
Interfacing Neuronal Cultures to a Computer Generated Virtual World

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Abstract

Current studies of learning typically focus on either the biological aspects of learning or on behavioral measures. The goal of the *Animat* Project is to bridge the gap between these two areas by developing a system in which the biology can behave in a virtual world. Using multi-electrode array technology (MEAs), rat cortical neurons cultured on a MEA have been given a simulated body which together form a neurally controlled *Animat* that can move or "behave" in a virtual environment created by a computer. The computer then acts as the *Animat's* senses, providing feedback in the form of electrical stimulation about the effects of interactions between the *Animat's* movements and the virtual world (e.g., navigating around a barrier). Because MEAs offer the possibility of the detailed study of neurons in culture, any changes in the *Animat's* behavior resulting from experience in the virtual environment can be studied in concert with the biological processes (e.g., neural plasticity) responsible for those changes.

Introduction

Neural encoding consists of the transformation of information from one representation (e.g., sensory input from neural connections to the eyes, ears, etc.) into another (a neural code). There has been a great deal of effort in the past to understand how such information is both processed and encoded by the brain. To date, most of what we have learned about the neural basis of information processing is based upon recordings from single neurons. However, beginning in the 1970's with the development of multi-electrode array (MEA) technology (e.g., Thomas et al. 1972; Gross, 1979; Pine, 1980) the possibility of detailed study of the activity of entire populations of neurons became possible. This ability has led to a rapid expansion of researchers interested in investigating the properties of information processing and neural encoding of distributed neural activity in brains, slices, and dissociated neural cultures. However, while implanted MEAs allow one to study intact neural tissue in the brain, the lack of accessibility makes it difficult to perform simultaneous detailed studies of the underlying morphological structures. Conversely, studying neural tissue in culture allows highly detailed study through extracellular, patch clamp, or standard microscopy techniques. The disadvantage is that these cultures now lack any meaningful sensory input or the means to produce any sort of behavioral output. Therefore, it is difficult to associate the changes in morphology that can be observed with these techniques with the changes in behavior that occur as a result of experience within an intact brain.

A new technique that combines the advantages of each approach is currently being developed by the Neurally-Controlled *ANIMAT* project at the California Institute of Technology. The goal of this project, which is illustrated in Figure 1, is to give a dissociated neuronal culture a body with which to behave and a sensory system with which to perceive. In other words, to create an artificial animal, an *ANIMAT* or simulated animal (Wilson, 1985, 1987), that is able to produce

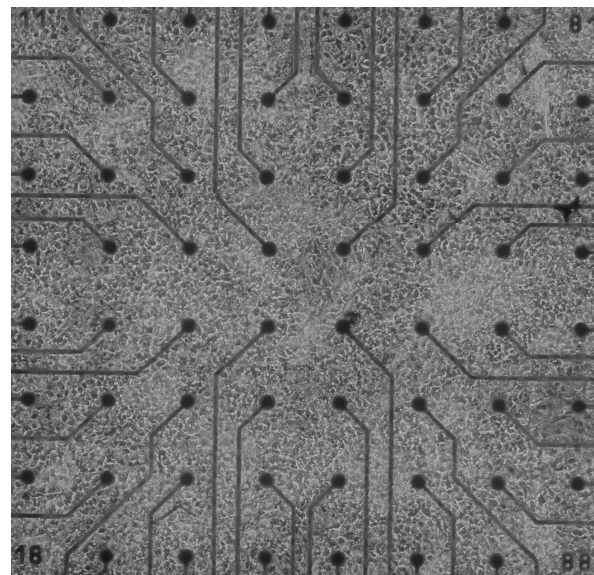
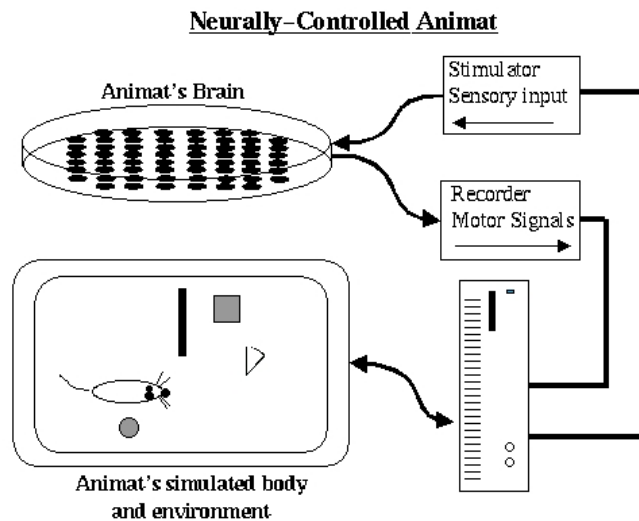
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movements using a simulated body in a virtual environment and receiving sensory information in the form of feedback (electrical stimulation) about the effects of its movements within this virtual world. Because both the system for sensory input and that producing the behavioral output are controlled by the experimenter, one can study how various stimuli (sensory information) are processed and encoded by these dissociated cultures producing changes in the behavioral output. Simultaneously, the biological processes responsible for any changes in the behavior of the *ANIMAT* can be examined in detail, either through changes in the distributed pattern of neural activity, or by conventional microscopy.

