Dynamic Clamp Analyses of Cardiac, Endocrine, and Neural Function

The dynamic clamp introduces artificial conductances into cells to simulate electrical coupling, voltage-dependent, leak, and synaptic conductances. This review describes how the dynamic clamp has been used to address various questions in the cardiac, endocrine, and nervous systems.

A common goal of many studies in physiological systems is to explain system behavior in terms of the behavior of their underlying components. In electrically excitable tissues such as those of the nervous, cardiac, and endocrine system, a classic problem is to understand how the properties of an individual cell depend on the interactions among its ion channels. In multi-cellular systems, it is also crucial to understand how the behavior of an organ or tissue depends on the properties of an individual cell and its interactions with its neighbors. The dynamic clamp is a set of related methods that allow investigators to address both the roles of individual membrane currents in cellular excitability and how the behavior of groups of cells depends on the properties of the cells and their interactions (50, 54, 59, 60). In this review, we use examples from cardiac, endocrine, and neuronal systems to illustrate the variety of configurations used in dynamic clamp experiments to illuminate important problems in physiology.

In a dynamic clamp experiment, the investigator records the membrane potential and then uses either a digital computer or an analog circuit to inject current into the cell in such a way as to construct an artificial conductance that is effectively in parallel with the other conductances in the cell (FIGURE 1). To construct an artificial voltage-dependent conductance, the conductance is first modeled as a set of differential equations, allowing the investigator to set the maximal conductance (g) (number of ion channels), the reversal potential (E), and the activation and inactivation properties of the channel so that the injected current fluctuates as the cell’s membrane potential changes. To construct an artificial electrical junction, the injected current depends on the artificial junctional conductance and the voltage difference between the two cells. To create an artificial chemical synapse, the presynaptic cell’s membrane potential is used to gate a modeled conductance in the postsynaptic cell (FIGURE 1). In all cases, the dynamic clamp allows the investigator to do “biological simulations” to assess the functional role of a given biophysical parameter on the system’s behavior.

Electrical Coupling

Creating artificial electrical connections between two cells is the easiest task that can be performed using the dynamic clamp. Since electrical coupling is one of the most critical features involved in the proper function of the cardiac syncytium, it is no surprise that the first artificial electrical junction was developed in 1979 to study the synchronization of clusters of beating ventricular cells in chicken (57). Soon thereafter, analog and digital systems were being used extensively in studies of cardiac function to artificially couple biological cells (25, 26, 32, 35, 63, 69) or to couple biological cells to model cells (27, 74, 76, 77, 79) (see Hybrid Networks). The artificial electrical coupling of cardiac muscle cells has been used to address questions such as 1) the occurrence of unidirectional block of action potential conduction between ventricular cells of different sizes (32, 69), 2) the conduction of the action potential at the junction between Purkinje and ventricular cells, 3) the basis of arrhythmogenesis (26), and 4) the involvement of voltage-dependent conductances in action potential conduction (25, 35, 63).

FIGURE 2A shows an example of the use of an artificial electrical junction and voltage clamp to determine the behavior of the calcium current during discontinuous conduction of the action potential between two ventricular cells of same size (35). After recording successes and failures of conduction for a high coupling resistance, the authors used the recorded waveforms as a voltage clamp command to define the precise magnitude and time course of the calcium current in both cells during the conduction of an action potential. Using this approach, they determined that the calcium current is strongly affected by the direction and the success or failure of conduction; the density of this current is always larger in the leader than in the follower cell. This asymmetry in the calcium current could be particularly significant when cells undergo high rates of stimulation and could lead to arrhythmogenesis.

