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Patch Voltage Clamp of Squid Axon Membrane

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Summary. A small area (patch) of the external surface of a squid axon can be "isolated" electrically from the surrounding bath by means of a pair of concentric glass pipettes. The seawater-filled inner pipette makes contact with the axon and constitutes the external access to the patch. The outer pipette is used to direct flowing sucrose solution over the area surrounding the patch of membrane underlying the inner pipette. Typically, sucrose isolated patches remain in good condition (spike amplitude >90 mV) for periods of approximately one half hour. Patches of axon membrane which had previously been exposed to sucrose solution were often excitable. Membrane survival of sucrose treatment apparently arises from an outflow of ions from the axon and perhaps satellite cells into the interstitial cell space surrounding the axolemma. Estimate of the total access resistance (electrode plus series resistance) to the patch is about 100 kΩ (7 Ω cm^2). Patch capacitance ranges from 10–100 pF, which suggests areas of 10^{-4} to 10^{-5} cm^2 and resting patch resistances of 10–100 MΩ. Shunt resistance through the interstitial space exposed to sucrose solution, which isolates the patch, is typically 1–2 MΩ. These parameters indicate that good potential control and response times can be achieved on a patch. Furthermore, spatial uniformity is demonstrated by measurement of an axoplasmic isopotential during voltage clamp of an axon patch. The method may be useful for other preparations in which limited membrane area is available or in special instances such as in the measurement of membrane conduction noise.

The implementation of potential control over excitable membranes, as conceived by Cole (1968), has produced a wide variety of "voltage-clamp" systems. The primary requirement of each system is adequate temporal and spatial control of potential over the area of membrane from which the current is measured. The particular membrane preparation under study often imposes restrictions on how this objective can be accomplished, and usually, in order to achieve adequate control, some system characteristics must be degraded. Consequently, a specific voltage-clamp system cannot be applied universally to all preparations. This problem is more evident in voltage-clamping muscle fibers (Johnson & Lieberman, 1971) and neural membranes other than large axons (Neher & Lux, 1969).

Ideally, if a small area of the external surface of a cell membrane could be voltage-clamped without internal electrodes, the application of accurate potential-control measurements would not be limited by accessibility, cell shape, and membrane electrical properties. Realistically, in order to achieve a clamp of a small patch of membrane, some compromise in performance or applicability is inevitable. Nevertheless, some useful attempts have been made (Strickholm, 1961; Hencek, Nonner & Stämpfli, 1969; Neher & Lux, 1969).

This communication describes a patch-voltage clamp method which uses a flowing sucrose solution and a pair of coaxial pipettes to isolate a small area of the external surface of squid axons. The prime motivation for developing this method was the requirement of a small area of membrane for low extraneous noise measurement of fluctuations relating to ion movements (Fishman, 1973*c*; Fishman, 1975; Fishman, Poussart & Moore, 1975). The technique is simple, effective, and may be applicable to isolated cells or intact bundles of small or large single cells provided that the preparation can tolerate sucrose solution. A preliminary account of this work has been presented (Fishman, 1973*b*).

Preparation

All measurements were made on single giant (400–650 μ diameter) axons of the squid *Loligo pealei*, which were supplied live at the Marine Biological Laboratory, Woods Hole, Mass. The procedure for dissecting axons from squid has been described (Rosenberg, 1973). The axon chamber used in this work has also been described (Fishman, 1970). Natural flowing seawater, which was conveyed through an ice bath for cooling, was used as the standard external solution.

Isolation and Construction of Recording Electrode

Coaxial pipettes have been developed and used previously in small cells to record simultaneously intra- and extracellular potentials (Tomita, 1956; Freygang & Frank, 1959); to record simultaneously intracellular potential and apply molecules externally to the surface membrane (Curtis, Phillis & Watkins, 1959); and to apply current and measure potential simultaneously within cells (Tomita & Kaneko, 1965). In the present experiments an electrode, which consisted of a pair of coaxial glass pipettes, was developed and used externally to isolate and measure potential or current simultaneously in small areas of membrane (Fig. 1). A description of the electrode construction follows. A small glass (Pyrex) capillary

