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Californica Dreaming: Neurotransmitter 'Doze' Response and Neuronal Signal Analysis of *Aplysia californica*

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Abstract-The invertebrate central nervous system is ideally suited for the study of neurophysiology, as the comparative size of the individual neuron is rather large; on the order of 20 to 200 microns. Intracellular recordings of these cells are accomplished with a modicum of difficulty to an investigator familiar with the anatomy of the specified invertebrate. Once a microelectrode is inserted in the cell, neurotransmitters, such as 5-hydroxytryptamine (5-HT) and acetylcholine (ACh), can be released into the surrounding tissue bath, and the response to those neurotransmitters are observed as changes in the neuronal output. The engineering aspect of this investigation lies within the signal processing of the observed response. This research examines the dissection process of *Aplysia californica*, the cellular response of the central nervous system to 5-HT and ACh, and the signal processing techniques of the resulting waveforms.

***Index Terms*— Biomedical engineering, electrophysiology, Aplysia californica, neurophysiology, neurotransmitter.**

I. Introduction

APLYSIA californica are an invertebrate of the phylum mollusca and the class gastropoda [1]. They are found, as one might expect, in the cold pacific waters off the coast of California. They have a simple, yet elegant, central nervous system which consists of several key ganglia, including the buccal, cerebral, pleural, pedal and abdominal ganglion. It is this last ganglion which is the focus of this research.

The abdominal ganglion, also referred to as the visceral ganglion, is positioned in the abdominal region, in close proximity to the sex organ, the opaline gland, and the heart. Among its many functions, it serves as the origin of the command signal for one the *Aplysia* defense mechanisms: inking. This signal is carried to the inking gland, also known as the purple gland, via the branchial nerve. With the exception of the errant stimulation of the branchial nerve, and consequently the purple gland, the dissection is straightforward. The large neurons in the abdominal ganglion have been extensively mapped and identification is palpable[2]. Individual neurons are selected based on the type of neuronal activity they exhibit and the types of neurotransmitters that elicit a response, either excitatory or inhibitory, from the neuron.

The output neuronal action potentials are recorded and the time series analysis of the signal is performed using MATLAB [3].

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II. Methods

Dissection of Aplysia californica

The *Aplysia californica* preparation begins with the administration of approximately 20ccs of 0.36M MgCl to anesthetize the animal. (The adult juvenile *Aplysia* has a mass that ranges from 100 to 200 grams so the amount of anesthetic will vary.) A transverse incision is made along the hind quarter of the foot, providing entry to the visceral cavity. Using the thumb to create a pocket, scissors are used to make a cut along the long axis of the animal. The incision is stopped just before reaching the buccal mass. With the visceral cavity operative, the digestive organs are excised and the abdominal ganglion is isolated [4].

Removing the abdominal ganglion without stimulating the purple gland requires careful dissection technique. The branchial nerve descends sharply through the abdominal wall, terminating at the purple gland and the gill. Small, deliberate cuts are required to expose the entire nerve, and in a decisive manner, the branchial nerve is severed at the gill [4]. The remaining neural connectives are cut and the ganglion is removed. It is then transferred to a 4cm diameter Sylgard lined Petri dish filled with filtered seawater.

Intracellular Recording from R15

Among the identified neurons in the abdominal ganglion, R15 is located on the right side in the dorsal view (see figure 1). It is known to be a bursting neuron, meaning the action

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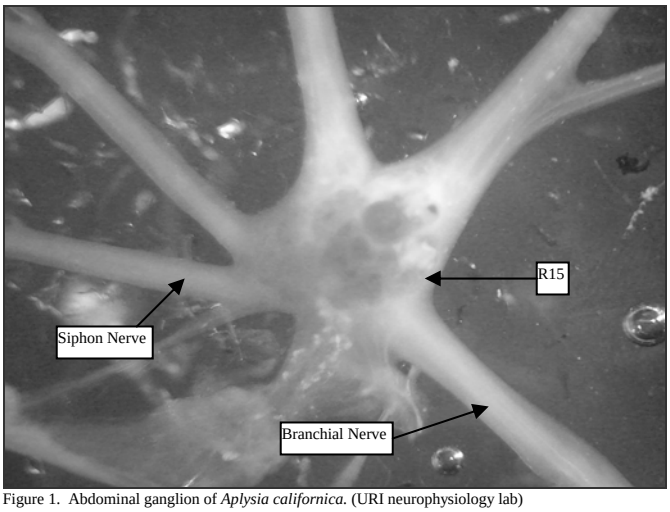


