intention is to emphasize that scientists must be aware of their biases, because these biases can have a dramatic effect on the outcome of their research. If technical progress is to be made in the understanding of the possible genetic bases of mental illnesses, then it will be essential that the biases be explicitly acknowledged and that extreme efforts be made to overcome their effects on scientific research.

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techniques

The dynamic clamp: artificial conductances in biological neurons

Andrew A. Sharp, Michael B. O'Neil, L. F. Abbott and Eve Marder

The dynamic clamp is a novel method that uses computer simulation to introduce conductances into biological neurons. This method can be used to study the role of various conductances in shaping the activity of single neurons, or neurons within networks. The dynamic clamp can also be used to form circuits from previously unconnected neurons. This approach makes computer simulation an interactive experimental tool, and will be useful in many applications where the role of synaptic strengths and intrinsic properties in neuronal and network dynamics is of interest.

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A basic goal of neuroscience is to understand how membrane and synaptic conductances combine and interact to produce the behavior of neurons and neural circuits. The conventional method for altering the activity of single neurons or perturbing their activity in networks is to inject constant current using the current clamp. This allows the investigator to either depolarize or hyperpolarize a neuron, but does not correctly replicate the conductance changes produced by synaptic inputs or modified by neuromodulators. The primary tools used to determine the characteristics of individual neuronal conductances are the voltage clamp and the patch clamp. While essential for understanding the voltage- and time-dependence of a conductance, these methods are less useful for studying the interplay of conductances that determine how neurons act individually or in circuits. Most voltage- and patch-clamp experiments halt the normal voltage excursions of the clamped neuron and they often employ pharmacological agents to isolate a single conductance. For these reasons, conventional voltage clamp methods do not allow the evaluation of the role of conductances during the normal dynamic evolution of the membrane potential. The classical solution to this problem is to simulate the electrical activity of a neuron using mathematical descriptions of its measured conductances[1]. The limitation of this method is that it requires a detailed description of many, if not all, of the conductances in a neuron, and these data may be difficult or impossible to obtain.

The dynamic clamp is a new approach that allows an investigator to introduce artificial voltage- and time-dependent conductances into biological neurons (Refs 2–4 and Hutcheon, B. and Puil, E., unpublished observations). In a sense, the dynamic clamp uses biological neurons as simulators, allowing the investigator to evaluate the role of individual conductances in shaping the electrical activity of single neurons, as well as determining the consequences of synaptic strengths in networks. The dynamic clamp combines the control and flexibility of computer simulation with the accuracy and realism of electrophysiological recording, using computer modeling as an experimental tool.

The dynamic clamp produces changes in conductance

The basic set-up for the dynamic clamp is similar to a conventional voltage- or current-clamp rig. However, in the case of the dynamic clamp, the injected current is controlled by a computer program that duplicates the current that would flow through a real membrane or synaptic conductance (Box 1). Any conductance that can be modeled mathematically can be introduced into the neuron being studied. The capabilities and uses of the dynamic clamp are illustrated here, using examples from the stomatogastric ganglion (STG) of the crab *Cancer borealis*. The STG offers a small group of well-defined neurons, whose connections and circuit characteristics are well understood, including extrinsic inputs and neurotransmitters.

Unlike current-clamp injection, the dynamic clamp duplicates both the voltage and the conductance changes caused, for example, by a neurotransmitter. In Fig. 1, the dynamic clamp mimics the response of an STG neuron to rapid bath application of the neurotransmitter γ-aminobutyric acid (GABA). Hyperpolarizing constant current pulses were used to monitor the input impedance of the neuron. GABA increased the conductance of the neuron (seen as a decrease in the amplitude of the changes

Box 1. Components of the dynamic clamp

The dynamic clamp uses a standard electrophysiological set-up with computer interface to measure membrane potentials and control current injected through an intracellular electrode. The membrane potential V is recorded with an intracellular electrode and transmitted to the computer through an analog-to-digital converter. On the basis of V and the differential equations describing the desired conductance, the current I flowing through the simulated conductance is computed. This is converted back into an analog voltage V_1, which controls the current injected into the neuron by the recording amplifier (see Figure).

