A Fully Digital Implementation of Voltage, Current, and Dynamic Clamping Methodologies

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Abstract—**Standard electrophysiology experiments utilize a technique known as voltage clamping: the surface of the cellular membrane is held constant, or clamped, which allows the researcher to study the membrane permeability and ion exchange. This process is usually performed with commercially available analog equipment. While analog circuitry provides an accurate and reliable equipment medium, it does not allow for much in the way of real-time data processing. Additionally, there is the issue of data acquisition which must be performed using an analog to digital converter (A/D). A need exists to resolve these issues in one device. The Universal Clamp, a patent pending electronic device designed and built by Dr. Jiang Wu at the University of Rhode Island, addresses this need. The Universal Clamp, however, does much more than data acquisition (DAQ) and signal processing. As the name implies, it has the capability of performing voltage clamping, current clamping, and dynamic clamping, all in one device, with the aid of a digital signal processing (DSP) chip. This functionality provides researchers greater flexibility in the types of experiments they can conduct, as well as simplifying current standard methodologies. Using the visceral ganglion from *Aplysia californica*, the Universal Clamp has successfully performed voltage clamping, current clamping and dynamic clamping, while simultaneously executing data acquisition and signal processing algorithms.**

***Index Terms*— Biomedical engineering, electrophysiology, voltage clamp, current clamp, dynamic clamp.**

I. Introduction

In 1952, A.L. Hodgkin and A.F. Huxley published a series of four landmark papers describing the inward and outward currents of Na+ and K+ through the cell membrane [1]. The research was made possible by the voltage clamp, developed by George Marmont and Kenneth Cole (independently) in 1949 [2]. It has been transformed many times over since its inception, but the fundamental principal remains: undesired capacitive currents only flow when the membrane potential is changing and therefore the membrane potential must be held to some fixed value via a network of feedback amplifiers in order to isolate the ionic currents responsible for the action potential. Other methodologies soon followed, including the current clamp, in which current is passed directly into the cell at a fixed value, creating a change to the resting membrane potential. But it wasn't until 1993 when A.A. Sharp *et al*, introduced a new type of clamping, called dynamic clamping, that the inter-neuronal communicatory relationships were able to be directly measured and manipulated [3]. The Universal Clamp, a fully digital implementation, is the next in the series of indispensable electrophysiology instrumentation by combining all three types of clamping in one device. It is to date the only device of its kind, as even commercial digital implementations still utilize analog feedback.

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II. Methods

The *A. californica* preparation begins with the administration of approximately 40ccs of 0.36M MgCl to anesthetize the animal. As *A. californica* has a half open circulatory system, the anesthesia can be injected into the musculature of the foot. That is, once the blood is pumped from the heart, it flows through the body and returns through the tissue, not a capillary network. The anesthesia is taken into the circulation by this process. Filtered sea water is used to rinse and perfuse the animal throughout the dissection process in order to keep the tissues viable for an extended period of time. A two centimeter transverse incision is made across the hind quarter of the foot, providing entry to the abdominal cavity. Using the thumb to create a pocket at the hind, 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]. The neurons R2 and $L9_2$ were used in these experiments.

III. Results

Voltage and Current Clamping Using the Universal Clamp

Since the Universal Clamp is a digital device, it has capabilities that are just not possible using standard analog voltage clamps. The most notable advantage is the ability to perform voltage clamping using one electrode without the need for external switches. Using software to control switching between current injection and voltage measurement, the Universal Clamp eliminates the need for a current injection electrode and a voltage measurement electrode. This is only possible at extremely high sampling rates, in the range of 200k-500k samples per second, since current is passed into the cell and the resulting transient must subside before the voltage measurement can be acquired (Typically 10-20 microseconds). Positive and negative pulses of current are

passed at high frequency to maintain the voltage level (Fig. 1, right). By eliminating the second electrode, the device reduces the risk of damage to the cell, prolonging the viability of the tissue. In a typical voltage clamp design, this is performed using external switches which are fixed and specific to the device. The Universal Clamp uses software to perform the switching, so the number of switches is scalable.

Current clamping involves passing current into the cell to change the resting membrane potential. Once enough current is passed to raise the membrane potential past the threshold, the cellular response is to initiate a high frequency volley of action potentials. When the current is removed, the membrane potential is returned to its original level, but the cell has adapted to the higher potential and will not fire another action potential until the resting potential is restored through refractory (Fig. 1, left).