Although this idea may sound far fetched, there are a number of reasons why such a system is possible. For example, one of the first major hurdles is to be able to record from and respond to neural activity within a biologically plausible time frame. However, with the ever increasing computing power, such brain-computer interfaces are now already being used. For example, Chapin (Chapin et al., 1999) has recorded the ongoing neural activity from the motor cortex of rats, and used these signals to allow the rat to control a robotic arm to receive water. Secondly, MEA technology has already demonstrated its usefulness as a tool for investigating the distributed patterns of activity representing a neural code that might be used to move the *ANIMAT*. For example, work by Nicolelis (e.g., Nicolelis et al., 1998) has shown evidence of a distributed neural code involved in representing tactile information in monkeys. In addition, Georgopoulos (e.g., Georgopoulos et al., 1986) has shown that one can use distributed neural codes to predict the reaching movement of a monkey's arm based on the pattern of activity recorded from implanted arrays. The third hurdle, to be able to provide rapid feedback in the form of electrical stimulation and more importantly, to have that feedback affect ongoing neural activity, has already been accomplished with the well-known effect of long-term potentiation (LTP) or depression (LTD) using patch-clamp techniques (e.g., Bi & Poo, 1999) as well as with MEA technology (Jimbo et al. 1998). Furthermore, recent work in Jimbo's laboratory has shown that the effects of these stimulations on a MEA are not limited to a few isolated neurons but can in fact affect the efficacy/connectivity of a large number of neurons, enhancing or depressing activity along specific pathways across an entire population of dissociated cultured neurons (Jimbo et al., 1999). Thus, by combining each of these: 1) the ability to record and produce movement in a biologically plausible real-time manner, 2) the ability to detect ongoing neural patterns as they occur, and 3) the ability to then influence those patterns as result of feedback, we can create an artificial animal on a MEA platform that will allow extraordinary access to both the biology and behavior.

Figure 1. Diagram of the Animat concept.



The following describes the project's first effort to produce a functioning real-time simulated animal, the *ANIMAT*, controlled by dissociated rat cortical neurons, and interfaced to a computer-generated 2D environment. In this first incarnation, a simple algorithm was used in which the neural activity patterns recorded from neurons on four electrodes were used to produce movements in four directions within a simple room. Feedback was provided to the culture through four different electrodes upon collisions with one of the four walls in this room. If the feedback that resulted from collisions produced a change in the pattern of neural activity in the culture, either in its connectivity or through synaptic efficacy, then these changes should produce a change in the movements of the *ANIMAT* (e.g., a change in time spent in various quadrants of the room, etc.) relative to a nonstimulated control condition.

