Adaptive Flight Control With Living Neuronal Networks on Microelectrode Arrays

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Abstract- The brain is perhaps one of the most robust and fault tolerant computational devices in existence and yet little is known about its mechanisms. Microelectrode arrays have recently been developed in which the computational properties of networks of living neurons can be studied in detail. In this paper we report work investigating the ability of living neurons to act as a set of neuronal weights which were used to control the flight of a simulated aircraft. These weights were manipulated via high frequency stimulation inputs to produce a system in which a living neuronal network would "learn" to control an aircraft for straight and level flight.

I. Introduction

Research into the computational properties of living neuronal networks has seen a rapid explosion in interest of the last two decades. This interest has been fostered by the advent of technology able to simultaneously measure neural activity from hundreds of neurons both in vivo [1-3] and in vitro [4-8]. However, many of the computational properties exhibited by these networks remain unclear.

Our approach is to use a system where we can measure, stimulate, and therefore manipulate activity across a grid of 60 electrodes using a planar microelectrode array (MEA). These arrays, shown in Figure 1 and Figure 2, consist of electrodes embedded under the surface of what is essentially a tissue culture dish in which a wide variety of neuronal tissue can be grown[4, 9]. Hence, the MEA makes the computational properties of cultured neuronal networks accessible for investigation using electrophysiological, optical, and pharmacological techniques.

In this paper, we report the results of an experiment using a living neuronal network as a matrix of weights that we can measure and manipulate in a real-time feedback control system to stabilize the flight of a simulated aircraft. The system, illustrated in Figure 1, consists of rat cortical neurons cultured on an MEA that are stimulated periodically to measure the weights from two different locations (stimulation sites) in the network. Proportional feedback as the result of errors in the aircraft's attitude (pitch and roll) is computed using the current synaptic weights measured between neurons within the rat cortical network. These weights were modified during each evolution based on the flight trajectory information, measurement of weights, and proportional feedback, to optimize the aircraft's stability. In other words, this living neuronal network essentially "learned" to act as an autopilot adjusting the aircrafts control surfaces to maintain straight and level flight.

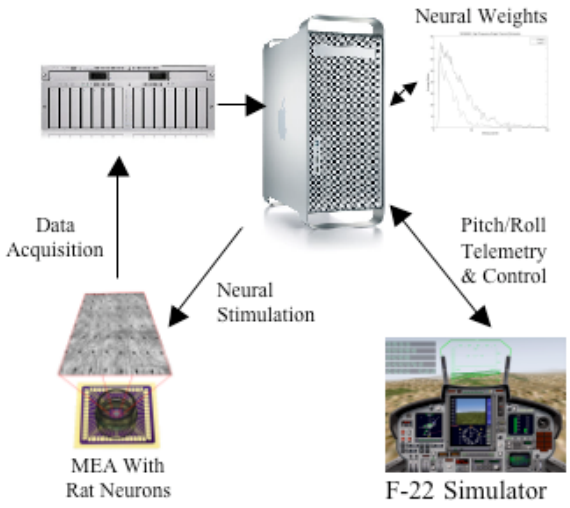

Fig. 1. Schematic of Neural Flight Control System using living rat cortical neurons for pitch and roll control.

II. Method

Rat embryonic (day 18) cortical hemispheres, obtained from Brain Bits™, were enzymatically digested with papain and mechanically triturated to remove connective tissue using the Papain Dissociation Kit from BioWorthington (cat# LK003150) producing a cell suspension of neurons and glia. The surface of each MEA was treated with polyethylene-amine (PEI) and laminin to improve cell adhesion, growth, and promote a uniform monolayer culture [10]. Twenty µl of this suspension containing approximately 25,000 cells was placed over the MEA electrodes shown in Figure 2 and supported with an additional 1 ml of culture media (DMEM supplemented w/10% Equine Serum- Hyclone).

Neurons in these cultures become spontaneously active within 3 to 5 days as connectivity between neurons is re-established [6, 11-14]. After 10 days the neural activity becomes synchronized producing spontaneous semi periodic bursts which will continue through out the network's lifetime. Measurements of neural activity were conducted using Multichannel System's data acquisition hardware and custom software on an Apple XServe with 3.5 Terabytes of XRaid disk storage. Raw electrical activity was recorded for each of

