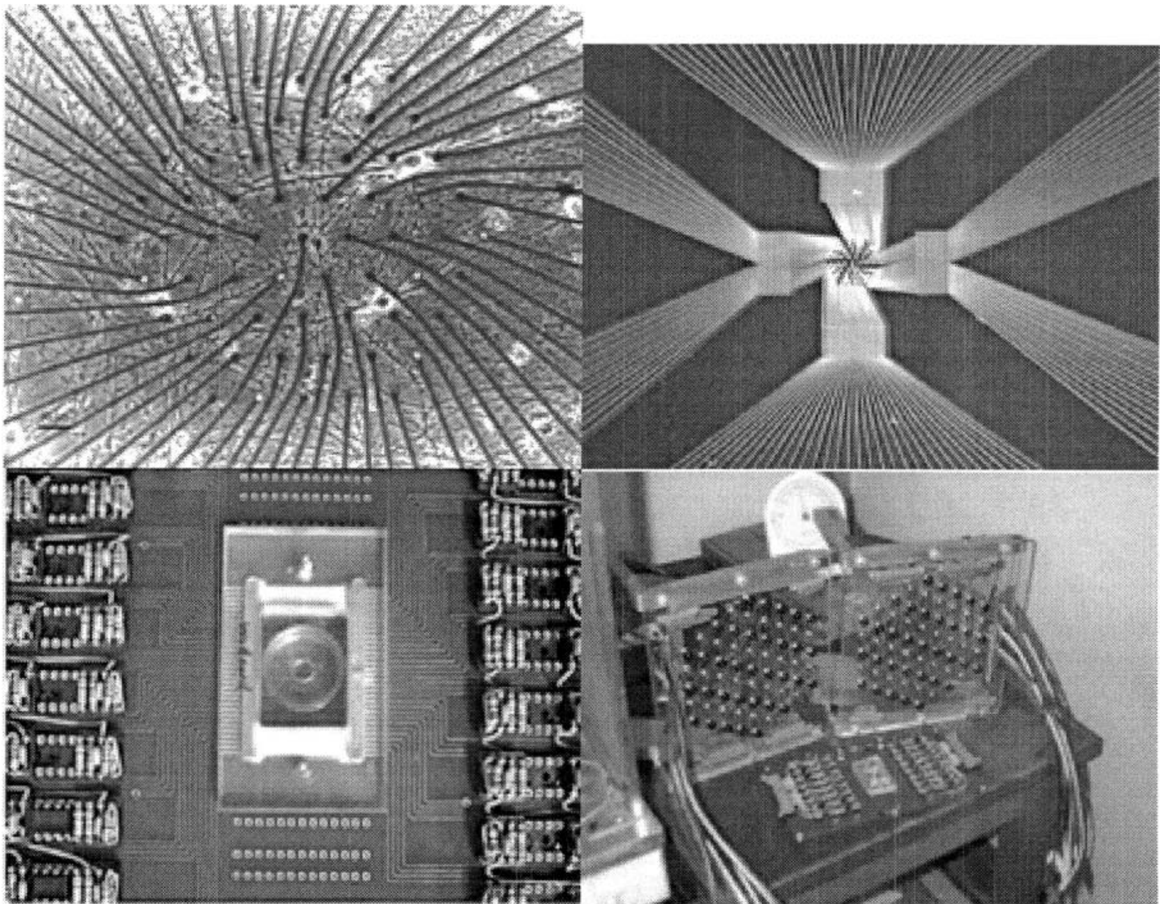
Therefore, we have developed three key technologies aimed at manipulating and observing many neurons at a time:

- A 61-electrode Petri dish (multi-electrode array, MEA) for non-invasive (extracellular) recording and stimulation of cultured rat hippocampal neurons.
- A custom 1000-frame/sec CCD camera to observe the electrical activity in cultures stained with voltage-sensitive dyes.
- A 2-photon laser-scanning microscope, which provides submicron resolution in living cells with far less photodamage than confocal microscopy. This allows us to study fine morphological features that subserve single-neuron and network activity, and long-term plasticity.

The combination of these three technologies offers the promise of correlating neuronal structure and function within a living neural network. To demonstrate that the network is functional, it will be linked to a real-time processor for stimulation and recording via the 61 electrodes. We will provide the cultured neural network with a simulated environment in which network activity controls the behavior of a simulated animal, or "animat." As the network's firing patterns command the animat to explore its environment, the computer will translate its sensory inputs into patterns of electrical stimulation designed to emulate those that arise spontaneously in these cultures. This "Animat in a Petri Dish" will allow us to determine the minimum parameters necessary to support learning in vitro. The detailed study of a working, embodied biological neuronal network should guide the construction of more biological artificial neural networks, and provide insight into the function of intact nervous systems.

