

Computation Within Cultured Neural Networks

T. DeMarse, A. Cadotte, P. Douglas, P. He, V. Trinh
Department of Biomedical Engineering, University of Florida, Gainesville, Florida

Abstract—In this paper we present three related areas of research we are pursuing to study neural computation in vitro. Rat cortical neurons cultured on 60 channel multielectrode array (MEA) allow the researcher to measure from and stimulate sixty different sites across a small population of neurons grown in vitro. Using this system we can send stimulation patterns into the network and study how these living neural networks compute by measuring its outputs. Our first series of studies uses chaotic control techniques to study the dynamics and potentially control the behavior of cortical network. At the same time, we are beginning to apply a model of computation called the liquid state machine or LSM model developed by Wolfgang Maass to provide a firm mathematical framework from which to proceed with our investigations. Each of these components is integrated into a third area investigating the role of computation and feedback using a real-time sensory-motor feedback robotic flight system.

Keywords—MEA; multielectrode array; neural computation; neuron; rat, cortex; cultured networks; stimulation; chaos control; nonlinear dynamics

I. INTRODUCTION

The brain is perhaps one of the most powerful and perhaps robust computers in existence. But how do the primary computing elements of the brain, neural cells or neurons, perform their computational feats? This is a difficult question to answer for a number of reasons. One is of course the brain's complexity. However, a more fundamental problem is the difficulty measuring these computational processes directly as they occur in a living brain. Furthermore, current data strongly points to our need to not only understand the properties of individual neurons, but the necessity of understand how thousands and perhaps millions of neurons interact as a population to produce computation. Fortunately, the technology has recently become available that may help to answer some of these questions.

Multielectrode arrays are specialized tissue culture dishes in which living neurons can be grown over multiple stimulation and recording electrodes. These electrodes permit the investigator to measure the activity of a small living neural network as well as manipulate that activity to study how information is processed, encoded, and translated into the network's outputs.

At the Neural Computation and Neural Robotics (NCNR) laboratory in the Biomedical Engineering Department at the University of Florida, we study how these

living networks, small populations of neurons perform their computations to unlock the mystery of how the brain performs it's computations.

II. METHODOLOGY

Living cortical neural networks are cultured on 60 channel multielectrode arrays (MEAs from MultiChannel Systems). Rat embryonic day 18 cortex (obtained from Brainbits) is dissociated by papain digestion followed by mechanical trituration. Ten to 20 ul of cell suspension containing approximately 50,000 neurons from this process are plated onto the surface of the MEAs which are coated with polyethylene-imine and laminin. Neurons that are grown on the MEAs are covered with a gas-permeable membrane which permits repeated observations without risk of infection from bacteria, etc. [1].

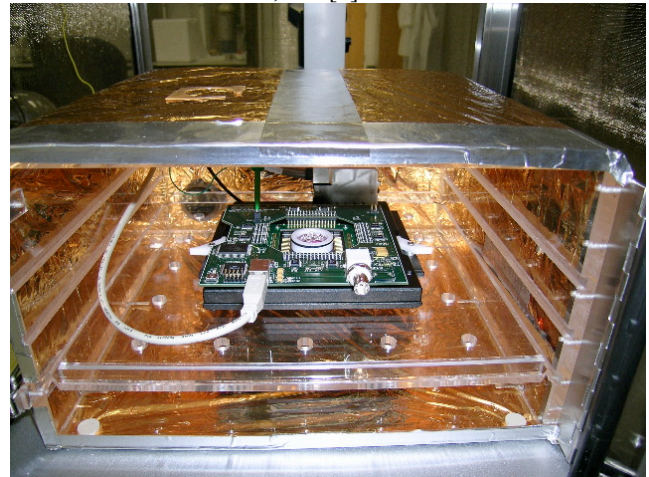


Fig 1. MEA1060 data acquisition system with 64 channel stimulator inside the environmentally controlled chamber.

