Experiential Neurophysiology Course for Biomedical Engineers

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ABSTRACT

An interdisciplinary course at the senior/graduate level has been developed and implemented to provide engineers with neurophysiological training. The contemporary engineering curricula generally consist of a significant amount of theory and mathematics. For biomedical engineering students this often results in limited hands-on experiences with live tissue samples and biological experimental techniques. In the Biomedical Engineering Program at the University of Rhode Island, this issue is addressed to some extent by implementing an experiential neurophysiology laboratory. The standard curriculum includes fundamental biology courses including Mammalian Physiology and Human Anatomy; however the underlying electrophysiological relationship is obfuscated. The two-semester project course establishes this relationship and provides laboratory skills in dissection, data acquisition with current physiological instrumentation, and physiological measurements. Additionally, the course serves as an electrical engineering design elective by incorporating electrical engineering applications, such as signal processing and electrical modeling, with neuroscience. Two experiments were chosen to elucidate neurophysiological principals as well as providing a physiological template to construct an electrical model: 1) microelectrode recording of neuronal action potentials from the central nervous system (CNS) of the pond snail (*Lymnaea stagnalis*), 2) microelectrode recording from R15 in the abdominal ganglion of the gastropod *Aplysia californica* while introducing the neurotransmitters acetyl choline (ACh) and 5-Hydroxytryptamine (5-HT). A circuit has been designed and constructed that emulates the action potential output of the neuron RPD1, located in the right parietal ganglion, of *Lymnaea stagnalis*. Signal conditioning and processing techniques have been incorporated to aid in the data analysis of the neuronal signal output of both specimens. This laboratory has proven to be an effective way to provide undergraduate biomedical engineering students with invaluable live tissue experimentation skills in neuroscience and electrophysiology, while maintaining electrical engineering design applications.

INTRODUCTION

BIOMEDICAL engineering education may come from within a traditional engineering curriculum. This is the case at the Electrical Engineering Department of the University of Rhode Island. The program focuses on electrical engineering as it pertains to biomedical applications. A need exists to integrate the traditional engineering training with the intricacies of biological systems. Engineers are better prepared to solve problems when they have a first-hand understanding of the problem as well as its cause. For medical education during the last decade, the pedagogical style has also shifted from factual teaching towards contextual, or problem-based, learning [1]. To this end, we have developed a laboratory course to address these issues and give the biomedical engineering students the insight and research skills necessary to understand and help solve biological and physiological problems. Specific issues such as neurophysiolgy, data acquisition, data analysis and interpretation, and electrical modeling are addressed.

METHODS

Action Potential Recording Using Microelectrode Methodology: Lymnaea stagnalis preparation

In 1952, Hodgkin and Huxley published a series of four papers describing the inward and outward currents of Na+ and K+ through the cell membrane [2]. These papers were the result of years of experimentation with the squid giant axon and the voltage clamp. In order to measure the influx of sodium ions and the outward flow of potassium ions, the membrane potential must be held fixed, or clamped, at a certain value. Therefore, any change in current, i.e. the flow of specific ions, which underlies an action potential, can be detected.

One of the neurophysiologic experiments conducted at our self- constructed lab at the University of Rhode Island involves the central nervous system of the pond snail, *Lymnaea stagnalis*. Although the ganglia are small, on the order of 200 to 400µm, the individual neurons are comparatively large (figure 1). In fact, they are large enough to record the action potential with a pulled glass pipette microelectrode. However, removing the ganglia intact requires terrific skill and a great deal of practice since the ganglia are completely obstructed, positioned inferior to the buccal muscle. Once the buccal muscle is moved, the ganglia are exposed and the process of meticulously separating the ganglia from the surrounding tissue begins [3].

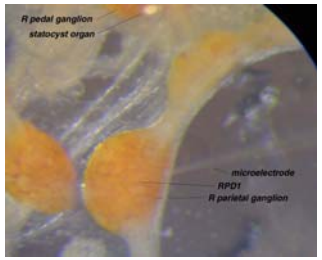


Figure1-*Lymnaea stagnalis* R. Parietal Ganglion and RPD1

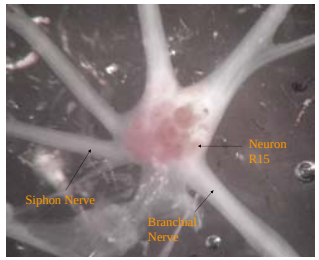


Figure 2-*Aplysia califonica Abdominal ganglion and R15*

Intracellular Recording from R15 in Abdominal Ganglion of Aplysia californica

The dissection of Aplysia californica is equally challenging, but significant research has been done on this animal that facilitates key anatomical knowlegde.

Among the identified neurons in the abdominal ganglion, R15 is located on the right side in the dorsal view (figure 2). It is known to be a bursting neuron, meaning the action potentials are generated in bursts of multiple action potentials followed by a period of hyperpolarization.

The microelectrode is created using a filament capillary tube (1mm O.D., 0.58mm I.D.). Using a horizontal manual microelectrode puller, the electrode is melted and stretched to a tapered end. The glass microelectrode is then filled with 3M KCl and the tip resistance is measured at 10-30 Mega Ohms.

