Out of control in the dendrites

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Although voltage-clamp recordings remain a favorite method for studying synaptic transmission, the space-clamp problems that are associated with somatic voltage-clamp recordings have never been directly measured. A study by Williams and Mitchell in this issue now measures the experimental errors associated with this technique.

When Hodgkin and Huxley performed their classic experiments using the voltage clamp to analyze the conductances underlying the action potential[1], they understood the importance of inserting wires into the squid giant axon to control the transmembrane voltage along its entire length (called 'space clamping'). The procedure was undoubtedly difficult and tedious, but it was necessary to accurately measure membrane conductance. Without the internal wires, the voltage imposed at one end would decay along the length of the axon. Furthermore, currents in distant portions of the axon would not be adequately detected, so the amplifier would not pass the current necessary to keep the voltage constant. As a result, uncontrolled activation of the voltage-gated conductances would occur, defeating the purpose of the voltage clamp and rendering the data impossible to interpret.

For two decades now, for better or for worse, voltage clamping has been the method of choice for neurophysiologists studying voltage-gated conductances and synaptic transmission. Many studies have attempted to voltage clamp synapses on dendrites using electrodes in the soma. Although the so-called space-clamp problems that are associated with this procedure were (and are) usually acknowledged, the magnitude of the expected error has not been directly measured. On page 790 of this issue, Williams and Mitchell[2] now tackle this problem head on by measuring voltage-clamp errors directly in layer 5 pyramidal neurons in neocortical slices, with simultaneous whole-cell patch-clamp recordings from the soma and dendrites.

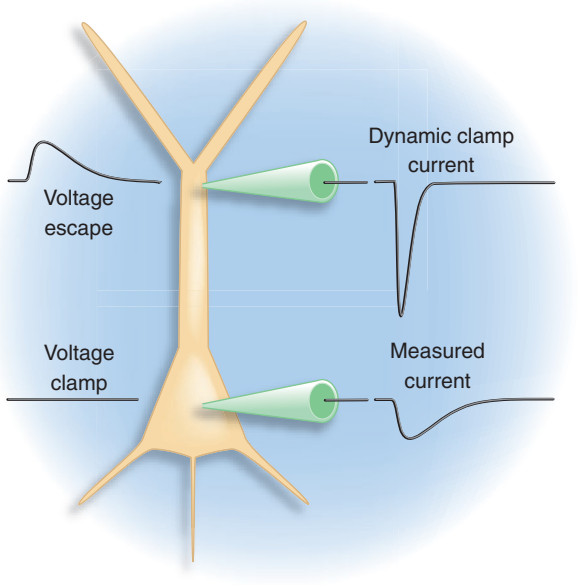

Figure 1 Diagram depicting the poor space clamp of dendritic conductances from the experiments of Williams and Mitchell[2]. A whole-cell patch electrode was placed on the soma and on the dendrites at varying distances from the soma. The trace at the upper right depicts the current injected by the dynamic clamp into the dendrites representing an excitatory synaptic input. The trace in the lower right depicts the current measured in the soma from this dendritic input. Note the smaller amplitude and slower kinetics as a result of poor space clamp. The trace in the upper left depicts the change in the local membrane potential during the synaptic conductance change even though the somatic membrane potential is 'clamped' (trace in lower left)

Since the experiments of Hodgkin and Huxley, others have devised additional methods to address the problem of space clamping; these include the three-electrode voltage clamp for muscle fibers[3], the sucrose gap clamp for small axons[4] and the suction clamp for measuring current over a restricted region of the nerve membrane[5]. The inventors of patch-clamp recording also understood the importance of space clamp, as they first used the method on small patches of membrane (cell-attached or excised patch recordings) or on small cells whose voltage could be controlled from a single point at the tip of the electrode[6]. Lured by the fascination of studying synapses that terminate on dendrites, many investigators applied the patch-clamp technique to carry out whole-cell recordings from other cell types, some taking pains to minimize the concomitant problems and others just ignoring them. Modeling studies have indicated that the errors are likely to be substantial[7,8], but such error estimates are subject to uncertainty because of the unknown accuracy of the models. Williams and Mitchell[2] now provide a more accurate estimate of these errors.

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Williams and Mitchell[2] harnessed a simple, but elegant, approach to measure space-clamp errors. They used a technique called dynamic clamp[9], whereby they injected a known conductance change into a neuron via a dendritic recording electrode. The injected conductances were intended to mimic the amplitude and kinetics of synaptic conductances measured in well-clamped neurons, but the study used a range of values to consider other possibilities. A second electrode, placed on the soma of the same cell, was used to voltage clamp the neuron while the dynamic clamp from the first electrode was activated. When the dynamic-clamp electrode was placed at the soma, the somatic voltage-clamp electrode captured the expected current almost perfectly. When the dynamic clamp electrode was moved away from the soma, however, the space-clamp errors became evident (**Fig. 1**). By varying the position along the dendrites of the dynamic clamp, the current measured in the soma could be compared with the ideal response and quantified in a systematic way.

