ON THE SQUID AXON MEMBRANE'S RESPONSE TO SEQUENTIAL VOLTAGE AND CURRENT CLAMPS

JOHN W. MOORE, NORMAN STOCKBRIDGE, AND STEVEN J. SCHIFF
Department of Physiology, Duke University Medical Center, Durham, North Carolina 27710

ABSTRACT Starzak and Starzak (1978. *IEEE Trans. Biomed. Eng.* 25:201–204.) proposed that, in cyclic application of current and voltage-clamps, the fidelity of the match of the output current with the original stimulus could be used to measure the spatial uniformity of voltage in a membrane. However, they failed to find such a match in experiments on either squid axons or an electronic model of a membrane patch. Computer simulations of such experiments show that the failure to return the initial pattern may arise from shortcomings of the instruments or instability of membrane characteristics. Logical arguments show that such cyclic experiments are not able to provide information about spatial gradients of membrane voltage.

INTRODUCTION

Starzak and Starzak (1978) proposed a novel approach to the analysis of the spatial homogeneity in voltage-clamp of axon membranes. Subsequent attempts (motivated by K. S. Cole [1980]) to apply and understand this approach led to questions of whether such information was obtainable and what the practical limitations of techniques used to realize this new method were.

Briefly stated, the questions are:

(*a*) The Starzaks' experiment. If one faithfully records a membrane action potential resulting from some current source, and a short time later applies to that same membrane patch the recorded voltage as the voltage-clamp command, does one record a current with the same shape as the original current stimulus?

(*b*) The Cole experiment. Does a voltage-clamp current record used as the command in a current clamp result in the original command potential?

(*c*) If the answers to the above questions are negative, what does this tell us about the uniformity of the membrane potential and the experimental technique?

For these experimental propositions to give an affirmative answer, in addition to the original requirement of spatial uniformity of the voltage, explicitly stated by the Starzaks, there are several implicit but critical assumptions that must be made:

(*a*) The current and voltage are measured in the same way in both current and voltage-clamp.

(*b*) The voltage and current clamps must approach ideal in speed and accuracy. The output impedance of the voltage-clamp circuit must be very low compared with the minimum membrane resistance encountered throughout the range of voltages to be controlled (Cole and Moore 1960). The current-control circuit must have a resistance that is very high compared with the maximum membrane resistance encountered (Cole and Moore, 1960).

(*c*) The recording and playback equipment must return a faithful reproduction of the input signal.

(*d*) The membrane characteristics do not change between the application of the first and second halves of the cycle.

In their experiments on squid axons, the Starzaks were unable to recover the input current pattern. There were large and erratic current disturbances during the action potential. They eliminated the possibility of biological variability by repeating the experiment and getting the same answer on J. Y. Lettvin's electronic model (Meta-Metric Corp., Carlisle, MA) of an axon membrane. Cole (1980) persuaded R. FitzHugh to simulate such a circular experiment with a membrane patch on a computer and the result was a current output pattern similar to that observed by the Starzaks. One of us (Dr. Moore) suggested to Cole that some—if not all—of the "hash" in the current output must be due to the discrete steps in the digitization of the action potential that, when applied as a command in a good voltage clamp, will cause transient capacity currents to flow through the membrane. It was proposed that a smoother continuous recording medium would be better for the Starzak experiment and this was confirmed by Fishman (see Fig. 2 of Cole, 1980).

Cole (1980) then proposed experiments using the reverse cycle-record currents under voltage-clamp steps and driving these in a current clamp. He notes that this problem is still to be resolved.

We have undertaken an examination of the instrumentation requirements by carrying out computer simulations to evaluate the magnitude and kinds of errors in the cycle output that arise from inadequate resolution in digitalization in amplitude and time. We have also examined the sensitivity of the cycle output to membrane parameter changes. We find that the Starzak experiment can be carried out satisfactorily with high resolution digital instruments. The Cole cycle experiment is so unstable that it can probably be carried out only on a computer.

METHODS

An isopotential patch of active membrane, described by the Hodgkin and Huxley (1952) equations was used throughout. The current and voltage-clamps were represented as having ideal source characteristics (i.e., having infinite and zero source impedances, respectively), but with a realistic first-order lag transient response and a time constant of 10 μs. This time constant is usually observed (e.g., Hodgkin et al., 1952) in squid axon voltage clamps and normally results from the resistance in series with the membrane. Nevertheless, for simplicity, we have taken the resistance in series with the membrane to be zero.