Materials and Methods

Embryonic day 18 rat cortical tissue was dissociated by trituration and digestion in papain, and cultured on a 60 channel multi-electrode array from Multichannel Systems[®] coated with polyethyleneimine from a 5% solution (PEI- Sigma #cc-4195 & cc-4196) and 1 μ g per ml of laminin (Sigma #L-2020). Electrodes were arranged in a 1.6 mm \times 8 \times 8 grid. Each 10 μ m diameter titanium-nitride electrode was separated by 200 μ m. 50,000 cells were densely plated over the surface of the array. After 2-3 days, the neurons began to reconnect and produce action potentials. After 2-3 weeks spontaneous burst of activity were recorded (i.e., bursts) (Kamioka, 1996).

Culture medium consisted of Dulbecco's modified Eagle's medium (DMEM) (Irvine Scientific #9024) with 10% horse serum (Hy-clone), and 2.5 μ g per ml insulin. Half of the culture medium was exchanged twice each week.

Cultures were enclosed with Teflon lids containing a gas-permeable 0.5 mil Teflon membrane from Dupont[®], which allowed both long-term and repeated recordings without the risk of contamination or rapid changes in osmolarity or the pH of the medium.

Figure 2. An example of a densely plated MEA after approximately one month *in vitro*. Note the single monolayer of cells composed of cortical neurons and glia covering the surface of the 60 electrodes. Numbers in the corner represent electrode numbers in column \times row format.

Recording was conducted using Multichannel Systems' MEA 60⁺ amplifier and PCI A/D data acquisition board, which permitted simultaneous recording from 60 channels at 25 KHz. The temperature of the culture during recordings was thermostatically controlled at 35°C. The computer used to record data, to provide the virtual environment, and to control the delivery of feedback was a Silicon Graphics[®] 540 Visual Workstation with four Pentium 550 Mhz processors, 512 Meg of RAM, and running Linux Red Hat[®] v6.0 along with custom device drivers to record from Multichannel Systems' PCI data card and to operate the stimulation device. Stimulation voltages were produced with Multichannel Systems' STG1008 stimulator, and custom built hardware containing analog switches with a fiber-optic trigger connecting a single channel from the stimulator to one of four selectable electrodes on the MEA. Spike detection was accomplished via online bandpass filtering and spike detection.

The virtual environment consisted of a 2-dimensional 100 \times 100 unit world. Of the 60 electrodes, four were chosen for feedback and four to produce motor movements. They were selected at random among the set of electrodes in which action potentials were recorded. Neural activity on four of these channels was integrated over 200 ms producing a single step in four associated directions (i.e., left versus right, and/or up versus down) depending on which channels were the most active during that period. Each collision with one of the four walls produced a 400 mV 200 μ s single bi-phasic pulse on one of the four remaining electrodes.

The experiment was conducted with a single dish after 4 months *in vitro*. At this stage neural activity is relatively stable (Kamioka et al. 1996; Watanabe et al. 1996). In the first phase of the experiment, the dish and equipment were setup, followed by a few minutes of recording to choose which of the 60 electrodes would be used to produce movement and receive feedback. The stimulating wires were then attached and the *ANIMAT* was then put online for 5 minutes without the stimulator providing feedback. The stimulator was then activated and the *ANIMAT* was put online for another 5 minutes with feedback based on the *ANIMAT*'s movements within the

[®]Multichannel Systems at <http://www.multichannelsystems.com>

2D world.