Electrical coupling is also important in many regions of the nervous system, such as in populations of cortical interneurons in mammals. Merriam et al. (45) used the dynamic clamp to demonstrate that the interplay between electrical and chemical synapses can control the extent to which interneurons fire synchronously or asynchronously. A similar problem was studied using the oscillating neuronal networks in the stomatogastric ganglion (STG) of crustaceans (58, 68, 73). Varona et al. (73) used the dynamic clamp to modify the strength of coupling between the two electrical-
The dynamic clamp has been used effectively to understand the origin of the differences between the bursting properties displayed by pancreatic β-cells when isolated or part of an islet (5, 34, 83). When part of an islet, β-cells are electrically coupled and produce bursts of electrical activity at a cycle period of 10–60 s. When isolated, β-cells usually burst at faster (period of a few seconds) or slower frequency (period of a few minutes) but rarely at the intermediate islet-like frequency. These differences are associated with different patterns of calcium dynamics that track the electrical activity (FIGURE 3A). To determine whether these differences are related to a change in ionic channel composition or are the consequence of the loss of electrical coupling, Bertram et al. (5) used the dynamic clamp to show that isolated β-cells could be converted from fast spikers to slow bursters (similar to the islet cells) by injecting a small voltage-dependent inward current into the cell, resulting in a calcium signal similar to the islet cell calcium signal (FIGURE 3A). The very small conductance necessary for the conversion of the phenotype of the β-cells led the authors to suggest that the isolated cells have a stochastic behavior related to the small number of channels involved in producing their electrical activity. When electrically coupled, the stochastic differences between the cells are averaged to produce the typical slow bursting of islet cells. The injected conductance decreases the stochastic aspect of the electrical activity of isolated β-cells, thus rescuing the expected islet-like electrical behavior.

In the nervous system, the dynamic clamp has been used to mimic the effects of many currents, including hyperpolarization-activated inward current (I_{h}) (23, 28, 30, 33, 36, 72, 84), potassium currents (19, 36, 39, 41, 43, 56, 66, 70), calcium currents (19, 29), and voltage-dependent modulator-activated currents (3, 59, 67). Simulating these ionic channels has led to better understanding of the bursting and oscillatory properties of neurons (23, 28, 29, 33, 36), bistability (29), subthreshold resonance (30), regulation of excitability (19, 72, 84), action potential and high-frequency firing mechanisms (39, 41, 43, 56), integrative and short-term dynamic properties of neurons (66, 70), and the effect of neuromodulators on neuronal activity (3, 23, 60, 67).

Voltage-Dependent Conductances

The dynamic clamp has been used to create artificial conductances that mimic the action of voltage-dependent or voltage-independent ion channels. The injection of voltage-independent conductances has been used to study the effect of impalement-induced and physiological leak conductances in leech heart neurons (12, 20, 40), stretch-activated conductances in atrial cells (75), and the impact of shunting synaptic inhibition on the activity of cerebellar granule cells (46) and lumbar motoneurons (8). The intrinsic difficulty of understanding the role of voltage-dependent conductances on cell function has led many investigators to exploit the dynamic clamp in a variety of systems.

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For example, Lien and Jonas (39) developed a fast dynamic clamp system (50-kHz sampling frequency) to define the role of Kv3 channels in the fast-spiking phenotype of hippocampal GABAergic interneurons. Blocking this current induced spike broadening and a loss of their fast-spiking behavior. Replacing the biological current by the dynamic clamp-simulated current restored the fast-spiking behavior of the interneurons. Conversely, artificially subtracting the Kv3 current in a control cell mimicked the effect of pharmacological blockers in abolishing the fast-spiking behavior (FIGURE 3B).

Even more convincing was the observation that the injection of this artificial conductance converted regular-spiking pyramidal cells to fast-spiking cells. By manipulating the activation and inactivation properties of the artificial Kv3 conductance, these authors demonstrated that the effects of Kv3 channels on spiking properties are strongly dependent on the gating properties of these channels. This study shows that the dynamic clamp is a perfect tool for determining the “tuning” of biophysical properties to a specific type of electrical activity.