The results of Williams and Mitchell's analysis[2] confirmed the rather ominous predictions of earlier modeling studies. The measured 'synaptic' currents were significantly smaller and slower than the currents injected via the dynamic clamp. Using reasonable values for a fast excitatory synaptic conductance, the amplitude of the measured current was reduced to 50% of the actual value when the dynamic clamp was just 90 μm from the soma, a distance equivalent to about one-tenth the total length of the dendritic tree. Considerably slower conductances attenuated less, but still presented 50% attenuation at distances of 200–300 μm. These values are specific to the large, primary apical dendrites of layer 5 pyramidal neurons. As poor as the clamp is for these primary dendrites, the situation is expected to be even worse for the smaller-diameter dendrites where most synaptic input occurs.

Consistent with the inability of the somatic voltage clamp to control the dendritic membrane potential, the voltage at the site of the dynamic clamp was not controlled at the desired command potential. The resulting 'voltage escape' (**Fig. 1**) introduces its own set of errors, most notably the possibility that voltage-gated conductances could be activated at the input site. At 200 μm from the soma, voltage-escape was nearly complete, implying a total ineffectiveness of the somatic voltage clamp to control voltage beyond this distance.

Approaches have been employed to minimize space-clamp errors, including filling the cell with cesium to block resting conductances and cooling the cell to slow the rate of conductance changes. As predicted by earlier modeling studies, Williams and Mitchell[2] found that these approaches did not overcome space-clamp errors in real neurons. In fact, errors in the measured current amplitude and voltage escape with cesium-filled electrodes were very similar to those measured with a normal (potassium containing) internal solution. Under these conditions, voltage escape can be even larger than under normal conditions, as a result of the higher input resistance of the cesium-filled cell. Unfortunately, performing recordings at room temperature was also ineffective at overcoming space-clamp problems.

If a somatic voltage clamp is ineffective, should we give up hope of understanding synapses on dendrites? Certainly not! Some alternative approaches may allow for accurate determination of the properties of synaptic currents in dendrites, including various types of dendritic recordings[10], the voltage-jump method[11,12] and reverse engineering of distal conductances from somatic measurements combined with compartmental modeling[13]. With some of these indirect approaches, however, care must be taken to prevent problems that are associated with the activation of voltage-gated channels as a result of voltage escape in the dendrites. The use of somatic voltage clamping for studying voltage-gated conductances is particularly problematic, and there may be few direct alternatives beyond various types of membrane patch recordings and imaging.

The results obtained by Williams and Mitchell[2] do not signal an end to the usefulness of the voltage-clamp method for neurons with dendrites. For many, voltage-clamp recording will remain the method of choice for monitoring synaptic responses where absolute quantitative measures are not essential. For example, in experiments studying long-term potentiation (LTP, a common test thought to be important in some forms of memory), the measure of interest may simply be a relative change in synaptic current amplitude, not the actual amplitude or kinetics of the measured current (if inhibition is blocked, a better measure might be synaptic charge, as it is less sensitive to space clamp errors)[10]. The voltage clamp may actually have some advantages for experiments such as this, as it offers an easy way to hold the neuron at a fixed potential, preventing activation of voltage-gated channels near the soma. On the other hand, the apparent stability of this situation can be misleading, as fluctuations of voltage in the axon and dendrites will be invisible to the experimenter. Furthermore, investigators studying LTP should consider the possibility that changes in voltage-gated channels in dendrites, which have been shown to accompany LTP[14], could contribute to apparent changes in the synaptic current measured in the soma.

The take-home message for the synaptic physiologist is to use the voltage-clamp method with extreme caution. However tempting it may be to believe that space-clamp errors may be minor, Williams and Mitchell[2] demonstrate unequivocally that this is almost certainly wishful thinking in all but the most optimal situations (for example, neurons with very short dendrites, such as cerebellar granule cells, or inhibitory synapses on the soma). Readers should also be cautioned to view voltage-clamp experiments with a critical eye. For example, these results[2] (and others, see ref. 10) suggest that measures of spontaneous or miniature synaptic currents from the soma will reflect only a small fraction of the total synaptic input. Nonetheless, being critical does not mean being dismissive. Just as Hodgkin and Huxley took the time to thread wires down the length of the squid giant axon, modern day researchers will find creative ways to use the voltage clamp and other methods to unlock the secrets of synapses and voltage-gated conductances in dendrites. The all-important nature of these structures makes them irresistible subjects of study.

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