Results

At four months *in vitro*, the culture's spontaneous activity typically consisted of the bursting of action potentials on over 93% of the electrodes. Figure 3 shows a raster plot representing neural activity for each of the 60 channels during the 5 minutes the *ANIMAT* was online without feedback. Bursting of activity was often highly synchronous in the entire dish, appearing as columns of points in the raster plot (e.g., at 20 and 160 seconds). However, on some

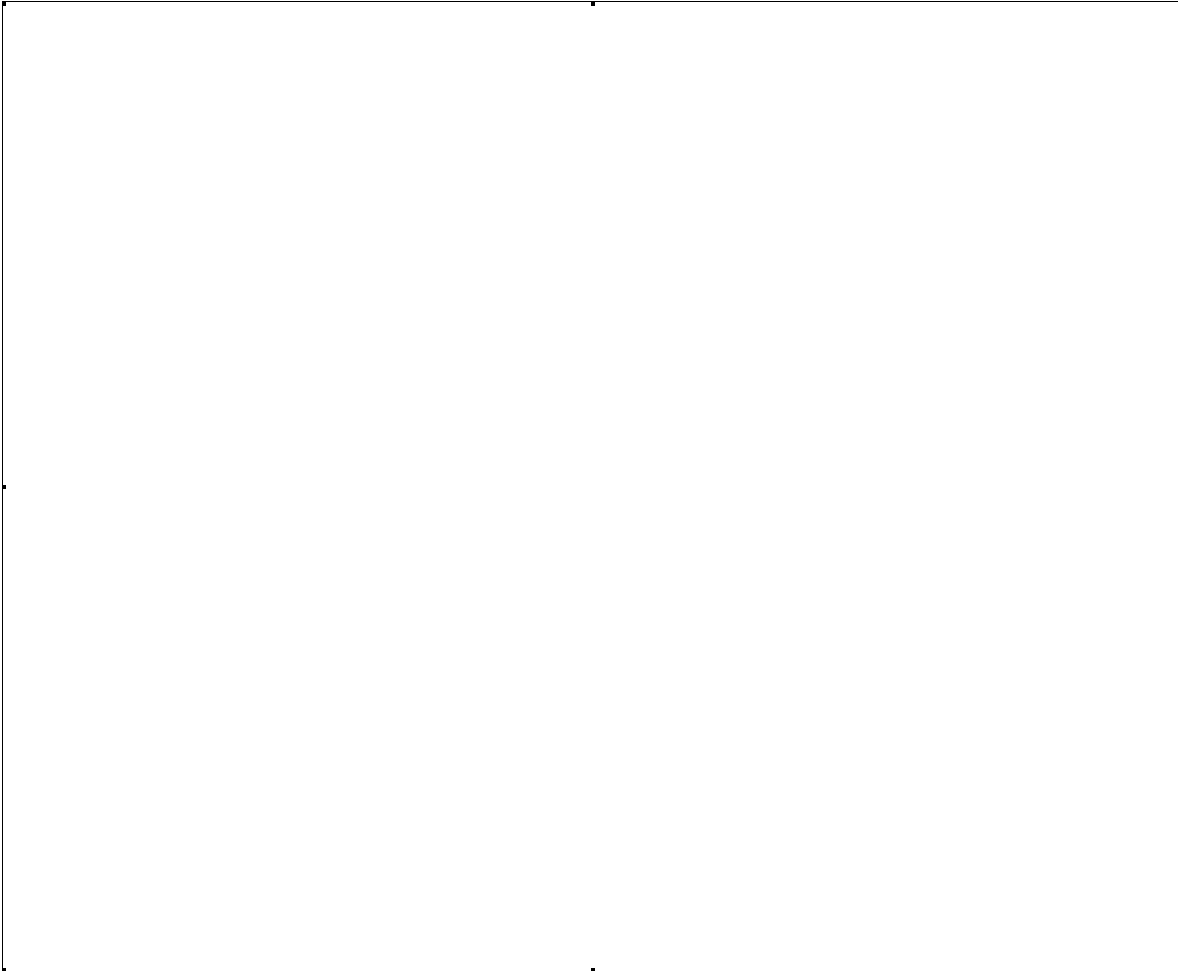


Figure 3. Raster plot of neural activity for each of the 60 channels over time. The top and bottom panels show activity with and without feedback, respectively. Dark columns of points in the lower panel represent occasions where stimulation occurred.

occasions, complex spontaneous activity would appear in which activity on one corner of the array would be followed by activity on the opposite corner.