A Narishige micromanipulator is used to position the tip of the electrode to the surface of the neuron. Slight tapping eases the tip into the cell and the recording begins.

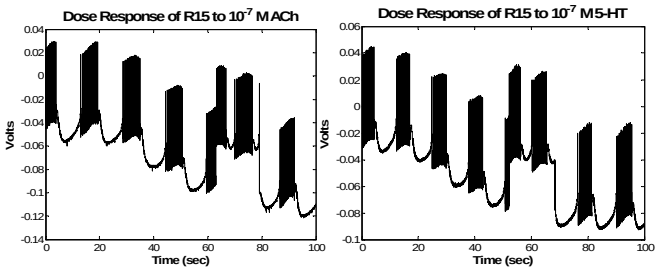
Neurotransmitters are slowly and methodically introduced using a perfusion system. This system, which is also used to provide a constant supply of fresh filtered sea water, minimizes the risk of generating a capacitance resulting from an abrupt change of the solution level.

RESULTS

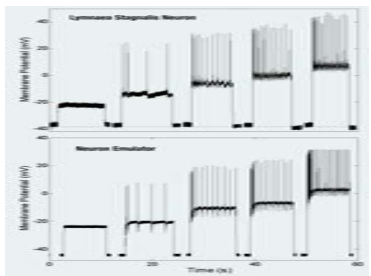
Since the biological experiments themselves have been well documented by those much more qualified than biomedical engineers, we focus our attention on what we know best: Circuit modeling, Data acquisition, and signal processing.

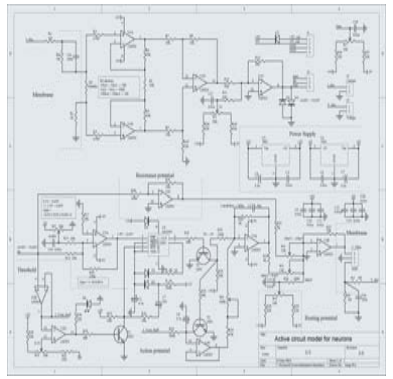
The neuron emulator (figures 3,4,and 5) was presented to the University of Rhode Island Intellectual Property Committee (IPC) and has subsequently been provided protection under U.S. provisional patent laws. The device will be made here at the University of Rhode Island, where further *Lymnaea stagnalis* experimentation is providing the inspiration for improved modeling and interactive controls.

Data acquisition of the biological signals is accomplished using the Axon Instruments Gene Clamp 500 amplifier and Measurement Computing's PMD-1608FS USB 2.0 data acquisition device (DAQ). Figures 6 and 7 (below) show dose response to 10^{-7} molar ACh and 5-HT (serotonin), respectively, as recorded using MATLAB's Data Acquisition Toolbox version 2.5.



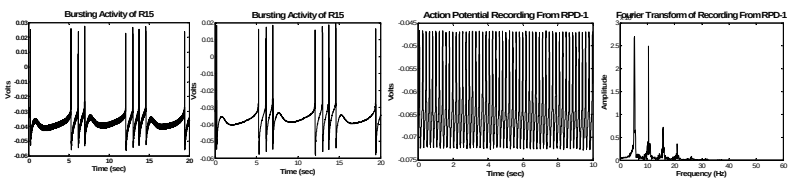
Figures 6 and 7. The figure on the left (**figure 6**) shows the steady hyperpolarization after the introduction of ACh. Similar results are achieved after adding serotonin (**right**).





The neuron emulator (**figure 3, above left**) performs real-time, interactive simulation of a neuron in terms of the following: 1) electrode and membrane resistance and capacitance for both current input and voltage output ports , 2) membrane resting potential when input current is zero, 3) firing of action potentials above a depolarization current threshold, 4) adaptation of the spiking response (**figure 4, left**), 5) response to voltage clamping, and 6) hyper-polarization following each spike. **Figure 5** (**above**) is the circuit schematic for the Neuron Emulator.

A critical issue in the data acquisition of biological signals obtained in this fashion is the susceptibility to noise. The electrode resistance is on the order of 30 Mega Ohms, so faint ambient electrical signals, as well as their harmonics, can be manifest in the recorded signal. This is a simple issue to rectify using MATLAB's powerful signal processing toolbox. Figures 8 and 9 show the dramatic reduction in 60Hz noise from a 20 second segment of the R15 bursting signal using a 6th order low pass elliptic filter with 0.1dB attenuation in the pass band. Further signal analysis yields the Fourier transform of a signal from Lymnaea stagnalis (RPD-1) is shown in figures 10 and 11.



Figures 8, 9, 10, 11 (Left to Right). 60Hz noise in the signal in **figure 8** contributes close to 5mV, where as **figure 8**, the filtered signal, is virtually noise free. Note the slight attenuation of the signal. Signal from RPD-1 (**figure 10**) and its corresponding Fourier transform (**figure 11**).

DISCUSSION

The experiments developed in this neurophysiology course have provided invaluable experimental training for the biomedical engineering students. Despite the specificity of the experiments, they exemplify advanced research in neuroscience and are technically challenging to motivate the students. As stated, a further goal of this course was to incorporate electrical engineering design skills. This was successfully accomplished with the design and manufacture of the Neuron Emulator, currently under the protection of U.S. Provisional Patent.

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