A mathematical model of the conductance being simulated is programmed into the computer that is controlling the current injected by the dynamic clamp. The model current may be explicitly developed from voltage-clamp data describing a current from the preparation being studied. Typically, the membrane current I is given by the classic form of the Hodgkin–Huxley model[a]:

$$I = gm^p h^q (V - E_r)$$

where g is the conductance, p and q are integers, E_r is the reversal potential of the current, V is the membrane potential, and m and h are activation and inactivation variables described by the differential equations:

$$\tau_m(V)\ \frac{dm}{dt} = m_\infty(V) - m$$

and

$$\tau_h(V)\ \frac{dh}{dt} = h_\infty(V) - h$$

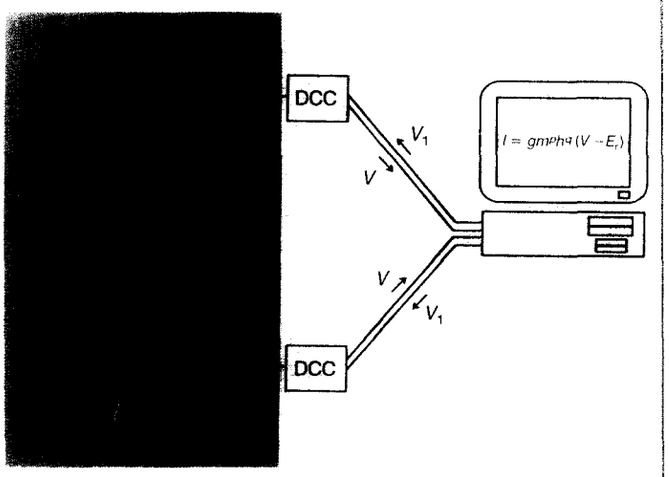
where τ_m, τ_h, m_∞ and h_∞ are measured functions of V. During the operation of the dynamic clamp these equations are integrated numerically in real time to generate the desired current.

To construct an artificial chemical synapse, the dynamic clamp must be programmed with a mathematical model of the synapse being simulated (a previous method[b] used presynaptic potential to trigger current injection). For the examples described in this article, the synaptic current is given by:

$$I = gs(V_{post} - E_r)$$

where E_r is the synaptic reversal potential and V_{post} is the membrane potential of the postsynaptic neuron. The synaptic activation variable s varies between 0 and 1 and is determined by:

$$[1 - s_\infty(V_{pre})]\ \tau_s\ \frac{ds}{dt} = s_\infty(V_{pre}) - s$$



where s_∞ is given as a function of the presynaptic potential V_{pre} by:

$$s_\infty(V_{pre}) = \tanh\left[\frac{(V_{pre} - V_{th})}{\Delta}\right]$$

if $V_{pre} > V_{th}$; otherwise $s_\infty = 0$. τ_s, V_{th} and Δ are constants. An electrical synapse of conductance g between neurons with potentials V_1 and V_2 can be simulated by injecting a current, $I = g(V_2 - V_1)$, into neuron 1 and a current of equal magnitude but opposite sign into neuron 2 (see Refs c and d for another approach to building electrical synapses with an analog circuit).

The configuration shown in the Figure allows the addition of artificial membrane conductances into either or both neurons, and construct on of artificial synapses between the two neurons. The dynamic clamp uses generally available hardware. In the system used for the examples cited in this article, analog-to-digital and digital-to-analog conversions were handled by a 'Scientific Solutions Lab Master DMA Board', and current calculations were performed by an Intel 80486DX-based PC running at 50 MHz. An 'Axoclamp-2A' (Axon Instruments) run in discontinuous current clamp (DCC in Figure) mode (sampling frequency of 5 kHz) was used to record membrane potential and control current injection through an intracellular electrode.