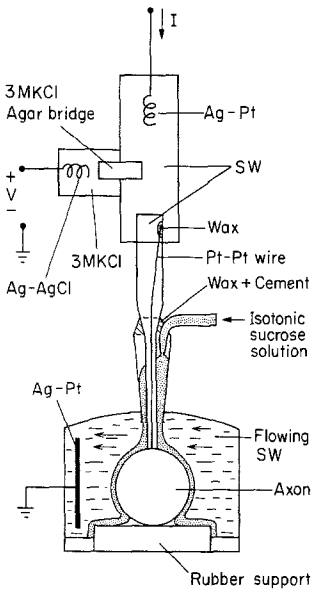

Fig. 1. Diagram of the coaxial pair of pipettes used to isolate and measure potential or current simultaneously in a small area of squid axon membrane. *See text* for details

(1.5 mm OD) was drawn in a vertical pipette puller by applying a constant force to one end of the heated capillary, while the other end was held fixed. The long tapered portion of the pipette was reduced to 2–3 cm in length by scoring the glass by hand with a diamond cutting wheel (Norton Abrasives, Worcester, Mass.) and tapping the glass lightly. This procedure produced a very smooth break at the scored point. Outside (tip) diameters (OD) of 30–100 μ were made by selection of filament current, which determined the taper. The tip could be fire-polished and reduced to an ID of 10 μ by approaching the tip with a heated filament during observation under a microscope. Another larger (3 mm OD) glass (Pyrex) capillary was drawn and fire-polished in the same way after it was scored and the tapered length of the shank reduced to 0.7 cm. The OD at the tip of this pipette was about 200–300 μ. The smaller pipette was inserted into the wide end of the large pipette and advanced until the tip of the small pipette extended 300–500 μ beyond the large pipette tip. The large capillary was scored with a file at a point slightly beyond the beginning of the taper of the smaller pipette to provide, eventually, the space for insertion of another pipette at that point. The small pipette was then removed from the larger one, and the large capillary was broken at the scored point. A tapered right-angle inlet pipette was prepared from the small glass capillary by bending and drawing by hand in a flame. The small pipette was again

inserted into the larger one until its tip was 300–500 μ beyond the tip of the large pipette. The inlet pipette was then inserted into the large capillary without disturbing the position of the previously inserted small pipette. The whole assembly was aligned so that the small pipette was approximately coaxial with the large pipette tip. Dental stickiwax was melted over and around all three pipettes at the wide end of the large capillary. Then cement (Du Pont, Duco) was applied over the wax to rigidify the whole assembly.

The final step in the coaxial-pipette assembly was the preparation and insertion of a platinized-platinum (Pt – Pt) wire within the length of the inner pipette. A 5 cm length of 25 μ platinum wire was platinized, using the square-wave current source method described by Moore and Cole (1963). The plated wire was inserted into the wide end of the small capillary (after the cemented assembly had dried and was rigid) and adjusted under the microscope so that the end of the wire was a few μ back from the tip. The other end of the wire was waxed to the inside surface of the wide end of the capillary to prevent the wire from moving into or out of the tip. The excess platinum wire was then cut. The completed assembly could be stored vertically in a dust-free box for an indefinite period. When used, the small capillary was filled with seawater (or whatever external solution was desired) and mounted in an airtight Plexiglas holder (Fig. 1), which was filled with seawater (SW) and contained two electrodes. One platinized-silver (Ag – Pt) wire (Cole & Kishimoto, 1962) was used for passing current through the small pipette; and the other, a Ag/AgCl wire in 3 M KCl, communicated with the seawater through a 3 M KCl agar-filled glass capillary and was used to record potential. In this arrangement the Pt – Pt wire within the small pipette was "floating" with respect to the potential of the current and voltage electrodes in the holder. The Pt – Pt wire within the small pipette has been shown (Fishman, 1973*a*) to provide a low impedance AC path which shunts the much higher resistance of the SW in the small pipette. A low access resistance is an essential feature of the described method of patch voltage clamping.