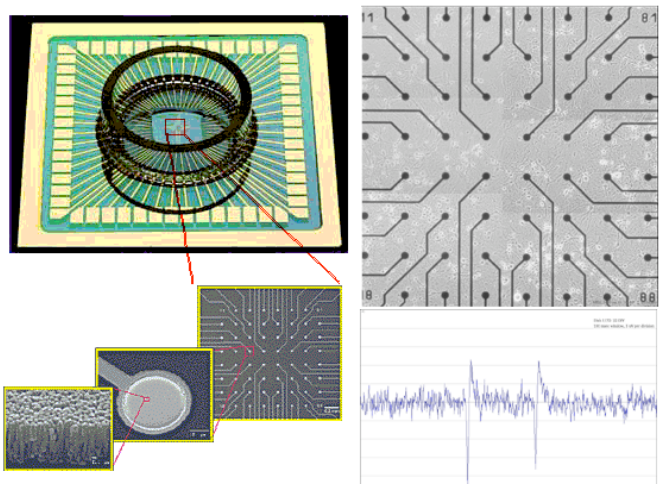

Fig. 2. Schematic of Neural Flight Control System using living rat cortical neurons for pitch and roll control.

the 60 channels on the MEA sampled and digitized at 25KHz per channel. This data was then streamed via TCP/IP to an Apple G5 client computer over a local gigabit network. The client then performed further data processing detecting action potentials (APs) (deviations in voltage above or below 5.0 x standard deviation of estimated noise per channel) and mapping telemetry from the flight simulator to schedule stimulations, while sending control commands to the aircraft, and logging the data.

An F-22 Raptor was simulated with the commercially available XPlane aircraft simulation software. The aircraft simulator was run on a separate computer (Dell PC) communicating with a client via UDP (transmitting flight telemetry: heading, speed, altitude, pitch and roll angle) every 200 ms. The simulator also received commands to adjust the angle of the aircraft's aileron and elevator control surfaces modifying the plane's in-flight roll and pitch angles, respectively.

Two of the 60 electrodes on the MEA where selected as stimulation sites representing the control for pitch and roll, respectively. These sites were randomly selected from sites that were both spontaneously active and could evoke activity with a stimulation pulse. Stimulations throughout this experiment consisted of a single 200 us/600 mV bipolar pulse. Each stimulation pulse evokes a response from neurons near the stimulating electrode which in turn propogates via synapses throughout the network resulting in a burst of activity for approximately 100 to 200 ms. The first 150 ms of that evoked response was recorded for each of the electrodes. Hence, stimulation of a single site permits the estimation of synaptic connectivity between neurons near the stimulation site to other neurons across the network (c.f. Jimbo, Tateno, & Robinson [15] for a similar technique used to assess network wide plasticity). Each stimulation site within the network produces a fairly reliable spatiotemporally rich response that varies in both number of APs produced per channel and the timing of those responses within the burst [16].

The weights within the network were modified using an effect originally reported by Eytan et al. (2002) in which low frequency (1/50 Hz) and high frequency (1/5 Hz) single site stimulation pulses where shown to selectively enhance or depress, the network wide response. For example, high frequency stimulations of a single site would, over the course of 15 minutes, reduce the number of evoked APs detected across the network. In contrast, low frequency stimulations delivered to a second stimulation site would result in a gradual increase in the number of APs.

More importantly, these stimulations could be conducted concurrently in the same network, increasing the evoked response from one stimulation site while simultaneously decreasing the response from another. Hence, the weights within the network could be systematically manipulated, measured, and used as a living neural analog of a set of weights to adjust the response of a simple flight control system.

In this experiment, two stimulation sites where chosen to represent the weights for pitch and roll control. The evoked response from one site was used for pitch control, while the evoked response from the second site was used for roll. The average number of APs 150 ms following each stimulation was recorded and divided into 1 ms bins producing a 150 element weight vector. The difference between the current weights and the initial weights established the current flight weight vector that was used to control the aircraft.

The current pitch and roll error from aircraft telemetry, ranging from straight and level (0 degrees) to a maximum of ±180 degrees (inverted flight), was mapped to the 150 ms interval. A proportional control signal was achieved by summation of the current flight weights from 0 ms to the millisecond value corresponding to the current error.

Each evolution began with a series of nine high frequency stimulation pulses delivered to both the pitch and roll channel. Following this, a single probe stimulation to the pitch and roll channel measured the current weights within the network for that channel. Every 200 ms during this period the telemetry from the aircraft was used to determine any corrective feedback. This feedback was based on the current flight weights determined from the prior evolution.

Initially, no difference is present between the weights measured before high frequency stimulation was delivered and the first few evolutions. Thus, any errors in pitch or roll will not result in any corrective feedback. However, as the network is modified the control signals (and correspondingly the movement of the control surfaces) should increase in magnitude. After, several minutes of flight, the network will slowly begins to correct for any errors in the flight path resulting in straight and level flight.