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in membrane potential produced by the current pulses). When programmed to mimic the effect of GABA, the dynamic clamp also decreased the change in membrane potential produced by the current pulses, demonstrating that the dynamic clamp effectively changes the conductance of the neuron. In fact, the dynamic clamp behaves as if the channels described by the programmed equations were located at the tip of the microelectrode.

Using the dynamic clamp to understand single neuron behavior

A large number of voltage- and time-dependent conductances may contribute to the activity of a neuron. In addition, many neuromodulatory substances either activate or influence voltage- and time-dependent conductances. Because these conductances all have different voltage- and time-dependent properties, it is often impossible to predict, without simulation, the effect on the activity of the neuron of changing one or more of these conductances. The dynamic clamp provides this tool directly. Figure 2 illustrates the use of the dynamic clamp to determine how a biophysically characterized, modulator-activated conductance influences the electrical activity of a single neuron. The peptide proctolin elicits a non-specific cation conductance[5] in certain STG neurons that is maximal at membrane

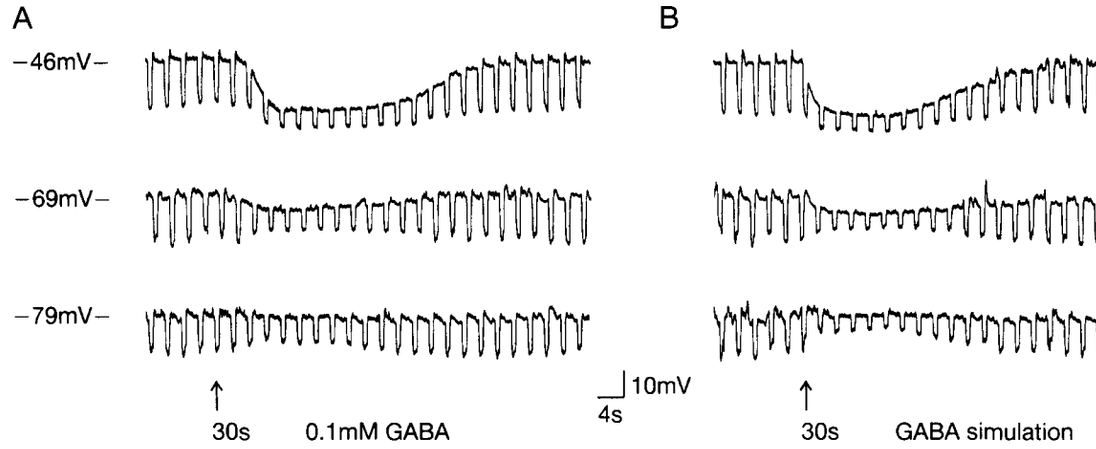


Fig. 1. *Artificial γ-aminobutyric acid (GABA) conductance.* **(A)** *Intracellular recordings from a crab stomatogastric ganglion (STG) neuron in primary cell culture. Hyperpolarizing current pulses (−0.05 nA) were applied every 3 s to monitor the input impedance of the neuron. At the arrow, the superfusion medium was changed from 30 s to one containing 0.1 mM GABA. Recordings at different baseline potentials show that the reversal potential for this response was around −80 mV.* **(B)** *Recordings from the same neuron shown in (A). At the arrow, the dynamic clamp was used to simulate the response of the neuron to GABA. The GABA response was modeled as having an 8 nS conductance with a reversal potential of −75 mV and an exponential rise (τ = 5s) and fall (τ = 15s).* (Taken from Ref. 2.)

potentials close to the resting potential, but that decreases at hyperpolarized potentials because of a voltage-dependent block by extracellular Ca^{2+}. The biophysical properties of this current are shown in Box 2.