Electrical Isolation of a Membrane Patch

The isolation of small areas of axon membrane at the external surface was achieved with a coaxial pair of pipettes and sucrose solution. The Plexiglas holder containing the electrodes and coaxial pipettes was mounted in a micromanipulator. The pipettes approached the axon sur-

face at an angle of 60° with the horizontal plane of the axon. This angle allowed direct observation from above through a microscope. The holder was positioned so that the tip of the SW-filled inner pipette was in contact with and slightly dimpled the axon. The inlet pipette was connected to a 30 ml syringe filled with isosmotic deionized sucrose-solution (0.8 M) located 30 cm above the chamber. The height of the sucrose reservoir produced sufficient pressure to give a flow rate of one or more drops of solution every 30 sec with enough volume for a two-hour experiment. The sucrose solution flow was established through the outer pipette which directed the solution over the portion of axon surrounding the axon area under the aperture of the inner pipette. The extension of the inner pipette 300 μ beyond the tip of the outer pipette assured spread of sucrose solution several millimeters along the axon and around the patch. The higher index of refraction and density of sucrose solution relative to SW enabled clear observation of the flow, which followed the contour of the axon without noticeable dispersion in the SW bath. The sucrose solution "fell" to the bottom of the chamber and was swept out by the continuous flow of SW past the axon, normal to its long axis.

Equivalent Circuit for the Patch Measurement

In the discussion of the patch measurement, the circuit in Fig. 2*A* is assumed to represent the important parameters. The element R_{el} is the equivalent resistance of the electrode access path from the potential-sensing electrode in the Plexiglas holder (Fig. 1) to the tip of the inner pipette. R_s is the usual resistance in series with any pair of internal and external electrodes used to measure membrane potential across a squid axon membrane. R_{sh} is the resistance from the patch through the surrounding sucrose solution to the chamber ground electrode (Fig. 1). R_p is

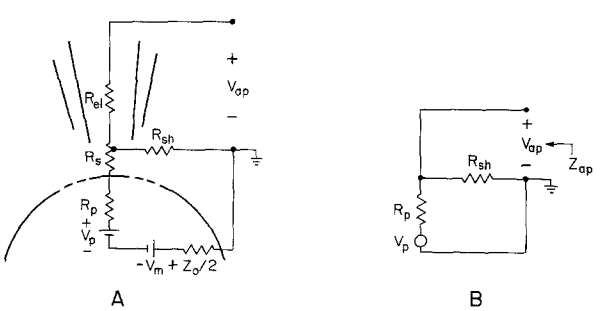

Fig. 2. Equivalent circuit of the pertinent parameters associated with patch potential and current measurements. Rest potential values are R_{el} (6–16 kΩ), R_s (40–90 kΩ), R_{sh} (1–2 MΩ), R_p (10–100 MΩ), $Z_0/2$ (5 kΩ). The circuit in *A* is shown to reduce to *B*

the resistance of the patch of axon which is in series with a resting transpatch potential V_p. The return path from within the axon to ground includes a resting membrane potential V_m in series with one-half of the cable impedance $Z_0/2$ (the parallel impedance of an infinite cable extending in both directions from the point of isolation).

Determination of Patch Parameters

The equivalent resistance of the inner pipette R_{el} was determined by a previously described method (Moore & Cole, 1963; Fishman, 1973*a*). A square wave of current from a current source (10 MΩ) was passed through a Ag wire into SW (in which the tip of the SW-filled inner pipette was immersed) and through the electrode to ground. The potential of the electrode from tip to ground was recorded with another Ag wire in the SW and observed on an oscilloscope. As shown in Fig. 3, there is a sudden change of potential at the beginning and termination of the current pulse. The resistance causing this initial change is designated the "zero-time resistance" and is 6 kΩ. The subsequent increase of potential which occurs during current flow is associated with an equivalent resistance as it is measured at various times. The 14 msec impedance is 16 kΩ. These low values of equivalent electrode resistance R_{el} are a consequence of the floating Pt – Pt wire within the inner pipette (Fishman, 1973*a*).

To estimate an upper bound (worst case) on the series resistance R_s, a rectangular pulse of current I from the 10 MΩ source was applied to the patch electrode (Fig. 1) after isolation of a patch. The time course of patch potential V (Fig. 1) was then recorded (Fig. 4) with the same patch electrode used in Fig. 3. The "zero-time" upward deflection of the trace before the generation of the spike reflects the pipette resistance plus series resistance