Wired Petri Dish

The Pine lab has pioneered the development of multielectrode substrates for neural cell cultures (Pine, 1980). These consist of a glass substrate onto which is patterned an array of microelectrodes and leads that carry signals to and from external electronics. The electrodes and leads are made from the transparent conductor, indium-tin oxide, used in liquid-crystal display (LCD) technology. This allows easy visualization of cultures of dissociated neurons growing on the MEA, using phase-contrast optics on an inverted microscope. The Pine arrays have been used to record daily changes in spontaneous activity in neurons from the suprachiasmatic nucleus, part of the brain that regulates the circadian rhythm (Welsh et al., 1995). They have also been used to record waves of spontaneous activity in the developing retina (Meister et al., 1991).



Multielectrode array dish and associated preamplifiers and patch panels. The arrays consist of leads and electrodes made of the transparent conductor indium-tin-oxide. The leads are insulated with silicon nitride, and the electrodes are plated with platinum to enhance the signal-to-noise ratio.

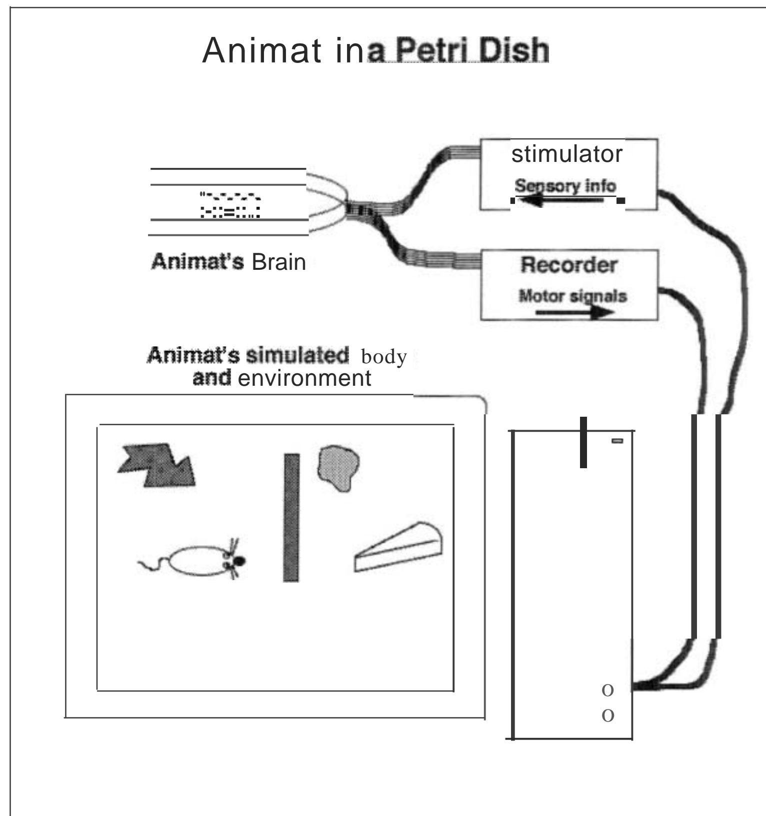
We have recently added the circuitry necessary to stimulate neurons with the MEA. The microelectrodes are connected to 61 preamplifiers and a pair of patch panels that allow us to stimulate and record from as many as sixteen

channels simultaneously. The MEA cultures are accessible to standard glass microelectrodes for comparison to whole-cell intracellular recording. They can be easily removed from the amplifier board and returned to the incubator for recording at a later date. Using similar MEA technology, the Gross lab has recorded from spinal cord cultures that were over a year old (Gross and Schwalm, 1994).

Learning in vitro

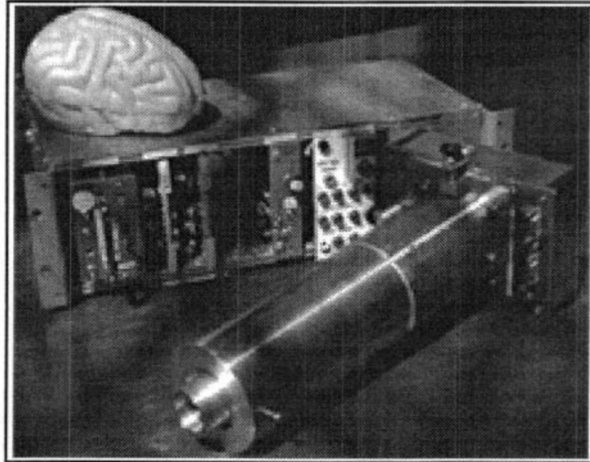
We would like to use the MEAs to study learning in a simplified system amenable to detailed optical analysis. Whether learning is possible in such a reduced preparation is still an open question, although long-term synaptic potentiation has been demonstrated in dissociated hippocampal cultures (Malgaroli et al., 1995). To make the definition of "learning in vitro" more concrete, we will provide the cultured network with the means to interact with a simulated environment. We will embody the network in the form of a simulated animal (animat) situated within that environment. First we will observe the spatio-temporal patterns of spontaneous activity that develop in cultures as they mature. We will map members of the ensemble of recurring patterns onto specific behaviors of the animat. The mapping could be arbitrary, or perhaps based on some somatotopic or environment-centered topography. For example, if neurons on the right side of the dish fire, the animat will move to the right at a speed proportional to the mean firing rate.