Bath application of real proctolin to a pacemaker neuron of the STG, the anterior burster (AB) neuron, increases both the frequency and the amplitude of its bursts[6] (Fig. 2A). What features of the proctolin-activated current and the conductances of the AB neuron are important in these actions? Although proctolin adds a depolarizing inward current to the neuron, its actions are not adequately mimicked by merely depolarizing the neuron (right-hand panel in Fig. 2B). Note that depolarization with conventional current clamp duplicates the increase in burst frequency, but fails to replicate the increase in burst amplitude produced by proctolin and the artificial proctolin current. This is because the voltage dependence of the proctolin current interacts with the other currents present in the AB neuron, so that the neuron undergoes oscillatory swings in membrane potential that alternately activate and inactivate the proctolin current. For this reason, the proctolin-activated current increases both the amplitude and the frequency of the AB neuron burst. Because we do not have detailed descriptions of all the voltage-dependent conductances in the AB neuron, these results could not be obtained using conventional modeling techniques. However, by using the dynamic clamp in conjunction with the biological AB neuron, with all of its conductances intact, we were able to study the effects of the one conductance whose properties we had obtained by conventional voltage-clamp methods.

Using the dynamic clamp to understand circuit dynamics

Neural circuit activity depends on the intrinsic properties of all the constituent neurons, as well as the synaptic connections among them. Many neuromodulatory substances influence circuit

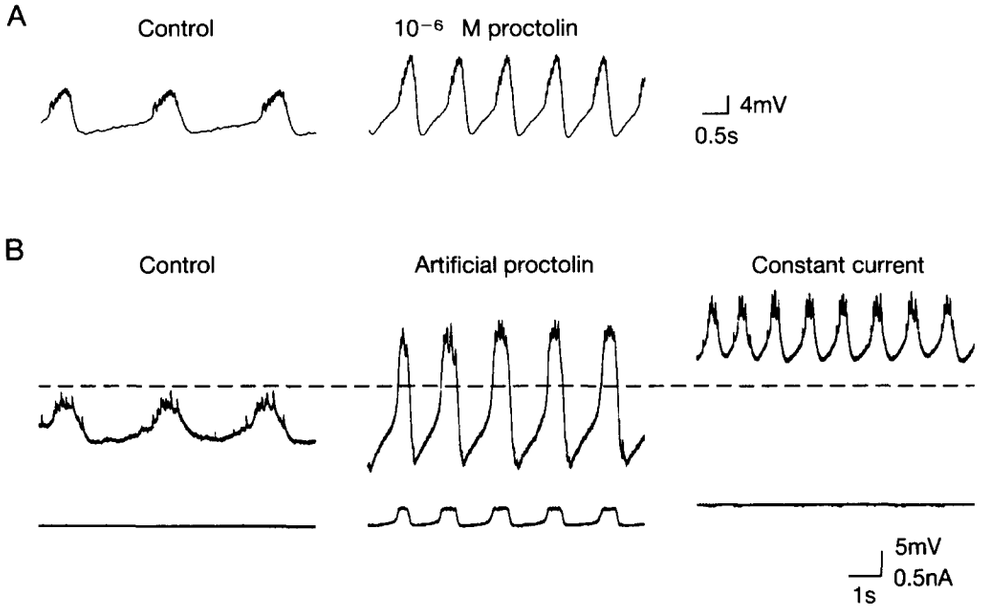
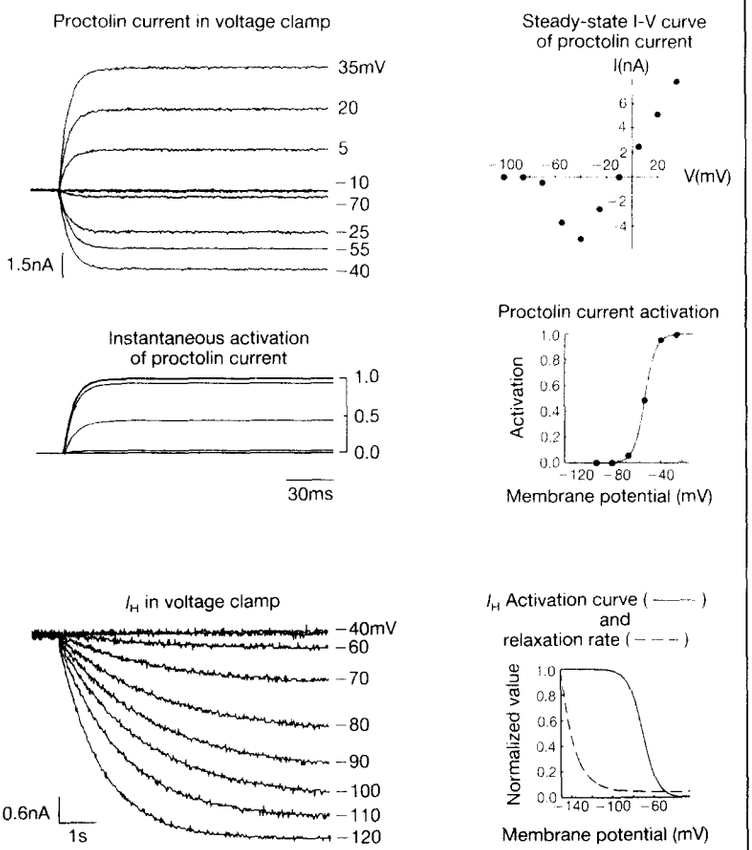