Recordings are conducted using the MEA1060 system from MultiChannel Systems which records neural activity at 25KHz per channel. This system permits long-term recordings as well as the development of real-time feedback systems custom Linux software. Neural stimulation is accomplished via a custom 64 channel neural stimulation hardware (64CNS) which interfaces directly to MultiChannel System's amplifier stage. The 64CNS system features onboard computer control interfaced to a host computer through a high speed USB connection. Bi-Phasic stimulation voltages ranging from 200 to 800 mV and 100 to 500 us in duration are provided by an onboard 16 bit digital to analog converter which is isolated from each channel on the MEA with serially controlled analog switches. These switches

isolate the noise inherent in the stimulation hardware (millivolt range) from the amplifiers which record neural activity (microvolts). The 64CNS allows the host to dynamically stimulate each of the channels on the MEA on the fly as the experiment progresses.



Fig 2. Data acquisition system including (from top left to bottom right) amplifier, MEA temperature controller, MEA, MEA amplifier base, 64 channel stimulator, and PCI data acquisition card from MultiChannel Systems.

The 64CNS also includes a second bank of analog (blanking) switches which can tie individual channels through a 33K resistance to ground during stimulations to reduce artifacts, or to remove a channel from recording due to excessive noise levels (e.g., poor contact, or a faulty electrode). Any remaining artifacts from stimulation are further removed through software using an artifact suppression routine [2]. Using this system we can continuously record both the spontaneous neural activity on each channel and measure the neural responses to stimulation within a few milliseconds after each stimulation pulse. These pulses can be programmed into various stimulation patterns either from set preprogrammed experimental protocols or dynamically in response to ongoing real-time neural activity measured by MEA system.

III. RESULTS AND DISCUSSION

A. Chaos Control

One of the most common patterns of spontaneous activity of neurons cultured on these arrays takes the form of spontaneous, network-wide bursts [3-5]. These bursts are semi periodic occurring every 5 to 15 seconds and are typically 100 to 1000 ms in duration. Stimulation pulses delivered to a network of cultured cortical or hippocampal neurons primarily result in the production of a network wide burst of activity rather than eliciting responses from only a few neurons. In fact, much of the data investigating plasticity using these networks is based on differences in the number of action potentials produced during these bursts (e.g., [6, 7]).

Unfortunately it is difficult to elicit responses from these networks within a few seconds due to an inherent refractory period following each burst. This creates an upper limit on the rate at which information (patterns of stimulation) can be input or read out from the network. This is especially problematic during experiments requiring real-time control of external robotic systems when information about the network's current state that is used to control an external system such as a robot can only be extracted every 5 to 15 seconds. Furthermore, studies probing the network's state typically do so using periodic delivery of stimulation pulses and given the network's aperiodic behavior, these probes result in highly variable responses during the bursts that they produce if there is any response at all. Therefore developing a method to transition these networks from a state of bursting to more aperiodic state consisting of individual asynchronous action potentials would be very useful.

We are currently embarking on a new strategy to control and perhaps eliminate bursting within these networks using nonlinear or chaos control techniques pioneered by William Ditto and his colleagues [8-11]. With this technique the current state of the network is analyzed in terms of a return map in which the time between successive bursts is plotted. Periodicity is present when the time between previous bursts I_{n-j} and the current burst I_n fall on a diagonal line plotting I_n vs I_{n-j} . These points represent unstable fixed points in that as the system approaches these points (states), the system will rapidly (exponentially) diverge into unstable directions or manifolds. Chaos control identifies the location of these unstable manifolds and attempts to gently perturb the system into a stable orbit. Using this technique, Ditto and others have shown that this technique can be used to control the seemingly chaotic arrhythmia in both cardiac and neural hippocampal slice tissue [9, 10]. Interestingly, they also demonstrated the inverse relationship, that of *anti-control* in which the system was specifically driven into an unstable state becoming aperiodic.

We are currently working in collaboration with Bill Ditto at the University of Florida to implement this technique within the dissociated neural networks we use on multielectrode arrays. Our goal is to be able control the a periodic burst behavior of these networks to initially produce periodic burst behavior such that the network can be probed during times that will reduce the variability and increase the reliability of the network's responses. A second goal is to investigate the use of anti-control in an effort to eventually perturb the system to trajectories in which bursting no longer occurs. This would remove the rate limitation we have with current techniques and increase the bandwidth that we can communicate, modify, and assess the computational abilities of these cortical networks.