A few studies have used the dynamic clamp to simulate ion channels in cardiac cells (4, 64, 75). Sun and Wang (64) simulated the potassium current $I_{\text{to}}$ in endocardial ventricular cells in an attempt to determine the biophysical differences between endocardial and epicardial cells, which could explain the transmural dispersion of the duration of the action potential. Subtracting $I_{\text{to}}$ from epicardial cells or adding it to endocardial cells affected the early phase of repolarization of the action potential, whereas no change in duration of the action potential was observed, ruling out the possibility that the difference in expression of this channel could be responsible for the transmural dispersion of the action potential duration.

In an elegant study, Berecki et al. (4) developed a new kind of dynamic clamp to study the consequence of long-QT syndrome-associated potassium human ether-a-go-go-related gene (hERG) channel

**FIGURE 2. Artificial electrical coupling**

A: two myocytes were coupled with an analog-based dynamic clamp system. First and third panels show voltage waveforms recorded in myocytes 1 and 2 after stimulation of myocyte 1 or myocyte 2, respectively. Second and fourth panels show pharmacologically isolated calcium currents elicited in response to these waveforms. The calcium current is larger in the stimulated cell (leader: myocyte 1 in first and second panels, myocyte 2 in third and fourth panels). Figure was modified from Ref. 35. B: PD cells electrically coupled using a digital dynamic clamp system. When the electrical coupling conductance was not modified (top; $g = 0.0 \text{ nS}$), the PD cells produced bursts synchronously and spikes asynchronously. When the electrical coupling conductance was decreased (middle; $g = -250 \text{ nS}$), the two cells produced bursts in anti-phase. When the electrical coupling conductance was increased (bottom; $g = +190 \text{ nS}$), both the bursts and the spikes were synchronized. Modified from Ref. 73.
mutation (R56Q) on the action potential dynamics. In this technique, called dynamic action potential clamp, currents were recorded from a HEK-293 cell expressing either a mutated channel or the wild-type channel. These currents were scaled and injected into an active ventricular cell where the $I_{Kr}$ potassium current had

![FIGURE 3. Artificial Ionic conductances](image)

A: simulation of inward conductances in isolated β-cells. The membrane potential and intracellular calcium signal were recorded in response to glucose application. Top pair shows isolated β-cell recordings showing the fast changes (top) or combined fast and slow changes (bottom) in calcium signal in response to glucose. Middle pair shows islet β-cell recordings showing the slow bursting profile in membrane potential (bottom) and calcium signal (top). Bottom pair shows that a dynamic-clamp injection of a small voltage-dependent conductance ($g = 0.008 \text{nS}$) into a fast-spiker isolated β-cell results in bursts of electrical activity and calcium signal. Modified from Ref. 5. B: hippocampal interneuron activity pattern obtained in response to a 0.5-nA current step injection. In control condition (black trace, 1st and 3rd panels), the cell fires at high frequency, and spike-frequency adaptation is observed (black line, 2nd and 4th panels). When the Kv3 channel is pharmacologically blocked (red trace, 1st and 3rd panels), the firing frequency and the spike-frequency adaptation decrease (red line, 2nd and 4th panels). Injecting an artificial Kv3 current (lower blue trace, 1st panel) using dynamic clamp rescues the original firing behavior of the cell (blue trace, 1st panel). The effect of pharmacological blockade of Kv3 channels can be mimicked by subtracting Kv3 artificial conductance (lower blue trace, 3rd panel) using dynamic clamp (blue trace, 3rd panel; blue line, 4th panel). C: voltage and current traces obtained in response to periodic stimulation of a myocyte at different frequencies (0.2, 2, and 5 Hz from left to right). The traces shown correspond to wild-type hERG channel (top 2 panels) or R56Q mutant hERG channel (bottom 2 panels). The action potentials and the corresponding currents last longer for the mutant than the wild-type current, and as a consequence only the wild-type current can follow high-frequency stimulation (5 Hz, traces on right). Modified from Ref. 4.
been previously pharmacologically blocked (Figure 3C). The mutated channel was associated with a delayed repolarization of the action potential in the tested cell type (rabbit left-ventricular myocyte). Because of the differences in gating properties between the wild-type and the R56Q channel, the