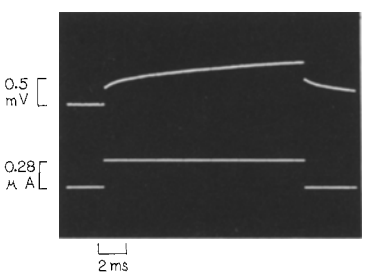

Fig. 3. The equivalent resistance (Moore & Cole, 1963; Fishman, 1973*a*) of the access pathway R_{el} through patch electrode from current electrode to inner pipette tip (Fig. 1) with the inner pipette filled and dipping into SW. The upper trace is the potential across the entire electrode in response to a constant current (lower trace). Zero-time resistance is 6 kΩ and the 16 msec impedance is 16 kΩ. 100 μ diameter tip

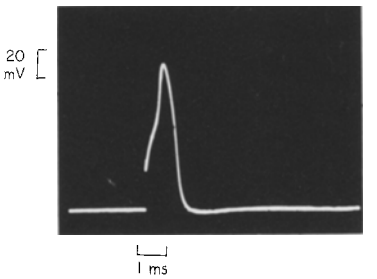

Fig. 4. Patch spike recorded by patch electrode for a short-duration (ends before spike) current pulse. The zero-time voltage displacement gives an estimate of the total access resistance ($R_{el}+R_s$). The current applied was 2.8×10^{-7} A through the same electrode used in Fig. 3

($R_{el}+R_s$) since the change in membrane potential should be negligible at this time due to the membrane capacitance. The initial deflection in Fig. 4 is 27 mV and the applied step of current was 2.8×10^{-7} A. $R_{el}+R_s$ is thus calculated to be 96.4 kΩ. Since R_{el} at "zero time" in Fig. 3 is 6 kΩ, R_s is estimated to be 90.4 kΩ. Assuming the patch area is approximately that of the 100 μ diameter aperture (area, 7.9×10^{-5} cm^2) of the pipette used in this measurement, a specific access resistance ($R_{el}+R_s$) of 7.6 Ω cm^2 and specific series resistance of 7.1 Ω cm^2 are calculated.

In order to assess the relative importance of the remaining elements in the circuit of Fig. 2*A*, the following data are presented. Fig. 5 (upper left) shows typical $V_{ap}(t)$ records (measured at "V" in Fig. 1) for constant current (hyperpolarizing) pulses applied to the inner pipette at electrode *I* (Fig. 1) during application of the electrode to the axon surface without sucrose flow (lowest visible trace). The sucrose solution flow was initiated, and the subsequent increasing responses in V_{ap} were recorded as the sucrose solution "isolated" a patch. In about a minute, a steady response was established. The vertical scale is calibrated as an equivalent resistance, shown as Z_{ap} in Fig. 5, and the 6 msec impedance is 2.5 MΩ. In general, the equivalent resistances which were measured ranged from 1–5 MΩ with 90% in the 1–2 MΩ range (over 500 patches). The stability of the isolation, as determined by measurement of the equivalent resistance, was maintained over several hours. Typically, the excitability of the patch was good for periods of at least one-half hour.

The equivalent resistance determined from Fig. 5 (upper left) is a measure of the apparent patch impedance Z_{ap}, i.e., the parallel combination of R_p and R_{sh} in Fig. 2*A*. The following points support this statement. 1) R_{el} plus R_s was estimated to be insignificant (96 kΩ) compared to the apparent patch impedance (2.5 MΩ). 2) An estimate of the $Z_0/2$ element

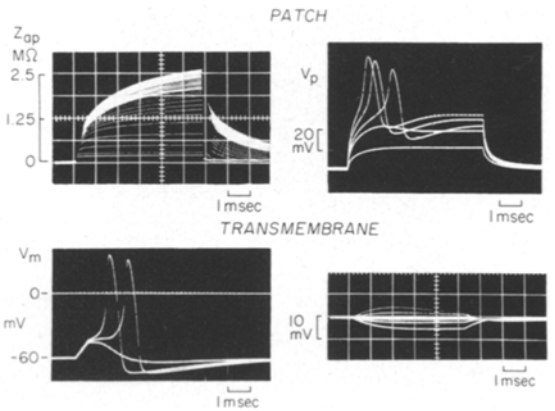

Fig. 5. *Upper left:* Voltage (apparent patch impedance, Z_{ap}) recorded by patch electrode in response to constant rectangular current pulses applied to patch electrode before and during the isolation of an axon patch with sucrose solution. *Upper right:* Patch spikes elicited by long-duration constant current pulses. *Lower left:* Transmembrane spikes recorded by a potential electrode inside the axon. *Lower right:* Potential difference between internal potential electrode and external bath ground during current stimulation of patch spikes. The transmembrane rest potential V_m in the membrane exclusive of the patch is relatively undisturbed by currents which excite the patch and return to ground through the rest of the axon in the chamber

