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. Currently, 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 still 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. This poster will present the relevant experiential justification for asserting the effectiveness of the Universal Clamp, the different types of clamping and their purpose, as well as the algorithms employed which make the Universal Clamp such a unique device. The broader impact to the physiological community, including research in neural prosthesis, spinal cord injuries, and the brain-machine interface, will also be discussed.

INTRODUCTION

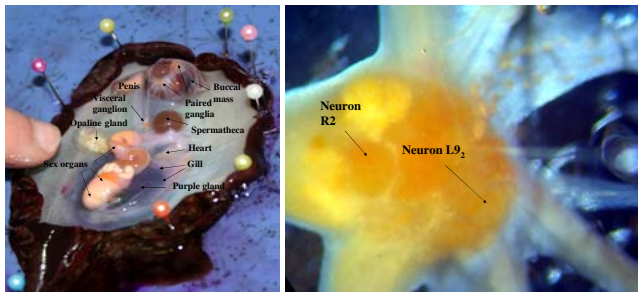
In 1952, A.L. Hodgkin and A.F. Huxley published a series of four landmark papers describing the inward and outward currents of Na+ and K+ through the cell membrane [1]. These papers were the result of years of experimentation with the squid giant axon and the voltage clamp. The ingenious voltage clamp, developed by George Marmont and Kenneth Cole in 1949, 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. 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. The functionality, however, extends far beyond the clamping capabilities by providing researchers with signal and data processing options previously unavailable in one complete device. The Universal Clamp is marketed under the name Digital Clamp One and is supported by NIH SBIR grant #1 R43 NS48682-01A1 in collaboration with MyNeuroLab (St. Louis, MO). The proposal for the SBIR PHASE II grant is currently in draft.

METHODS

Action Potential Recording Using Microelectrode Methodology

As the goal of this presentation is to illustrate the functionality of the Universal Clamp, only a brief outline of the dissection process is provided for scientific validation. Further information regarding *Aplysia californica* can be found in (2).

The *Aplysia* preparation begins with the administration of approximately 40ccs 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.) As the *Aplysia* 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 will be 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. Care must be exercised so that no internal organs are severed.



Figures 1 and 2. The fully exposed visceral cavity of *Aplysia californica* (Left) and the isolated visceral ganglion (Right)

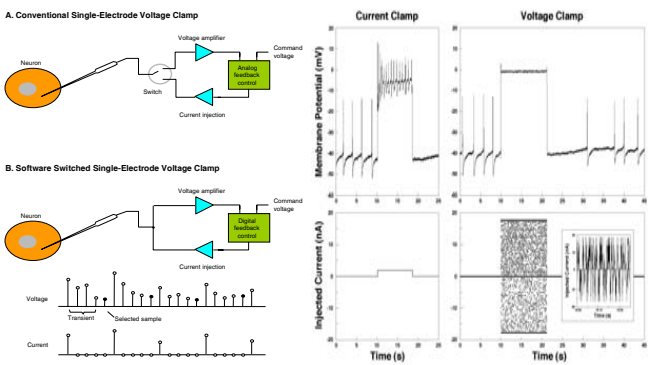
Using the thumb to create a pocket at the hind, scissors are used to make a cut along the long axis of the animal. The incision is stopped just before reaching the buccal mass. With the visceral cavity operative, the digestive organs are excised and the abdominal ganglion is isolated [3]. The neurons R2 and L9$_2$ were used in these experiments.[3]

RESULTS

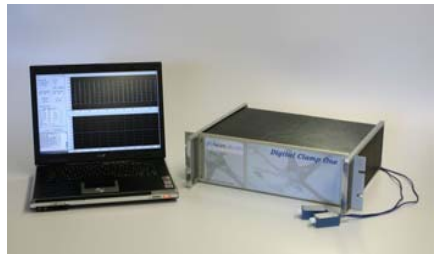
Voltage and Current Clamping Using the Universal Clamp

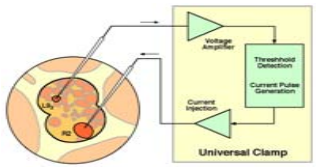
Since the Universal Clamp is a digital device, it has capabilities that are just not possible using standard analog voltage clamps. The most notable advantage is the ability to perform voltage clamping with one electrode without the need for external switches. Using software to control switching between 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). (Figure 3, below, left) . Positive and negative pulses of current are passed at high frequency to maintain the voltage level. (Figure 4, below, 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. Because the Universal Clamp uses software to perform the switching, the number of switches is scalable, providing researchers great flexibility in the design of the experiment.