III. Results

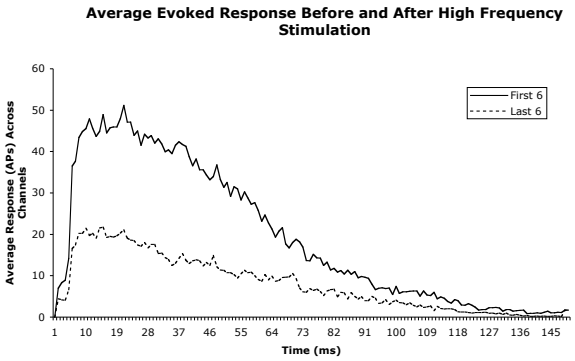

Fig. 3. Average number of APs across channels 150 ms following 6 probes before and after 15 minutes of high frequency stimulation (n = 4 cultures).

The effect of high frequency stimulation was assessed by comparing the network's response to single site stimulation during the first versus the last six stimulation pulses. Figure 3 shows the average number of evoked APs, 150 ms following each stimulation, before and after high frequency stimulation. This average represents the response across all 60 electrodes. There was a significant decrease in the number of APs between the first 6 and last 6 stimulation probes (ANOVA, $F(1,149) = 161.59$, $p < 0.01$) which is consistent with the effect originally reported by Eytan and Marom[5].

As the network's response is modified over time resulting in an increase in the flight weights, the system will begin to correct for errors in the aircrafts pitch and roll. Figure 4 shows the pitch and roll angles of the aircraft during the last eight minutes of flight for one culture. The typical performance of the network's control was within 10 degrees of desired for both pitch and roll. However, as the high frequency stimulations increase the weights used to fly the aircraft these weights will eventually become too large resulting in over corrections from even the smallest in errors. Moreover, the rate at which pitch and roll channels change (as a result of the high frequency stimulation) over time can lead to differences in control. This over correction is apparent in the data for the roll angles whose flight weights caused the aircraft to begin to oscillate.

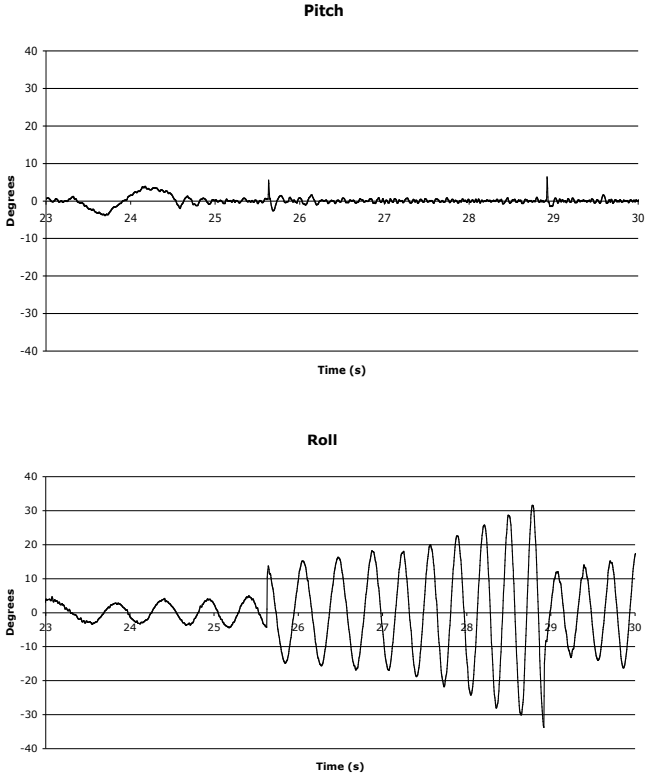

Fig. 4. Pitch and roll error during the last 8 minutes of a 30 min experimental run for one culture. Note the oscillations present in the roll axis. With continued high frequency stimulation, the response from the network associated with roll channel will decrease to the point of overcorrecting for small errors.

IV. Discussion

A system was created in which a network of living rat cortical neurons were slowly adapted to control an aircrafts flight trajectory. This was accomplished by using high frequency stimulation pulses delivered to two independent channels, one for pitch, and one for roll. This relatively simple system was able to control the pitch and roll of a simulated aircraft.

However, the system is susceptible to continued increases in flight weights as long as high frequency stimulation is present leading eventually to over corrections. This problem can be alleviated by simply removing high frequency stimulation when the weights are optimized or by switching to low frequency stimulation. This would then reverse the effect, and therefore, decrease the weights.

In this experiment, the network's response was manipulated from only two of the sixty possible sites. However, if the network's response for each channel can be selectively increased or decreased, dependant on the frequency of stimulation, it may be possible to adjust the response of the network at all sixty sites. In other words, perhaps much more of the network can be controlled, essentially treating the network as a set of living neuronal weights and manipulating those weights in much the same way as artificial neural networks (ANN) do during training.