A FORTRAN program describing the membrane and voltage clamp by methods previously described (Moore et al., 1975) was run on a PDP-11/34 computer (Digital Equipment Corp. Maynard, MA). The time courses of the current and voltage were calculated in double precision arithmetic (17 decimal digits). Integration was carried out by the Euler method using time step sizes of 5 μs or less. We found it crucial to evaluate both halves of the cycle with precisely the same time step. Such synchrony assures precise correspondence in the two phases. A Taylor expansion was used to evaluate the Hodgkin-Huxley rate constants near their indeterminate points.

With such precautions, precision, and synchrony of time scales in the two half cycles, all four critical assumptions were met and it was possible to simulate both the Starzak and Cole cyclic experiments and recover the initiating signal.

It then remained to evaluate the sensitivity of the two cycles to (*a*) small changes in membrane parameters (e.g., Cm, $\bar{G}_{Na}$, $\bar{G}_K$) halfway through a cycle, and (*b*) degradation of the recorded signal. For the latter case we degraded the recorded (and played back) signal by processing it through an alogrithm that mimicked an analog-to-digital (ADC) converter whose amplitude and time resolution could be specified and altered.

RESULTS

Starzak Cycle

A Hodgkin-Huxley membrane patch of 1 cm^2 at 6.3°C was subjected to a 10-μA pulse of current for 1 ms and the resulting action potential was calculated (to double precision). This action potential was then used to drive a voltage clamp of (*a*) the identical patch, (*b*) a patch with altered membrane capacitance, and (*c*) a membrane patch with altered ionic channel densities. Fig. 1 shows that the initial current pattern is adequately recovered when the voltage-clamped patch is identical to that used in the current clamp.

Membrane Parameter Changes. The output current from the voltage clamp is relatively insensitive to a change in membrane capacitance but quite sensitive to small changes in the density of ionic channels ($\bar{G}_{Na}$ and $\bar{G}_K$) as can be seen in Fig 1, also.

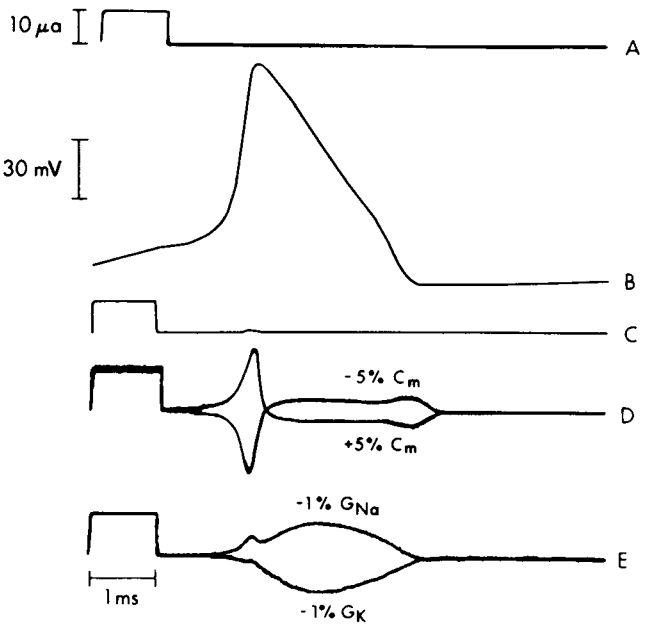

FIGURE 1 In this simulation, as in Fig. 2, a Hodgkin-Huxley membrane patch was current clamped with a 10-μA 1-ms pulse (*A*), and the resulting membrane action potential (*B*) was used as the command to a voltage clamp for (*C*) an identical membrane patch, or one in which capacitance (*D*) or conductance (*E*) was changed. *D* shows the results of +5% and −5% changes in the membrane capacitance of the second patch; *E* shows the results for −1% changes in sodium and potassium conductances (with leakage to give zero current at rest).

Degradation of Signal by Digitalization. The action potential calculated in a current clamp as above was processed to simulate sampling by an ADC. At discrete time intervals (corresponding to the ADC sampling rate) the value of the membrane potential was rounded off to a new value depending on the chosen amplitude resolution of ADC to be simulated.

This new "stair-step" representation of the current clamped action potential was used as the driving command for a voltage clamp of an identical 1 cm^2 of Hodgkin-Huxley membrane. Fig. 2, showing the voltage-clamp current output, demonstrates that a good representation of the original current pattern can be obtained with a superb ADC (amplitude resolution of 1/65,000 or 16 bits at a sample rate of 1 μs). However, when a more conventional ADC with less resolution (1/4,096 or 12 bits) is used, there is significant degradation of the current pattern. The degradation becomes unacceptable when the ADC sample rate is reduced twofold (to 2 μs intervals).