in Fig. 2*A* indicates it is also negligible compared to 2.5 MΩ. The DC expression for cable impedance (*see* Cole, 1968, p. 69; Strickholm, 1961, p. 1081) is

$$Z_0 = [R_m R_i / 2\pi^2 a^3]^{\frac{1}{2}}$$

where R_m is the membrane specific resistance (1 000 Ω cm^2); R_i is the axoplasmic resistivity (30 Ω cm); a is the axon radius (2.5×10^{-2} cm). Using the values in parentheses, $Z_0/2 = 4931$ Ω. 3) The voltage source, V_m, in Fig. 2*A*, which represents the resting potential in the remainder of the axon (exclusive of the patch), is relatively unaffected by patch currents sufficient to produce excitation in the patch. Fig. 5 (upper right) shows spikes elicited at the patch for long-duration applied current pulses. Fig. 5 (lower left) shows transmembrane spikes elicited by an external stimulus and recorded between a pipette within the axon and an external electrode. Fig. 5 (lower right) shows the voltage response recorded by the same internal pipette when the current applied to the patch is sufficient to produce excitation (Fig. 5, upper right). These data indicate (1) that currents through the patch which elicit spikes in the patch do not significantly alter the resting potential V_m in the remainder of the axon; (2) patch spikes are nonpropagating.

The preceding measurements and discussion suggest that for electrical stimulation of the patch the important parameters are R_p, V_p and R_{sh}. Consequently, the circuit in Fig. 2*A* can be simplified to the one shown in Fig. 2*B*.

Estimate of Patch Area by Capacitance Measurement

The best estimate of patch area is through a measurement of patch capacitance since axon membrane capacitance is known to vary no more than $\pm 10\%$, whereas membrane resistance can vary considerably from axon to axon. Patch capacitance was determined by integration of the charging current during a step-voltage clamp (Hodgkin, Huxley & Katz, 1952). Fig. 6 shows the current response (upper trace) to a 20 mV step change in patch potential (lower trace). A capacitance of 67 pF is computed from these data. Since this capacitance is small, stray capacitance cannot be assumed to be negligible. A stray capacitance of 15 pF was measured by placing the electrode in air with all leads connected to it. The actual patch capacitance is then 52 pF. Assuming a membrane specific capacitance of 1 μF/cm^2, the estimated patch area is 5.2×10^{-5} cm^2. The area of the aperture (70 μ diameter) of the inner pipette used to make these measurements was 3.9×10^{-5} cm^2, which indicates that the isolated area is actually 25% larger than the pipette-tip area. The spread in area is not unexpected, since the tip of the inner pipette actually makes contact with the Schwann cell covering, which is 7–28 μ thick (Cole & Hodgkin, 1939), rather than with the axon surface. Generally the patch areas calculated from capacitance measurements ranged from 10^{-4} to 10^{-5} cm^2 for inner-pipette tip diameters of 50 to 100 μ.

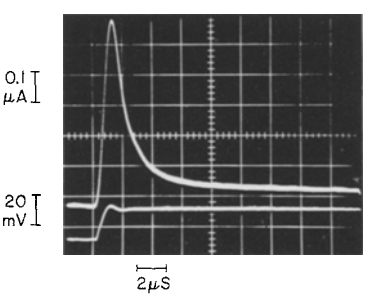

Fig. 6. Capacitive current in response to a fast step-voltage clamp. A patch capacitance of 67 pF is calculated from the integral of the charging current and the voltage step. Correction for stray capacitance yields a patch C of 52 pF. The time constant associated with the decaying portion of the current is 1.4 μsec

Estimate of R_p and R_{sh}

From the estimate of patch area and the assumption of a specific membrane resistance, R_p can be determined. The major source of error in this determination is the particular value of specific resistance assumed, since it can vary considerably. Nevertheless, an estimate provides some indication of R_p and R_{sh}, which is useful in assessing patch voltage-clamp performance. If a resting membrane specific resistance of $1000\,\Omega\,\text{cm}^2$ (Cole, 1968) is assumed together with the patch area estimates of 10^{-4} to 10^{-5} cm^2, the resting value of R_p ranges from 10–100 MΩ. Z_{ap} is typically 1–2 MΩ (Fig. 5, upper left). Since the estimate of R_p at rest is much larger than Z_{ap}, it appears that the 1–2 MΩ apparent patch-impedance is approximately R_{sh}.