Figure 4 shows a surface plot representing neural activity at approximately 295 seconds over the 8x8 grid of electrodes arranged horizontally across the floor of the plot. The height represents integrated activity. Channels corresponding to the motor and feedback systems were selected among the active channels. Channels 27, 45, 56, and 87 (column x row) were chosen to produce movements, while channels 51, 54, 62, and 76 were chosen to receive electrical feedback.

Figure 5 shows the trajectory of the *ANIMAT*'s steps within the 100 x 100 unit world. Over the course of the experiment, the *ANIMAT* moved in each of the four directions with steps often occurring in rapid succession as a result of spontaneous bursting in the culture. The direction of movement, however, gradually progressed towards the lower left corner of the world followed by a series of collisions and movement along the boundary walls. This is to be expected since without feedback, there should be no possibility of network modification due to feedback. Hence,

Column Row

Figure 4. Surface plot representing neural activity over the surface of the MEA.

the trajectory should represent nothing more than Brownian motion within this environment.

Of more interest is the trajectory of the *ANIMAT* when feedback was applied, which is shown on the right panel of Figure 5. Since feedback occurred only following collisions with one of the four walls, the effect of feedback should be apparent only after a collision had occurred. Initially, the *ANIMAT* demonstrated the same pattern of movement as before, moving in a random walk towards the lower left of the 2D environment. However, even after feedback commenced as

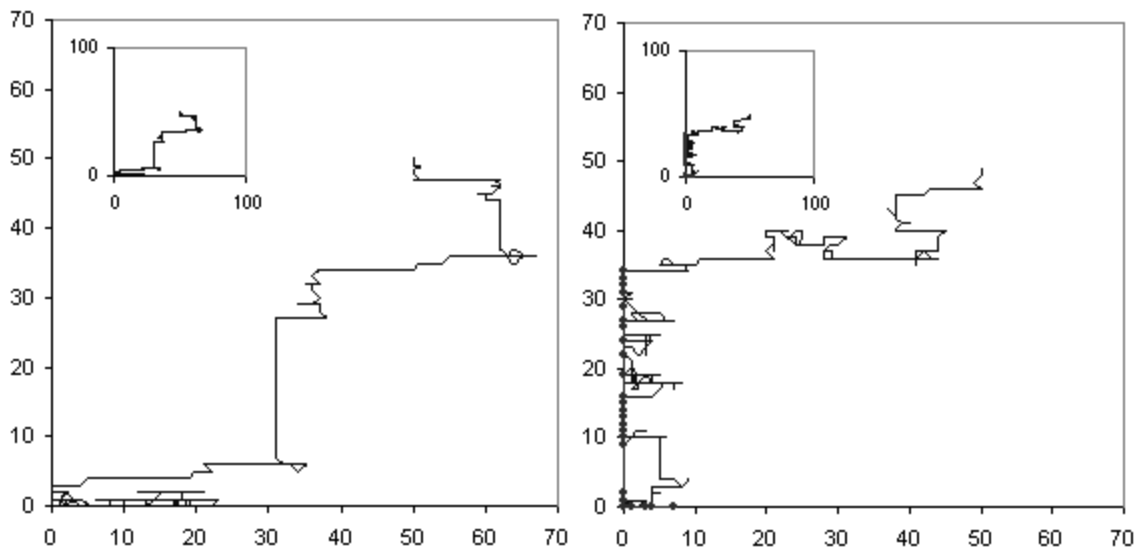


Figure 5. Trajectory of the Animat's movements in the 2D world during feedback (right panel) and no feedback (left panel). Dots in the right panel indicate occasions on which a stimulation pulse (feedback) was delivered.

a result of collisions with the left wall, the trajectory of movement remained similar to that observed without feedback. That is, the movement once again settled along a border, albeit a different one and with more deviations from the wall. A comparison of the data shown in the raster plots (cf Figure 3) with and without feedback indicated that although bursting appeared to be more frequent during the session with feedback, this effect was apparent even *before*

stimulations had commenced after approximately 97 seconds. Thus, this difference may be due to the time of measurement rather than any effect of stimulation.