No creature functions in isolation from the outside world. In order for the behaviors of the animat to develop meaning (to the researcher), there must be feedback from the environment to the animat that can shape behavior. Therefore, we will stimulate the culture with patterns of activity similar to those that occur spontaneously. This stimulation will be triggered by the actions of the animat, and will represent (to the researcher) sensory input to the animat. For example, if the animat bumps into a wall, its bump sensor is activated and a specific pattern of stimulation is played across a given set of electrodes in the MEA. Only when this creature-environment feedback loop (see drawing, next page) is closed can learning be clearly defined (and easily observed) as an enduring change in the patterns of behavior of the animat as a function of past experience.

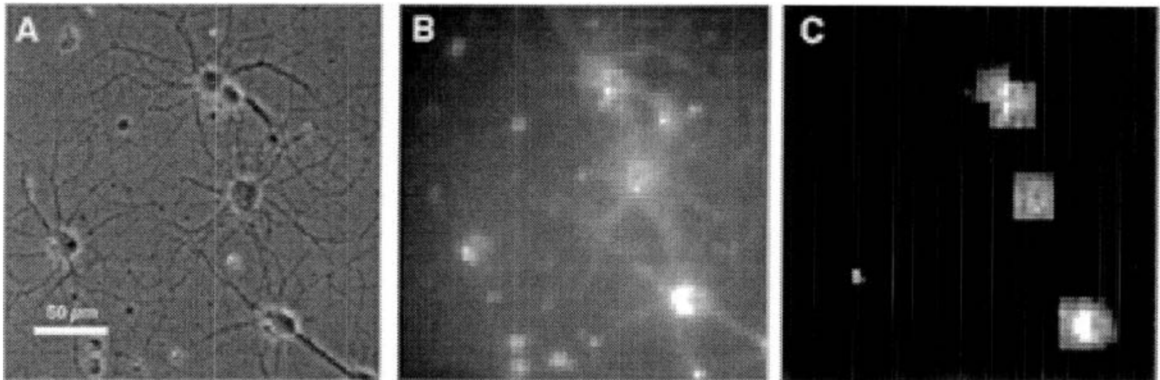


Imaging Neural Activity

In the best possible world, all 61 electrodes in the MEA will be able to record from and stimulate at least one neuron. However, our present 16-channel system will miss a lot of that activity. Furthermore, most hippocampal cultures extend past the active region of the MEA. In order to provide a more complete view of the spatio-temporal patterns of spontaneous activity in hippocampal cultures, we designed and built a high-speed CCD camera (Potter *et al., 1997)*. The camera detects the minute changes in fluorescence from neurons stained with voltage-sensitive dyes when they fire action potentials. The camera's imager is a Tektronix TK064 CCD chip, a 64 x 64 pixel array with large wells (500,000 photoelectrons), that can be read out at 10 MHz. To allow imaging at over 1000 frames per second, the camera can be instructed to discard uninteresting pixels, digitizing only a set of user-selected regions. These could be the somata of neurons in a network, or different dendritic regions of a single neuron. The High-speed CCD Camera (HSCCD) could also be used to image changes in intracellular calcium that occur at rapid time scales. The HSCCD provides significantly better spatial resolution than the photodiode arrays commonly used for voltage imaging. It also provides significantly better temporal resolution than the scientific CCD cameras commonly used for calcium imaging.



High-Speed CCD camera and associated electronics.



A. Three-week old hippocampal culture, imaged using a cheap *video* camera with phase-contrast optics and a 20x objective. B. The same cells, labeled with voltage-sensitive fluorescent dye, imaged using our high-speed CCD camera in full-frame mode (200 frames/sec). C. Subregions were selected to image the somata at 1000 frames/sec.