Another indirect way of estimating the value of R_p and R_{sh} is from the amplitude of the patch spike. From the circuit of Fig. 2*B*, it can be seen that a change in patch potential V_p, recorded as V_{ap} across R_{sh}, will be attenuated by the factor $R_{sh}/R_{sh}+R_p$. Therefore, if Z_{ap} (at rest) is taken as a measure of R_{sh} (which is assumed to remain constant), the ratio of recorded patch spike height to actual spike amplitude can be used to calculate R_p during the peak of a spike. An extrapolation of the resting value of R_p can then be made since membrane specific resistance is known to decrease 100-fold from rest to the peak of a spike (Cole, 1968, pp. 74 and 143). As a typical example, the apparent patch spike amplitude for a short-duration current pulse shown in Fig. 4 is 104 mV. The patch potential during the measurement in Fig. 4 was 20 mV hyperpolarized from rest potential, due to the sucrose flow (Julian, Moore & Goldman, 1962). If a maximum spike amplitude of 120 mV is assumed and the resting hyperpolarization of 20 mV is taken into account, the actual spike amplitude in this case should be 140 mV. The ratio of measured to assumed spike amplitude is then 0.74. However, spike amplitudes of 120 mV have been recorded (Fig. 8, Fishman, 1975) which would raise this ratio to 0.86. For a measured $Z_{ap} \simeq R_{sh} = 1$ MΩ, R_p, at the peak of the spike, is thus estimated to range from 160 kΩ to 350 kΩ. The extrapolated resting value of R_p is then two orders higher or 16–35 MΩ, which is within the 10–100 MΩ range estimated from the capacitance and patch area calculation.

Evaluation of Patch Voltage Clamp from Patch Parameters

The most important parameter with respect to voltage clamping of a patch of membrane is the access resistance to the patch. The "*IR*" drop across this resistance causes an error in the actual potential to which the

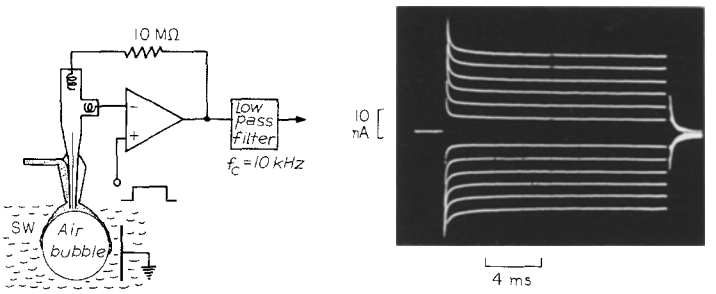

Fig. 7. Voltage clamp currents from an "isolated" patch of a 500 μ diameter air bubble in SW. The current records correspond to steps of ±50, ±100, ±150, ±200, ±250 and ±300 mV from a holding potential of zero. The current records were low-pass filtered (f_c = 10 kHz, 24 db/octave) to eliminate noise on the traces

patch is clamped. Furthermore, since R_p changes with potential and time, I is not a constant and the IR drop becomes time dependent, which can obscure the kinetics associated with the patch ion-currents. The access resistance to the patch was measured to be about 100 kΩ, of which about 10 kΩ (at the time of the minimum value for R_p) is due to the pipette. The access path through the pipette thus introduces only a 10% increase of resistance in series with the patch. In spite of this relatively small increase, it appears, however, that the specific access resistance ($R_{el}+R_s$, 7.6 Ω cm^2) in the patch method is comparable to the specific series resistance (7 Ω cm^2) measured by axial wire technique (Hodgkin *et al.*, 1952). With respect to access resistance, then, the patch method is no worse than an axial-wire technique. In addition, the method of feedback compensation (Hodgkin *et al.*, 1952) can also be applied to the patch (Fishman *et al.*, 1975) to reduce access-resistance errors.