Fig. 2. *Artificial proctolin conductance.* **(A)** *Intracellular recording from a lobster AB neuron (a pacemaker neuron of the stomatogastric ganglion) in control saline and in 10^{-6} M proctolin.* (Taken from Ref. 6.) **(B)** *Intracellular recordings from a crab AB neuron (−45 mV at dotted line). The middle panel shows the effect of 20 nS of simulated proctolin current added to the cell. The final panel shows depolarization with constant current. The bottom traces are simultaneous recordings of the injected current.*

Box 2. Testing the implementation of the dynamic clamp

After the dynamic clamp has been programmed to mimic a given conductance it is important to test the accuracy of the program with a voltage-clamp experiment. A useful way to do this is to replace the neuron with a resistance–capacitance (RC) circuit. This approach retains the RC components of the cell membrane, but eliminates all the voltage- and time-dependent conductances other than those created by the dynamic-clamp. The Figure illustrates this process for the proctolin current and for the hyperpolarization-activated inward current I_H. The dynamic-clamp programs for these two conductances are based on mathematical descriptions[a,b] previously derived from voltage-clamp data obtained from the stomatogastric ganglion (STG)[c,d]. For the proctolin conductance, current traces (after leak subtraction) are shown after steps from a holding potential of −100 mV to the eight test potentials listed at the right-hand side of the traces. Simultaneous recordings of the activation variable are shown below this. The steady-state *I–V* relationship measured from these recordings (plotted on the right) are in good agreement with those induced by real proctolin in the STG. The steady-state activation of the proctolin current from this experiment (data points in the proctolin current activation plot) fall properly on the theoretical curve. For I_H, current traces produced by voltage steps from a −40 mV holding potential are also shown. These current traces accurately represent the theoretical time- and voltage-dependencies of activation plotted on the right.



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dynamics[7]. To understand how this occurs it is necessary to study the effects of changes in synaptic strength, and of modifications of the properties of individual neurons. The use of the dynamic clamp to understand the role of a particular membrane current in network dynamics is shown in Fig. 3. The current studied here is I_H, a hyperpolarization-activated inward current that is present in stomatogastric ganglion neurons[8,9]. The dynamic-clamp description of this current is illustrated in Box 2.