FIGURE 4. Artificial synapses
A: simulation of synaptic background in rat cortical cells. Top left: when a simple current step is injected into the cell, the spike timing is highly variable between trials (bottom, raster plot). Bottom left: response to a fluctuating Poisson-like AMPA conductance. The number of spikes is decreased, but the spike timing is more reliable. Top right: response to combined Poisson-like AMPA and NMDA excitation. The spike-timing reliability is strong only when the NMDA conductance reaches steady state. Bottom right: response to combined fluctuating Poisson-like AMPA and GABA conductances. The spike-timing reliability is very strong independent of time during the stimulation. For all panels, top traces represent voltage responses (black is first trial, red is following trials), middle traces represent the conductance stimulus used to elicit firing of the cell, and bottom traces are raster plots of the spiking response of the cells. Modified from Ref. 24. B: synaptic coupling of two GM cells of the stomatogastric ganglion. Increasing the synaptic conductance increases the cycle period of the half-center oscillator (left column; synaptic conductance increases for traces from top to bottom). Increasing Ih conductance decreases the cycle period of the oscillator (right column; Ih conductance increases for traces from top to bottom). Modified from Ref. 61.
duration of the action potential and its dependence on the frequency stimulation were modified (FIGURE 3C). This creative implementation of the dynamic clamp illustrates the evolution of dynamic clamp methodologies to address a variety of questions.
Chemical Synapses

The number of synapses received by a single neuron can vary from a few to tens of thousands. The dynamic clamp has been used to simulate one synapse (2, 3, 9, 10, 15, 42, 51, 54, 68, 71), a small number of synapses (6, 9, 13, 18, 21, 22, 31, 81, 82), or thousands of synapses (1, 9, 11, 14, 16, 17, 24, 31, 44, 46, 55, 62, 65, 80, 85), and to determine their effect on neuronal activity. In some dynamic clamp experiments, an artificial synaptic conductance is injected into the neuron in response to a command given by the experimenter to mimic only the postsynaptic action of released neurotransmitter in an open-loop configuration. In a second type of experiment, the dynamic clamp is used to artificially couple two or three biological neurons by using the membrane potential waveform of the presynaptic neuron to control an artificial postsynaptic conductance.

A large number of studies have employed the dynamic clamp to simulate “synaptic noise” or “background activity,” mimicking the thousands of inputs received by neurons in the mammalian brain (1, 9, 11, 14, 16, 17, 24, 31, 44, 46, 55, 62, 65, 80, 85). This approach consists of recording neurons in vitro while simulating the highly variable total synaptic input (excitatory and/or inhibitory) received by these neurons in vivo. This type of simulation has been used to address different questions about the integrative properties of neurons in vivo. Several studies have shown that increasing the variability of the synaptic signal, but not its mean, is sufficient to increase the gain of the response of the neuron to this input (1, 11, 46, 62). This is true not only for excitatory inputs but also for inhibitory inputs: for a constant excitatory input, increasing the variability of simulated GABAergic events decreases their average inhibitory influence on the firing of the neuron, demonstrating that the heterogeneity of the inhibitory synaptic inputs to a neuron could play a major role in setting the gain of the neuron’s response to excitatory synaptic inputs (1).