In this system the weights measured from two of the sixty possible sites were used to control the aircraft. However, if more sites can be manipulated it may be possible to adjust the weights within this network on a much broader scale than what was accomplished here. For example, imagine an image containing sixty pixels in which each pixel was mapped as a desired weight onto the 60 sites of the MEA. If the network's weights were then modified to represent those weights using a combination of high and low frequency stimulations, we could embed those weights into the network and examine whether degraded images (e.g., missing pixels) are "filled in" by the network. In other words, examine the potential for pattern

recognition within these networks. This question, and many others are among those we are currently pursuing in our research in an effort to study the computational properties of these living neuronal networks.

References

[1] Chapin, J.K., K.A. Moxon, R.S. Markowitz, and M.A.L. Nicolelis "Real-time control of a robot arm using simultaneously recorded neurons in the motor cortex," *Nature Neuroscience*, vol. 2(7), 664-670, 1999.

[2] Chapin, J.K., K.A. Moxon, R.S. Markowitz, and M.A. Nicolelis "Real-time control of a robot arm using simultaneously recorded neurons in the motor cortex," *Nat Neurosci*, vol. 2(7), 664-70, 1999.

[3] Chapin, J.K. and M.A. Nicolelis "Neural network mechanisms of oscillatory brain states: characterization using simultaneous multi-single neuron recordings," *Electroencephalogr Clin Neurophysiol Suppl*, vol. 45, 113-22, 1996.

[4] Egert, U., B. Schlosshauer, S. Fennrich, W. Nisch, M. Fejtl, T. Knott, T. Muller, and H. Hammerle "A novel organotypic long-term culture of the rat hippocampus on substrate-integrated multielectrode arrays," *Brain Res Brain Res Protoc*, vol. 2(4), 229-42, 1998.

[5] Eytan, D., Brenner, N., and Marom, S. "Selective Adaptation in Networks of Cortical Neurons," *Journal of Neuroscience*, vol. 23(28), 9349-9356, 2003.

[6] Gross, G.W., and Kowolski, J. "Origins of activity patterns in self-organizing neuronal networks in vitro," *Journal of Intelligent Material Systems and Structures*, vol. 10, 558-564, 1999.

[7] Jimbo, Y., A. Kawana, P. Parodi, and V. Torre "The dynamics of a neuronal culture of dissociated cortical neurons of neonatal rats," *Biological Cybernetics*, vol. 83, 1-20, 2000.

[8] Potter, S.M. and T.B. DeMarse "A new approach to neural cell culture for long-term studies," *J. Neurosci. Methods*, vol. 110, 17-24, 2001.

[9] Novak, J.L. and B.C. Wheeler "Two-dimensional current source density analysis of propagation delays for components of epileptiform bursts in rat hippocampal slices," *Brain Res*, vol. 497, 223-30, 1989.

[10] LeLong, I.H., V. Petegnief, and G. Rebel "Neuronal cells mature faster on Polyethyleneimine coated plates than on plylysine coated plates," *Journal of Neuroscience Research*, vol. 32, 562-568, 1992.

[11] Segev, R., M. Benveniste, E. Hulata, N. Cohen, A. Palevski, E. Kapon, Y. Shapira, and E. Ben-Jacob "Long term Behavior of lithographically prepared in vitro neuronal networks," *Physical Review Letters*, vol. 88(11), 118102, 2002.

[12] Kamioka, H., E. Maeda, Y. Jimbo, H.P. Robinson, and A. Kawana "Spontaneous periodic synchronized bursting during formation of mature patterns of connections in cortical cultures," *Neurosci Lett*, vol. 206(2-3), 109-12, 1996.

[13] Habets, A.M., A.M. Van Dongen, F. Van Huizen, and M.A. Corner "Spontaneous neuronal firing patterns in fetal rat cortical networks during development in vitro: a quantitative analysis," *Exp Brain Res*, vol. 69(1), 43-52, 1987.

[14] Canepari, M., M. Bove, E. Maeda, M. Cappello, and A. Kawana "Experimental analysis of neuronal dynamics in cultured cortical networks and transitions between different patterns of activity," *Biological Cybernetics*, vol. 77, 153-162, 1997.

[15] Jimbo, Y., Tateno, T., and Robinson, H. P. C. "Simultaneous Induction of Pathway-Specific Potentiation and Depression in Networks of Cortical Neurons," *Biophysical Journal*, vol. 76, 670-678, 1999.

[16] Mainen, Z.F. and T.J. Sejnowski "Reliability of spike timing in neocortical neurons," *Science*, vol. 268, 1503-1506, 1995.