Cole Cycle

The current flow in a 1 cm^2 patch of Hodgkin-Huxley membrane at 6.3°C undergoing a step change from rest to a depolarization of 40 mV was calculated (with double

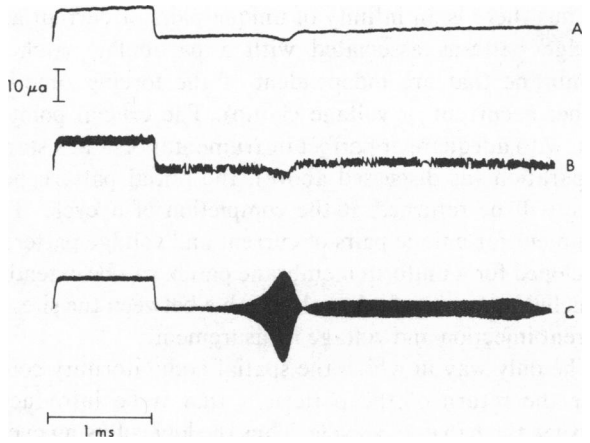

FIGURE 2 In this simulation, a Hodgkin-Huxley membrane patch was current clamped, with a 10 μA pulse of 1 ms duration. The resulting membrane action potential was sampled with an ADC and used to command a voltage-clamp of an identical Hodgkin-Huxley membrane. The output current records obtained are shown in Figs. *A*, *B*, and *C*. In *A*, the ADC sampled at 1 μs intervals and used 16 bits to span the voltage range from −15 to +120 mV. If (*B*) the resolution was reduced to 12 bits for the same 1 μs sample rate, considerable noise was generated. Alternatively, if the 16-bit resolution was retained but the sample rate was reduced to 2 μs (C), the error became intolerable.

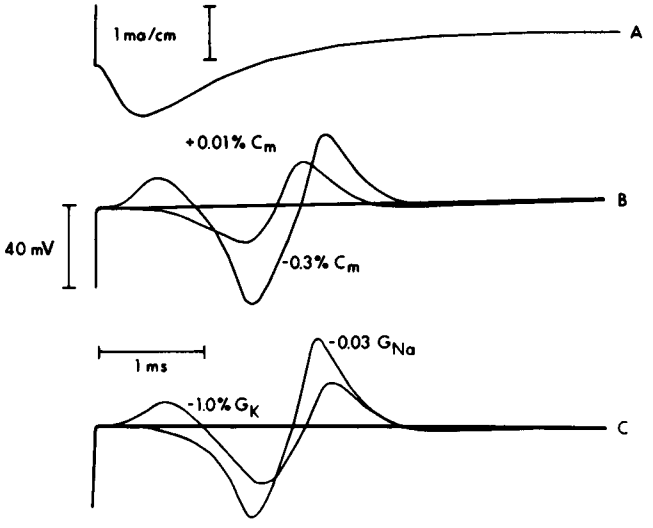

FIGURE 3 In this simulation, a 40 mV depolarizing pulse was used to command a voltage-clamp of a Hodgkin-Huxley membrane patch. The familiar current record obtained appears in *A*. When this current record is used to current-clamp an identical Hodgkin-Huxley membrane patch, the result is the original 40 mV step (flat curves in *B* and *C*). *B* shows the effect of the indicated percentage changes in membrane capacitance of this second patch, and *C* shows the sensitivity to conductance changes in the second patch (the leakage again being adjusted to produce zero current at rest).

precision) at 1 μs time intervals and used to drive a current clamp of an identical patch, an accurate reproduction of the original voltage step across the membrane was obtained as shown in Fig. 3 (flat records in *B* and *C*).

Membrane Parameter Changes. When either the membrane capacitance or sodium channel density was changed by a very small percentage, wild deviations in the membrane potential are observed, e.g., 20–40 mV when $\bar{G}_{Na}$ was changed by as little as 0.1% or $\bar{G}_K$ was changed by 1.0% from its original value. Similar excursions were also produced by small perturbations of the membrane capacitance.

Degradation by Digitalization. When the voltage-clamped current pattern was digitized into discrete voltage levels and time steps before being used to drive an identical patch with the current clamp, similar errors in the output voltage pattern were observed.

This was probably because the resolution (in time and amplitude) was not sufficient to give an accurate representation of the capacitive current transient associated with the voltage step. When this signal is used to drive a current clamp, the membrane potential fails to arrive at the 40 mV of depolarization. After this initial failure the membrane potential can never become stable.