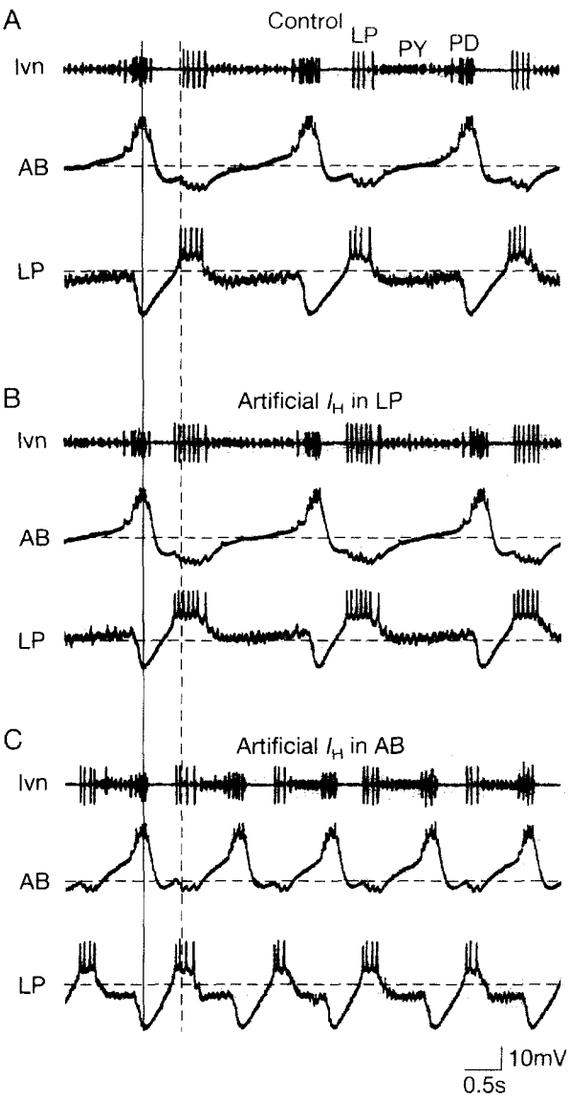

Fig. 3. *Artificial* I_H. **(A)** *Control recordings from an intact stomatogastric nervous system. The top trace is an extracellular recording from the lateral ventricular nerve (lvn), which shows the periodic activity of the LP, PY and PD neurons. The next two traces are intracellular recordings made from the AB and LP neurons.* **(B)** *Recordings from the same neuron as in (A) with 50 nS of artificial* I_H *added to the LP neuron.* I_H *was modeled as shown in Box 2. Notice that the LP neuron fires earlier and for a longer period of time than in the control.* **(C)** *Recordings from the same neuron as in (A) with 50 nS of* I_H *added to the AB neuron. Notice that the frequency of the network has increased. The horizontal dashed lines indicate a membrane potential of −50 mV.*

The pyloric rhythm of the stomatogastric ganglion is shown in Fig. 3. The top extracellular nerve recording in Fig. 3A shows the rhythmic activity of the lateral pyloric (LP), pyloric (PY) and pyloric dilator (PD) motoneurons. The other two traces in Fig. 3A are intracellular recordings from the AB neuron (which fires with and is electrically coupled to the PD neurons) and from the LP neuron. The LP and PY neurons are inhibited by the AB and PD neurons. What determines when the LP and PY neurons fire? Previous work had suggested that the properties of both I_A (the fast transient outward K^+ current) and the time-courses and strengths of the inhibitory synapses[10–12] played a role. Figure 3 shows that I_H is also important. Increasing I_H in the LP neuron caused it to fire earlier and longer in the pyloric rhythm (Fig. 3B). Note that increasing I_H in the AB neuron increases the frequency of the pyloric rhythm (Fig. 3C). I_H has different effects on the AB and LP neurons because they have different intrinsic voltage- and time-dependent conductances. Systematic comparisons of the role of I_H, I_A and the strength and time-courses of the synapses in determining when the LP and other neurons fire are now possible using the dynamic clamp.

Hutcheon and Puil (unpublished observations) have used an independently developed system to study the role of I_H in rat neocortical neurons in slice preparations. Preliminary dynamic clamp experiments with the leech heartbeat preparation (another well-defined ganglionic circuit) (Calabrese, R. H. and Sharp, A. A., unpublished observations) indicate that the strength and voltage-dependence of I_H are crucial in generating the rhythm of the leech heartbeat oscillator, as well as contributing to its period.