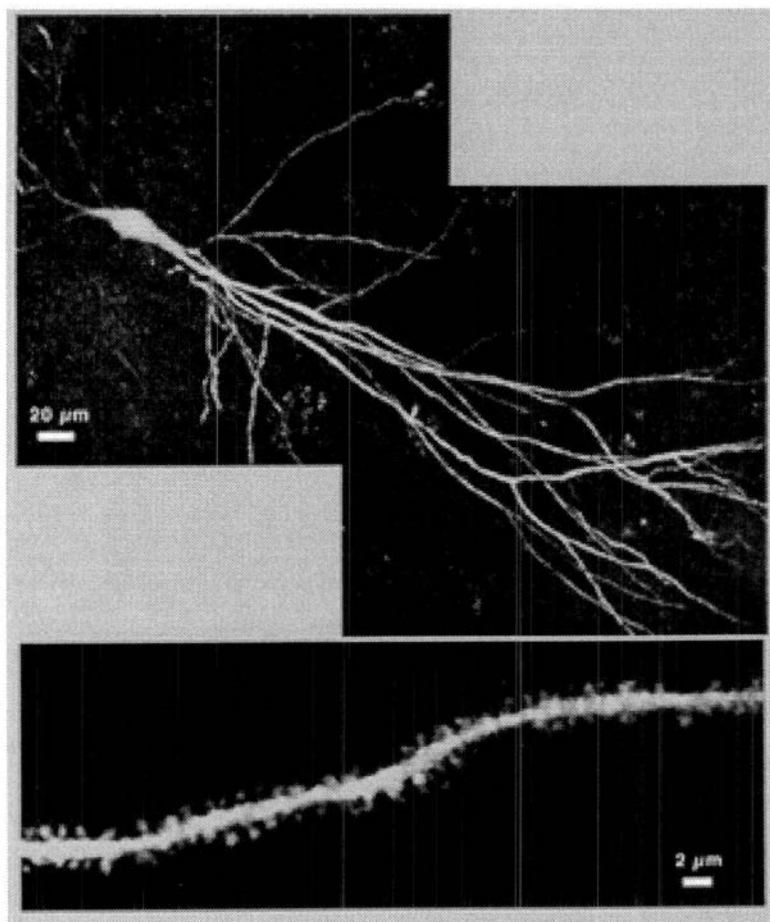
Eventually we will use the HSCCD to image both spontaneous and evoked neural activity in networks grown on the MEAs. The system is still undergoing software development and testing using control cultures stimulated by standard microelectrodes. We can detect evoked action potentials with trials averaging, but we need to improve optical signals to be able to observe single-shot spontaneous activity or subthreshold responses.

2-Photon Microscopy

There is considerable evidence that changes in the morphology of neurons underlie learning and memory (Wallace et al., 1991). Most of this evidence has been obtained by comparing fixed tissue from different animals using electron microscopy. We are interested in observing these morphological

changes in living neurons while they are occurring. This would eliminate artifacts associated with tissue fixation, and would allow the dynamics of the whole process to be followed. Unfortunately, high-resolution optical imaging with a confocal microscope is often lethal to living cells. It is also greatly limited by photobleaching of the dye used to label the cells.

To allow us to observe living neurons at high resolution for extended periods of time, we constructed a 2-photon laser-scanning microscope (Denk et aL, 1990; Potter and Fraser, 1995; Potter et aL, 1996a; Potter et aL., 1996b; Potter et aL, 1996c). This technique is similar to confocal microscopy, except that a pulsed infrared laser is used to excite dyes normally excited by visible light (Potter, 1996). It provides a number of advantages over confocal imaging, notably a greatly reduced light dose that is not harmful to the cells or the dye molecules used to label them. Two-photon excitation is limited to the focus of the laser beam within the specimen, where there is a high probability that two IR photons will hit a fluorophore at the same moment. Because no confocal pinhole aperture is necessary to eliminate unwanted fluorescence, 2-photon microscopy enjoys a greater signal-to-noise ratio than confocal microscopy, which allows repeated imaging of submicron structures such as dendritic spines.



Living hippocampal pyramidal neuron from area CA1, labeled by intracellular injection of fluorescein dextran. Top: Low-resolution 3D reconstruction of the apical dendrites from a series of optical sections. Bottom: High-resolution optical section of one living dendrite, showing numerous spines.

If we are successful in getting the Animat to learn something, it will be of great interest to image the neural culture at high resolution to determine the morphological correlates of the learning process. The 2-photon microscope is ideal for carrying out this imaging in a non-destructive manner.

Conclusion

We have developed a powerful set of new technologies aimed at observing learning in vitro. It is likely that distributed patterns of activity are crucial to the functioning of even simple neural networks, so we have focused on technologies that allow the observation of many neurons at a time. With the multielectrode arrays and the high-speed CCD camera, we can observe network dynamics on the millisecond time scale. Using the 2-photon microscope, we can correlate changes in these dynamics with gross or minute changes in neuron morphology. We hope that these three technologies will shed light on the larger field of neural computation, and provide neural modelers with information about which parameters of biological neural networks are most important to include in their models.

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