Current clamping involves passing current into the cell to change the resting membrane potential. If positive current is passed, this raises the membrane potential, which is usually -40 to -90 mV. Once enough current is passed to raise the membrane potential past the threshold potential, the voltage needed to be reached before any action potential is fired, the cellular response is to initiate a high frequency volley of action potentials. When the current stimulus is removed, the membrane potential is returned to its original level, but the cell has adapted to the higher potential and it therefore behaves as if the membrane potential is lower than its original level. (Figure 4, below, right.)



Figures 3 and 4. Figure 3, left, diagrams the single electrode voltage clamp mechanism. The duration from the initial current stimulus to the end of the transient is 12 microseconds. Figure 4, right, shows the response of the cell to the current and voltage clamps, respectively. Not e in the voltage clamping the inset of injected current is continuously and automatically adjusted to compensate for the time varying capacitance of the cell.





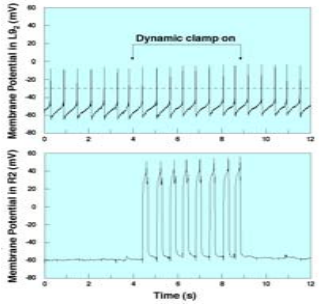


Figure 5, above, and Figure 6, right. **The Universal Clamp**, figure 5, above, marketed under the proprietary name Digital Clamp One, in its present state. The **NIH SBIR PHASE I grant (1 R43 NS48682-01A1)** was awarded to the University of Rhode Island and MyNeuro Lab of St. Louis, MO, for the ingenuity of the design and the ability of My Neuro Lab to bring it to market. The draft proposal for the NIH SBIR PHASE II grant is presently underway. **Dynamic clamping,** as performed by the Universal Clamp, figure 6, right, is perhaps the most important tool for understanding cellular communication. Neuron L9$_2$ is a known beating cell [2]. That is, it has a regular firing pattern. Neuron R2 is known to be silent. By using one electrode to acquire the signal from L9$_2$ and pass that signal on to R2, it is possible to elicit an action potential (AP) response from an otherwise silent cell. This is the basis of dynamic clamping, providing an artificial synapse to two otherwise electrically decoupled cells. The applications are quite wide reaching, including neural prosthetics, spinal cord injury patients, and the brain-machine interface (BMI). Because of the drastic difference in size between the two neurons, a significant amount of current needed to be passed the larger neuron, R2, in order to raise its resting potential high enough to trigger action potentials.

Dynamic Clamping Using the Universal Clamp

The dynamic clamp, introduced by A.A. Sharp *et al* in 1993, has rapidly become the instrument of choice in electrophysiological research [4]. In effect, the digital clamp provides researchers the ability to insert an artificial synapse, connecting two, or more, uncoupled neurons.

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 (8 nA, 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. The reason for the seemingly high amount of current is that the cell being stimulated was much larger, relativistically speaking, than the cell providing the signal. Figure 6, above right, shows the accurate scale of the cells as well as the resulting wave forms from each cell. The results show that the Universal Clamp is capable of forcing a silent neuron to fire action potentials in synchronization with another beating neuron. The on-board digital signal processor (DSP) chip of the Universal Clamp makes it possible to control fast neuronal action potentials.

The true power of the device lies in its ability to perform real-time signal processing. Filters can be applied to reduce noise or isolate specific frequency components. This is absolutely necessary in the treatment of spinal cord injuries as 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. This is just not possible with analog instrumentation or slow DSPs.

DISCUSSION

The Biomedical Engineering Program at the University of Rhode Island has produced a completely unique digital electrophysiology instrument. The device, the Universal Clamp, provides researchers extremely advanced data acquisition and data processing capabilities previously unavailable in one device. 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, St. Louis, MO, in the SBIR Phase I grant has been a successful one indeed, and has established legitimacy for future grant proposals including the SBIR Phase II grant.

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