In order to assess the properties of the patch electrode and the sucrose isolation, voltage-clamp measurements were made on a substitute preparation. A 500 μ diameter air bubble was produced by blowing air through a fine-tipped capillary immersed in SW. The patch electrode was applied to the surface of the air bubble and a patch was isolated (with sucrose) and voltage clamped. Fig. 7 shows the clamp currents in response to both positive and negative steps of potential. The current responses were passed through a low-pass filter with a corner frequency of 10 kHz and roll off of 24 db/octave to reduce the "noisiness" of the traces. Consequently, the early "capacitive" transient appears slower than without the filter. After the transient, the current records are reasonably flat, which indicates that patch-electrode polarization effects are insignificant during the pulse duration. In addition, the linearity of the responses with respect to the

applied potentials demonstrates that the isolation provided by the sucrose solution is ohmic. Furthermore, since the air bubble is nonconducting, the resistance (100 MΩ) determined from the steady-state current-voltage relation is the isolation resistance. The fact that R_{sh} is about two orders lower in the isolation of a squid axon patch suggests that the sucrose solution does not displace entirely the solution in the space between Schwann cell membranes and the axon surface. This situation could be due to the continuous outflow of ions from the portion of axon exposed to sucrose solution and perhaps the satellite cells as a result of the concentration gradient, and would account for the relatively nondeleterious effect of sucrose solution which was observed in these experiments. In spite of the lack of Ca or other ions in the sucrose solution, regions of axon which had been exposed to sucrose solution were often excitable when patched, i.e., after the inner pipette, filled with SW, was placed in contact with the previously exposed region and the new area was isolated. Ion outflow would account for (1) the relatively high shunt leakage of the membrane patch, (2) a hyperpolarized rest potential, and (3) a gradual increase in axoplasmic resistance. The latter condition can be eliminated by internal perfusion.

The shunt resistance through the interstitial space of the satellite cells which are exposed to sucrose solution is another possible source of aberration in the patch method. This shunt is in parallel with the patch (Fig. 2*B*). Consequently, during voltage clamp the current recorded contains the patch current summed with the shunt current. The assessment of the relative values of R_p and R_{sh} has indicated that R_{sh} is quite significant. The effect produced by R_{sh} is analogous to that produced by the leakage conductance in the patch. Fig. 8*A* shows a set of step-clamp currents in which the shunt effect is obvious. Fortunately, the shunt appears ohmic, and can be removed from the records by subtracting a step function, which is proportional to the step command. This was done by applying the patch current-response to one input of a differential amplifier and a proportionate amount of the step command to the other input. A depolarizing and hyperpolarizing step of a few mV's is applied successively to the patch, and the gain from the command to the input terminal of the differential amplifier is adjusted so that the currents, in response to depolarizing and hyperpolarizing pulses, decay to baseline (overcompensation produces a crossing of the baseline for a hyperpolarizing pulse). This procedure gives apparently normal current records (Fig. 8*B*) and suggests that the sucrose shunt about the axon patch is ohmic. Nevertheless, it is clear that patch leakage-conductance cannot be measured

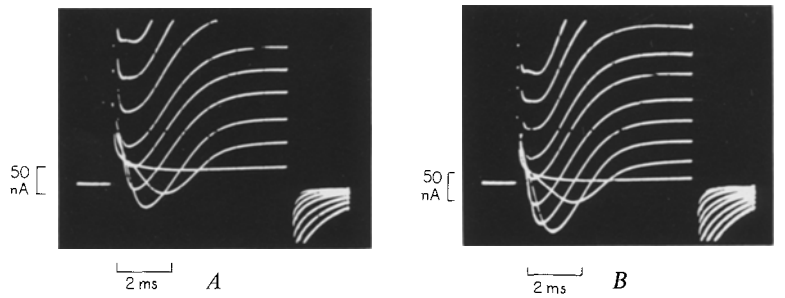

Fig. 8. (*A*) Patch voltage-clamp currents from squid axon including the sucrose shunt current. (*B*) The same as *A* but with the shunt current removed electronically from each current record

easily or with certainty because (1) the sucrose shunt conductance is relatively large, and (2) it is difficult to distinguish between the two leakages. Furthermore, calculations from the Hodgkin-Huxley equations show that the presence of an exaggerated leakage does not alter the kinetics of the voltage-dependent ionic conductances derived from clamp currents, and the magnitude of conductances can be corrected for leakage.