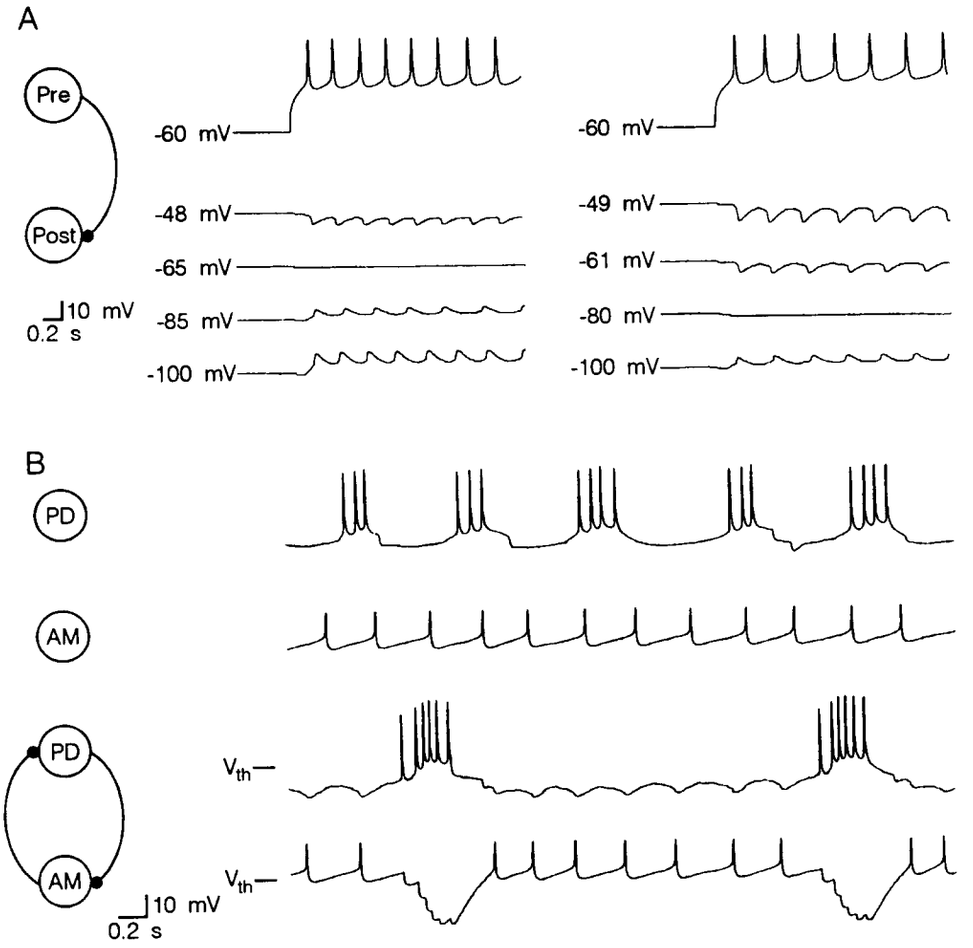

Fig. 4. *Artificial chemical synapses.* **(A)** *Construction of a single inhibitory synapse between two stomatogastric ganglion (STG) neurons. Depolarization of the presynaptic neuron generates a train of action potentials, which in turn generate IPSPs in the postsynaptic neuron. The postsynaptic response is shown at several potentials. The synaptic reversal potential is programmed at −65 mV for the left panel and −80 mV on the right.* **(B)** *Recordings from the PD and AM neurons in the intact STG. There are no synaptic interactions between these neurons under control conditions (top). The bottom traces show the result of coupling these neurons with reciprocal inhibitory synapses created using the dynamic clamp (V_{th} for PD = −45 mV, V_{th} for AM = −30 mV; for both neurons g = 100 nS, E_r = −80 mV, and Δ = −40 mV).* (Taken from Ref. 2.)