Simulating synaptic background activity has also been very useful in analyzing the reliability and temporal precision of neuronal responses to different kinds of synaptic stimuli (24, 31, 65, 85). For example, Harsch and Robinson (24) used this approach to determine the influence of different components of the total synaptic input on the firing properties of rat cortical neurons. They separated the synaptic input into three components: the excitatory AMPA receptor-mediated conductance, the excitatory conductance mediated by the NMDA receptor, and the GABA<sub>α</sub> receptor-mediated inhibitory conductance. The AMPA and NMDA components differ in their voltage dependence and in their kinetics; the NMDA component is slower than the AMPA component. When depolarized by a regular current injection, the neuron responds with little temporal precision (FIGURE 4A). When the neuron is stimulated with AMPA-like synaptic noise, spikes are produced with great temporal precision. The addition of the NMDA component reduces the temporal precision and confers a time dependence to this property: in the activating phase of the NMDA conductance, the temporal precision is poor and the spike production is unreliable, whereas the temporal precision becomes stronger and the spike production more reliable when the NMDA conductance reaches steady state (end of stimulation protocol). Adding an inhibitory component to the synaptic background decreases the firing frequency, but spikes are more precise and are produced with a delay. This suggests that excitatory inputs might be critical for establishing the firing rate of cortical neurons, whereas inhibitory inputs might define the temporal precision of response.

In a recent study, Wolfart et al. (80) used the simulation of in vivo synaptic activity to demonstrate that the distinction between the canonical hyperpolarized “bursting” and depolarized “tonic” firing modes described for the relay thalamic neurons in vitro might not be as relevant when the neuron is given realistic synaptic inputs. When receiving synaptic noise, the neuron responds with a mix of single spikes and bursts of spikes independent of the membrane potential (80). In an elegant study (55), the same group developed a method for estimating the synaptic conductance changes occurring in cortical neurons when the network is activated. To perform this analysis, the authors recorded from cortical neurons during spontaneous activation of the network. The change in the voltage profile was then decomposed into an excitatory and inhibitory component, and the mean and variance of each component were determined. The validity of this analysis was tested by injecting the calculated component and reproducing the activity profile of the neuron observed during spontaneous activation of the network.

Closed-loop studies in which artificial networks are created are less common. In one study, a closed-loop approach was used to study the phase relationships...
between cortical interneurons coupled with synaptic inputs of different kinetics (45). In the STG, closed-loop approaches have been used extensively (2, 15, 42, 60, 61) to study the role of synaptic and intrinsic properties on network dynamics. Reciprocal inhibition is a common feature in many central pattern-generating networks, including those in the stomatogastric nervous system. Understanding this kind of symmetrical two-cell network was the subject of one of the early studies using dynamic clamp in the nervous system. Sharp et al. (61) coupled two gastric mill (GM) cells with synaptic conductances of different strengths and kinetics and studied the influence of these parameters on the behavior of the resulting half-center oscillators. Neurons in the STG release neurotransmitter as a graded function of membrane potential, and therefore the effect of changing the threshold of activation of the synapses was also tested. The simulation of an h-type conductance in the two GM cells was needed to induce stable oscillations of this half-center oscillator. FIGURE 4B summarizes the effects of changing the conductance of the reciprocal inhibitory synapses or the maximal conductance of the $I_h$ current. Increasing the conductance of the synapses produced an increase in the cycle period of the half-center oscillator, whereas increasing the $I_h$ maximal conductance decreased the cycle period of the oscillator (FIGURE 4B). Manipulation of other parameters, like the time constant of decay or the threshold of activation of the synaptic inputs, led to other network behaviors such as synchronous tonic spiking, synchronous bursting, or spontaneous switching between different states. This study was essentially a simulation study carried out with biological neurons, as extensive parameter searches were done.

**Hybrid Networks**

The dynamic clamp has also been used to create "hybrid networks" consisting of one or more biological neurons coupled to one or more analog or digital model cells. In this way, large numbers of model neurons can be studied, over and above the relatively small number of biological cells that can be simultaneously recorded intracellularly (two to four). In theory, the size of a hybrid network is limited only by the computation rate, which is dependent on whether analog or digital models are used, on processing speed, and on model complexity.