Figure 1. Abdominal ganglion of *Aplysia californica.* (URI neurophysiology lab)

potentials are generated in bursts of multiple action potentials followed by a period of hyperpolarization.

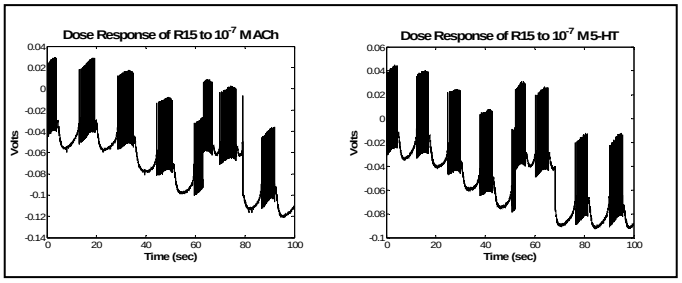
The microelectrode is created using a filament capillary tube (1mm O.D., 0.58mm I.D.). Using a horizontal manual microelectrode puller, the electrode is melted and stretched to a tapered end. The glass microelectrode is then filled with 3M KCl and the tip resistance is measured at 10-30 Mega Ohms.

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.

III. Results

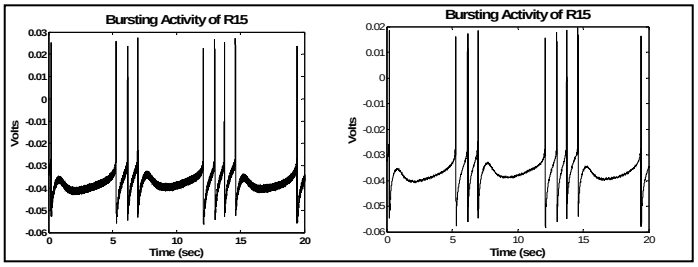
As neurotransmitters are extremely powerful compounds, we have introduced concentrations of 10^{-7},10^{-6} and 10^{-5} molar. To illustrate these effects, figures 2a and 2b show the response of R15 to 10^{-7} M ACh and 10^{-7} M 5-HT (serotonin), respectively. (The data was acquired using Measurement Computing's PMD-1608 and displayed using commands from the MATLAB data acquisition toolbox.)



Figures 2a and 2b. The left figure shows the steady hyperpolarization after the introduction of ACh. Similar results are achieved after adding serotonin (right).

Increasing the molar concentration increases the hyperpolarization trendline. It is steeper and the recovery to resting potential, approximately -40mV, is of longer duration.

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. Since the biological signals of interest have frequency components less than 60Hz, the frequency of oscillation of AC devices, MATLAB can create a low pass filter which removes all the high frequency components above a given threshold, in this case 60Hz. Figures 2a and 2b 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. Note the slight attenuation of the signal from figure 3a to figure 3b.



Figures 3a and 3b. 60Hz noise in the signal on the left (a) contributes close to 5mV, where as the figure on the right, the filtered signal, is virtually noise free. The cost is a slight attenuation of the signal

IV. Discussion

The neurophysiology lab at the University of Rhode Island has established an efficient and productive protocol for recording neuronal communication patterns from the mollusk *Aplysia californica.* In addition to providing critical hands on training in live tissue experimentation, the research conducted here has elucidated the complex nature of cellular communication, their ability to generate signals, and their response to neurotransmitters. Real world signals are analyzed and processed using familiar MATLAB techniques, while solutions to problems concerning data acquisition and noise are identified and solved.

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