Spatial Control of Potential over the Patch

To evaluate the adequacy of potential control over the patch, the method of Cole and Moore (1960) was used. An axon patch was isolated and voltage clamped. A potential-sensing pipette (Fishman, 1973*a*) was introduced into the axon (Fig. 9*A*) and used as a probe to provide an independent measure of the potential along the axon with respect to the potential in the patch electrode. Fig. 9*B* shows these results. A step voltage (60 mV) which produced maximum transient inward current was used. The patch-to-internal probe potential (lower traces) was then recorded as the probe was moved from a portion of axon remote from the patch, toward the patch. The highest baseline (*a*) and voltage pulse correspond to the voltage recorded with the probe remote (0.7 cm) from the patch; the intermediate baseline (*b*) and pulse for the probe under the portion of axon covered with sucrose solution; and, the lowest baseline (*c*) and pulse correspond to the probe directly under the patch. The change in baseline is expected, since it is known that sucrose solution produces a resting hyperpolarization (Julian *et al.*, 1962). From Fig. 9*B*, the resting hyperpolarization was 12 mV. The potential V_{ap} recorded from within the patch electrode to the external ground was 13 mV and thus V_{ap} is a measure of the extent to which the patch potential deviates from the actual transmembrane rest potential of the axon. Furthermore, there is

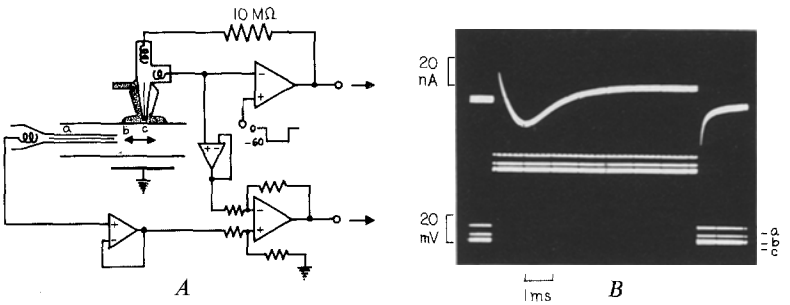

Fig. 9. Demonstration of the maintenance of an axoplasmic isopotential during a patch clamp which produced a maximum transient increase in conductance. (*A*) Measurement scheme. (*B*) Top traces: current (including sucrose shunt) in response to 60 mV clamp steps; lower traces: potential difference between internal electrode and patch electrode for the internal electrode remote from the patch (highest trace), within the sucrose fringe region (intermediate trace), directly under the patch (lowest trace)

no noticeable aberration (<1 mV) in the potential to which the patch is clamped for all positions of the internal probe along the axon. Therefore, these data suggest that the axoplasmic potential remains isopotential during a clamp pulse which produces a maximum conductance change in the patch.

Other Measurements

In addition to the measurements which are presented in this paper, tests of the adequacy of the patch voltage clamp at the level of spontaneous current fluctuations strongly support the results and inferences presented here (Fishman *et al.*, 1975). The noise measurements show that (1) the access resistance to the patch, when compensated by feedback, does not alter the form of current noise, and therefore does not produce significant error in clamping; (2) $Z_{ap} \simeq R_{sh}$ since the patch impedance does not show the resonance behavior which normally occurs in squid axon membrane. Over-damping of the resonance can only mean that $R_p > 5R_{sh}$ (Fishman, 1975; Fishman *et al.*, 1975); (3) adding capacitance to the patch does not alter the form of current noise, and therefore the clamp is adequate; (4) the axon membrane outside the patch does not contribute significant noise, which suggests good spatial control and isolation.

This work is a consequence of the subsequent axon membrane noise measurements, the importance of which was made evident to me by Dr. Kenneth S. Cole in 1969. I thank my colleagues, Dr. D.J.M. Poussart and Dr. L.E. Moore, for valuable suggestions with respect to tests of the patch technique. This work was supported in part by National Institutes of Health grants NS 09857 and NS 11764 and SUNY Research Foundation Awards during my affiliation with the Department of Biological Sciences, SUNY at Albany.

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