In the heart, two different types of hybrid networks have been developed: simple networks with one biological cell electrically coupled to one model cell (27, 74, 76, 78) and more complex networks in which one biological cell is connected to a two-dimensional sheet of electrically coupled model cells (77, 79). The two-cell networks were used to determine the critical intercellular coupling for frequency entrainment in rabbit sinoatrial cells (78), to study the influence of injury current on Purkinje myocyte activity (27), and to study the appearance of an ectopic focus of activity by elevation of extracellular potassium (74). Wagner et al. (76) combined the dynamic clamp with calcium imaging to show that the intracellular calcium dynamics of a myocyte was altered by its coupling, here to a model (FIGURE 5A). The calcium signal was larger and faster when propagation was successful than when it was not. However, the modification in the calcium signal was smaller than expected based on the dynamics of the L-type calcium current during successful propagation. The authors suggested that this discrepancy points to the involvement of the sarcoplasmic reticulum as a source of calcium during propagation of the action potential. In other studies, complex two-dimensional networks (square grids of cells) were developed that contained from 49 to 169 elements with only one central element being a biological cell (77, 79). By varying the coupling conductance within rows and within columns, the authors showed that anisotropy of the coupling within the network facilitates the development of ectopic foci and the spread of activity to the entire network. The authors suggested that the remodeling occurring in a peri-infarction zone could, by changing the anisotropy, facilitate or inhibit the development of arrhythmias.

In the nervous system, hybrid networks have been used mostly to address two types of problems: 1) the problem of phase relationships and/or synchronization in small closed-loop networks of bursting or spiking cells (36, 47–49), and 2) to study signal propagation (37, 52). LeMasson et al. (37) created a three-neuron network consisting of an analog-based retinal cell projecting onto a real thalamocortical neuron, itself connected by reciprocal synapses to a computer-generated inhibitory interneuron from the nucleus reticularis. The study focused on the relationship between retinal spikes and thalamocortical firing output. The authors showed that neuromodulation of thalamocortical cells and a decrease of inhibition from the nucleus reticularis neuron induced a switch in the behavior of thalamocortical cells from bursting to spiking. The authors suggest that this change in behavior would lead thalamocortical cells to be most effective only during an aroused state.

Reyes (52) developed an interesting strategy to study propagation of signals in a feedforward network (no recurrent connections) without having to record from more than one neuron in vitro (FIGURE 5B). The simulated network comprised $n$ layers each composed of $w$ neurons. All neurons in the first layer were modeled as firing repetitively and asynchronously at different frequencies. Each action potential resulted in a postsynaptic current (PSC). Ten percent of these trains of PSCs were randomly chosen and summed, and the sum was injected into the recorded neuron representing a neuron in layer 2. To simulate the response of the remaining $(w - 1)$ neurons in layer 2,
the same sequence was repeated \((w - 1)\) times. All the activity profiles generated by this operation were then used to create the PSC trains coming out of layer 2 neurons. Again, 10\% of the PSCs generated by these neurons were injected into the recorded neuron, which now represented a layer 3 neuron. These different operations were repeated \(m\) times to simulate the propagation of signals through all of the layers. The interesting result from this study is that the activity of all neurons within a layer became more synchronized as the signal propagated through the layers, reaching almost perfect synchrony by the 11th layer, although the activity of the neurons in the first layer was completely unorganized.

**General Conclusion**

The dynamic clamp technique has been used in a variety of different systems to answer a plethora of questions. We anticipate that the future will bring more innovative and creative uses of the dynamic clamp and hybrid circuits to understand the consequences of the nonlinearities central to most physiological processes. Because the dynamic clamp allows one to simulate networks of biological cells, the dynamic clamp theorist can allow the biological tissue itself to take care of many of the parameters that are still unknown or difficult to compute. It is a tremendous advantage to be able to do simulations in which the only parameters to be studied are under tight experimental control and the remaining parameters are by definition correct, because cells are themselves “computing” them in real time and space. As genetic manipulations provide tools to optically stimulate tissues (7, 38), one can imagine that hybrid optical devices will extend dynamic clamp-like methodologies to a larger number of neurons, allowing new and exciting ways to do network simulations with biological tissues.

**References**