DISCUSSION

The instrumentation requirements of this technique are easy to understand once one knows the how the role membrane capacitance plays in voltage clamp differs from that in current clamp. In the voltage clamp, the membrane capacitance produces an output current that is the time derivative of the membrane voltage, enhancing high-frequency noise. In the current clamp, the capacitance causes the output voltage to approach an integral where small errors can accumulate: low-frequency noise.

The major requirement, then, for the Starzak sequence is a fast and extraordinarily quiet signal playback. If the action potential record is digitized, stored, and played back, the command voltage staircase (steps between the discrete digitized levels) will cause capacitive current transients through the membrane that are certain to be largest during the rising phase of the recorded action potential. Their effect can be minimized by digitizing to a very high resolution at extremely small time intervals. However, most commercially available ADCs require a compromise between high speed and high amplitude resolution. Our computer simulations of a Starzak experiment, show that "very high resolution" is at least 16 bits (covering exactly the voltage range of the axon) and "extremely short time intervals" means no more than 1 μs. One might ask "why not smooth the steps in the digital staircase between the storage device and the voltage-clamp input?" The answer is that the time lag error thus introduced causes other serious deviations. A very quiet FM analog tape recorder might be the instrument of choice for this experiment, and has been successfully used by H. M. Fishman (Cole, 1980).

On the other hand, in the Cole sequence, digitized steps will be smoothed or filtered by the membrane's capaci-

tance, and might be tolerated. However, this experimental sequence requires an instrumentation system with very high time resolution for faithful reproduction of the fast capacitive current transient resulting from the command voltage step. If this record is distorted or attenuated, its integral will not drive the current-clamped membrane to the appropriate initial voltage level and the experiment will fail at the outset. The upper band-pass of most commercially available FM tape recorders is much too low for faithful reproduction of the few microseconds of capacitive current transient associated with a fast voltage-clamp step. On the other hand, fast digitization of the current record in the Cole cycle is not sufficient; amplitude accuracy requirements persist. The fact that very small computation errors cause the output voltage to undergo large deviations from the accurate value shows the insufficiency of normal digitization for storage and playback.

A major practical problem in attempting to carry out such cyclic experiments in a biological system is the stability of membrane parameters. In the Starzak sequence where errors do not accumulate, the output is less sensitive to small perturbations of membrane capacitance and conductance. Our examination of this problem by computer simulation showed that output of the Starzak sequence was much less sensitive than is the Cole sequence to small perturbations in the membrane properties. In a system that is sensitive to parameter changes below the normal experimental resolution, it seems unlikely that such an experiment could be carried out successfully. Such a conclusion was reached independently by John Rinzel (personal communication).

To address the question of what can be learned about uniformity of the membrane potential by such cyclic experiments, we need to extend the usual notion of a voltage clamp. A voltage clamp can be defined as a device that supplies the current necessary to maintain the desired potential across a cell membrane. Following the Starzaks' reasoning, if the chosen potential pattern is an action potential, the required current will be identical to that of the current clamp stimulus for the action potential.

Thus there is an infinity of unique pairs of current and voltage patterns associated with a particular patch of membrane that are independent of the forcing function (either a current or voltage clamp). The crucial point is that, with adequate or perfect instrumentation and a stable preparation (as discussed above), the initial pattern perforce will be returned at the completion of a cycle. The argument for unique pairs of current and voltage patterns, developed for a uniform membrane patch, can be extended to include the case of a length of cable between the sites of current injection and voltage measurement.

The only way in which the spatial nonuniformity could alter the return of the pattern is that it be introduced between the halves of a cycle. Thus the logic of using cyclic experiments to give information about spatial uniformity of voltage is flawed.

We conclude that in such cyclic current and voltage clamp experiments, failure to return the initial pattern may arise from shortcomings of the instruments or instability of membrane characteristics but not spatial gradients of membrane voltage.

Received for publication 8 November 1980 and in revised form 2 August 1982.

REFERENCES

Starzak, M. E., and R. J. Starzak. 1978. An action potential clamp to probe the effectiveness of space clamp in axons. *IEEE Trans. Biomed. Eng.* 25:201–204.

Cole, K. S. 1980. Digital operations can be deceptive:. squid giant axon membrane. *Biophys. J.* 31:433–434.

Cole, K. S., and J. W. Moore. 1960. Ionic current measurements in the squid giant axon membrane. *J. Gen. Physiol.*. 44:123–167.

Hodgkin, A. L., and A. F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.)*. 117:500–544.

Hodgkin, A. L., A. F. Huxley, and B. Katz. 1952. Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*. *J. Physiol. (Lond.)*. 116:424–448.

Moore, J. W., F. Ramon, and R. W. Joyner. 1975. Axon voltage-clamp simulations. I. Methods and tests. *Biophys. J.* 15:11–24.