The dynamic clamp can be used to construct circuits

The dynamic clamp can also be used to create artificial synapses between neurons. In order to construct an artificial chemical synapse, the dynamic clamp is programmed to modify the conductance of the postsynaptic neuron depending on the membrane potential of the presynaptic neuron (Box 1). Artificial synapses between STG neurons are shown in Fig. 4. The PD and anterior median (AM) neurons in the crab STG are not normally connected. When an artificial synapse was constructed between them, each PD neuron burst strongly inhibited the AM neuron, each AM neuron action potential elicited an IPSP in the PD neuron and, interestingly, the period of the PD burst was dramatically altered.

The modification of synaptic strengths is one of the primary ways that neural circuits are modified, either by activity or by neuromodulators. By building an artificial synapse in parallel with a real synapse, the synaptic strength can be increased or decreased in a controlled manner and the effect on network activity can be observed.

Artificial synapses can also be used to form novel circuits from otherwise unconnected neurons. Neurons grown in culture can develop natural synapses[13,14], and these circuits have been studied[15–17]. However, in these experiments the investigator is 'held hostage' by the serendipitous formation of synaptic contacts among the cultured neurons. By using the dynamic clamp to form

artificial synapses, circuits with precisely defined synaptic connectivity can be built and studied.

In addition to constructing artificial synapses between neurons, a computer model of an entire neuron can be coupled through artificial synapses to a biological network (see Ref. 4). This allows interesting hybrid computer–biological networks to be built and studied.

Accuracy and limitations of the dynamic clamp

The primary limitation of the dynamic-clamp method is the same as that of the voltage and current clamp: the problem of measuring and clamping the potential in situations where the neuron is not electrotonically compact. Because the dynamic clamp simulates a point source of conductance, it does not accurately describe the normal distribution of channels in the membrane. In an extended neuron, this may limit the use of the technique, especially for fast currents. On the other hand, if the neuron is reasonably electrotonically compact and if the conductances are fairly slow, the dynamic clamp should provide a good simulation. We have successfully used the dynamic clamp for several slow currents in STG neurons.

Use of the dynamic clamp technique requires that the current calculations are updated rapidly enough to simulate the real conductance while maintaining an accurate measurement of membrane potential. The maximum rate at which a conductance described by a single activation variable can be updated, with our present 50 MHz 486 computer, is about 5 kHz. This is sufficient to follow rapid voltage fluctuations such as action potentials. With our present system, the update rate for the simulation of eight conductances is ~1 kHz. Increases in hardware speed will increase these rates.

The dynamic clamp will not replicate the effects of second messengers elicited by Ca^{2+} currents. To simulate Ca^{2+} entry using the dynamic clamp Ca^{2+}-containing electrodes can be used, but this may not duplicate the secondary effects of Ca^{2+} because the dynamic clamp injects current at the site of the electrode tip rather than where the Ca^{2+} channels are located.

In principle, the dynamic clamp can be used to either add or subtract an existing conductance from a neuron. However, care must be exercised in the case of subtraction, especially if the investigator attempts to remove a conductance completely. If the conductance is not modeled accurately, a difference current with unpredictable properties may result from the mismatch between the real and the modeled conductance. Furthermore, adding a negative conductance that is too large will destabilize a neuron with disastrous consequences (for the neuron) if the maximum current output of the clamp is not restricted.

Future prospects

One of the most appealing features of this new method is the construction of novel circuits from isolated neurons in which the strength and time-course of each synaptic connection can be varied at will. The experimenter can now study 'model' circuits without worrying about the limitations of over-simplified model neurons. The biological unknowns are built into such hybrid modeling studies because the many biochemical and metabolic processes that control neuronal activity are handled correctly by the neurons themselves.

We expect the dynamic clamp will augment other conventional simulation, biophysical and pharmacological approaches. The dynamic clamp makes it possible to combine computer simulation with living neurons, and adds an interactive modeling technique to the tools available for studying neurons and nervous systems.

Acknowledgements

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