Dynamic Clamping Using the Universal Clamp

The beating neuron $L9_2$ in the visceral ganglion of *Aplysia californica* was used to drive a silent neuron, R2, in the same ganglion. The Universal Clamp algorithm is designed as follows: 1) Record action potentials via an electrode in the $L9_2$ neuron 2) Detect action potentials with a threshold set at -30 mV 3) Inject a current pulse (8nA, 0.3 s duration) immediately after each detected action potential via a second electrode into the R2 neuron. In the realm of cellular neurophysiology, 8nA is considered a moderate to high amount of current [5]. The justification for this amount of current is that the cell being stimulated was much larger, relatively speaking, than the cell providing the signal (Fig 2, left).

The results show that the Universal Clamp is capable of forcing a silent neuron to fire action potentials in synchronization with another beating neuron (Fig. 2, right). The on-board DSP of the Universal Clamp makes it possible to control fast neuronal action potentials. The power of the DSP means filters can be applied to reduce noise or isolate specific frequency components. In the treatment of spinal cord injuries, signals from the operative side of the injury must be preserved in real time and transferred to the non-operative side of the injury. Similarly, the brain-machine interface and neural prosthetics rely heavily on frequency encoding, which require Fourier Transform analysis to be performed in real time.

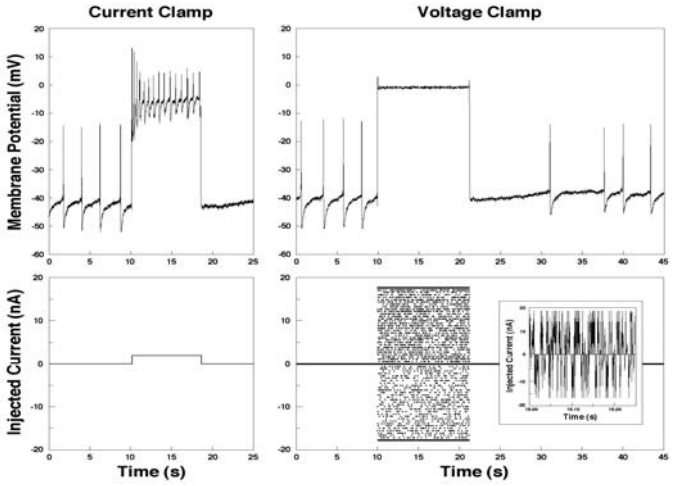

Figure 1. The response of the cell to the current and voltage clamps, from left to right, respectively. Note in the voltage clamp figure, the inset of injected current is continuously and automatically adjusted to compensate for the time varying capacitance of the cell.

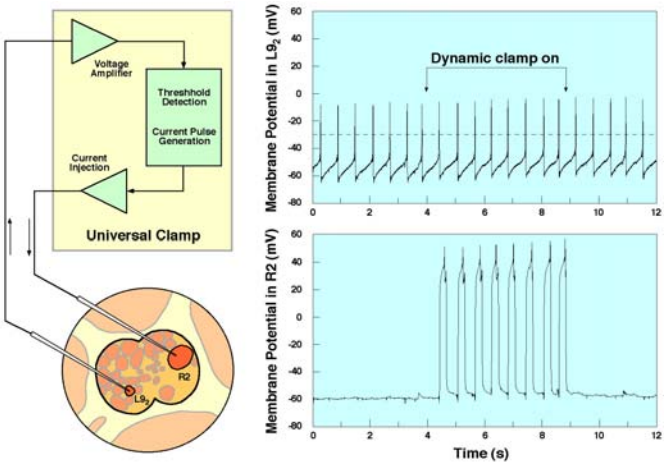

Figure 2. Left, a graphical representation of the visceral ganglion of the gastropod *A. californica* depicting two uncoupled neurons being connected by the Universal Clamp. Right, the device successfully forces a silent neuron to beat in synchrony with the driving neuron.

IV. Discussion

The Biomedical Engineering Program at the University of Rhode Island has produced a unique digital electrophysiology instrument. The Universal Clamp provides researchers an advanced data acquisition and data processing capabilities previously unavailable in one device. These capabilities have been demonstrated using the visceral ganglion of *A. californica.* Its efficient design makes it an attractive choice for researchers needing to streamline their instrumentation and concentrate on developing methodologies for physiological understanding. NIH funding has provided the means to improve the design and bring it to market. The partnership with myNeuroLab.com, St. Louis, MO, in the SBIR Phase I grant has established legitimacy for future grant proposals including the SBIR Phase II grant.

Acknowledgement

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