Discussion

One of the goals of the *ANIMAT* project is to study information processing *in vitro* by providing a dissociated culture of neurons a body with which to behave, and a world in which to behave in. We have succeeded in our first major goal: to read activity from the culture in real-time, and to respond with feedback based on interactions between the *ANIMAT*'s simulated body and the virtual environment. Over the five minutes the *ANIMAT* was online, its movements occurred in each of the directions provided in the environment. These movements also produced feedback in real-time whenever those movements collided with one of the four nearby walls.

The second goal was to demonstrate a systematic effect of the feedback on the movement of the *ANIMAT* resulting in a change in behavior as a result of experience in the 2D world. However, over the course of the experiment there was little evidence that the feedback delivered as a result of collisions with the walls changed the behavior (i.e., the pattern of movement) of the *ANIMAT*. The *ANIMAT*'s movements both with and without feedback gradually progressed to the lower left quadrant of the 2D world indicating that the stimulations used here appeared to do little to influence ongoing activity.

There could be several reasons why feedback had no effect here. For example, one potential problem may have been that feedback was delivered only rarely. After all, not only was the session with feedback only five minutes long, but the single electrical pulse used as feedback was provided only upon the *ANIMAT*'s collision with one of the four walls. Although this strategy allowed us to avoid problems with the electrical artifacts produced during a stimulation (cf. lower panel of Figure 3) that interfered with recording on neighboring channels, it may not be sufficient to produce changes in activity. Typically, studies producing effects such as LTP and LTD use prolonged pulse trains of stimulation (e.g., Jimbo et al. 1999) rather than a single pulse. In fact, the current arrangement essentially produces a situation analogous to an animal in a dark room that can only learn about the room when it bumps into the walls, which could be a rather slow process. Therefore, the next attempt will need to deliver feedback continuously, perhaps using more elaborate patterns of stimulation than those used here.

Another potential problem may be the simplicity of the algorithm used to move the *ANIMAT*. By using only four of the 60 electrodes to produce movement in four directions, any bias in the firing rates among those channels would naturally result in a bias for that direction in the 2D world. The result is a series of movements that will gradually bring the *ANIMAT* toward the wall associated with that direction, and unless feedback modifies that tendency, movements will continue in that direction. This highlights one of the potential problems of this approach. That is, how does one specify the mapping between the activity and the behavioral output? One solution is to use distributed patterns of activity across the entire dish to produce movements. However, whatever rule or algorithm is used, it will need to be simple enough so that any changes observed in the behavior of the *ANIMAT* will be the result of changes in the pattern of activity within the dish, and not simply an artifact or property of the algorithm used to produce that movement.

Nonetheless these results demonstrate that the neurally controlled *ANIMAT* is possible, and although several more hurdles need to be overcome, it will be interesting to see what directions this particular technique will take us. For example, one day we hope to study whether any changes that do occur as a result of experience within the virtual environments we create are similar to the changes one would expect to see from phenomena reflecting some basic forms of learning such as sensitization or habituation, stimulus generalization, or perhaps even classical conditioning (e.g., temporal effects, 2nd order conditioning, inhibition of delay, sensory preconditioning, or blocking). Although this is indeed ambitious, providing evidence of parallels between the biology and behavior of the *ANIMAT* with well known learning phenomena will be an important step connecting what we learn about neural coding from the *ANIMAT* to that learned from the animals from which it came.

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