B. Liquid State Machine

One of the difficulties of studying neural computation within these dissociated cortical networks is the lack of a sound model with which to investigate their computational properties. Recently, Wolfgang Mass [12-14] has developed a new model of computation called the liquid state machine (LSM) which is well suited for its application to these cortical neural networks. The LSM (illustrated in Fig. 3) is based on biologically realistic pulsed neural networks L^M with continuous time series inputs $u(\cdot)$, a readout function f^M , and continuous outputs $y(t)$.

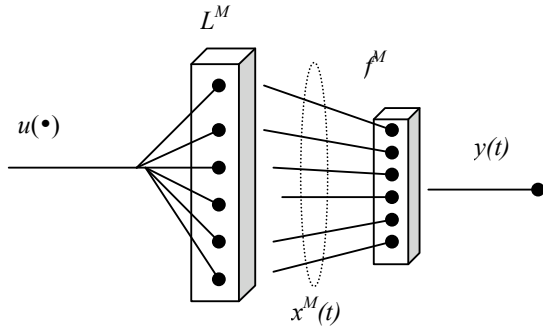


Fig 3. Architecture of the Liquid State Machine.

One of the most interesting features of this model is that the LSM is able to capture the dynamics of the liquid state and is capable of producing stable outputs even though the liquid state is a high-dimensional continuously varying pulse train similar to that of cultured cortical networks. Hence, we can read out the current computational state of the network and possibly map that to real world tasks. However, this model requires that inputs are separable/reliable in terms of

to explore and validate this model using dissociated cultured neural networks as a test bed in an effort to advance our understanding of the computational properties of how these networks process information.

C. Implementation of a Neurally Controlled Real-time Feedback and Control System

We are also investigating the use of these cortical networks as the computational unit for real-time control systems within autonomous robotic platforms. Our investigations are focused on creating a biologically controlled flight stabilization system in which living cortical neurons cultured on an MEA is interfaced to the flight control system of a small autonomous aircraft. This system, illustrated in Fig. 4 and Fig. 5., consists a remotely controlled aircraft with a forward mounted camera system, which transmits images of the horizon to a host computer on the ground. These images are then reduced to a 4x4 greyscale composite, which acts as the sensory input to the cortical culture. Each of the 16 gray scale pixel values are mapped to stimulations pulses on a corresponding channel in a multielectrode array containing cortical neurons. The timing between the delivery of each pulse is modulated by the 8 bit grayscale value (0 to 255) corresponding to 0 to 255 ms, respectively. The network's response is then read out and translated into control surface movements to stabilize the bank angle of the aircraft to maintain straight and level flight.

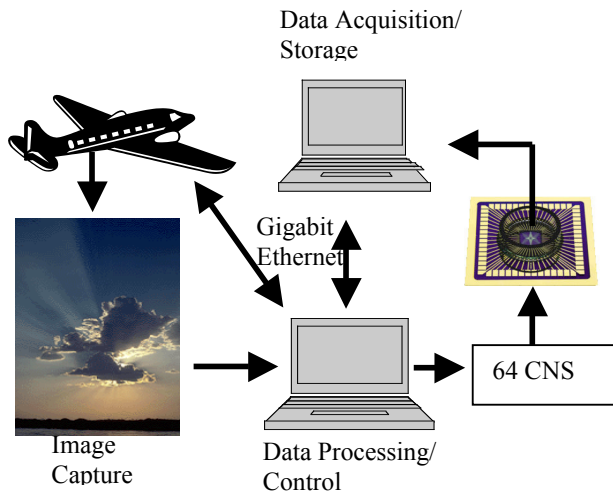


Fig 4. A cultured cortical network is used to control a robotic plane processing sensory information from an onboard camera to achieve stable flight.

the outputs they produce. In collaboration with Wolfgang Mass at the Technische Universitaet Graz in Austria, and Jose Principe at the University of Florida, we are beginning



IV. CONCLUSION

With the advent of multielectrode arrays it is now possible to study in detail how the brain computes. Our laboratory at the University of Florida is combining cutting edge technology with current theories and principles in nonlinear dynamics, modeling, and real-time neural data feedback and control test beds to further our understand of neural computation from the level of the individual neuron to population levels.

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