

An Optical Determination of the Series Resistance in *Loligo*

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ABSTRACT The resistance in series with the membrane capacitance in the giant axon of the squid *Loligo pealei* was measured using potentiometric probes that exhibit absorbance changes proportional to the voltage across the plasma membrane proper. The method relies upon the fact that a voltage drop across the series resistance produces a deviation in the true transmembrane voltage from that imposed by a voltage clamp. Optical measurement of the true transmembrane potential, together with electrical measurement of the ionic current, permits the immediate determination of the series resistance by Ohm's law. An alternative method monitored the amount of electronic series resistance compensation required to force the optical signal to match the shape of the reference potential. The value of the series resistance measured in artificial seawater was $3.78 \pm 0.95 \Omega \cdot \text{cm}^2$. The estimated value of the contribution of the Schwann cell layer to the series resistance was $2.57 \pm 0.89 \Omega \cdot \text{cm}^2$.

INTRODUCTION

A small resistance, electrically in series with the membrane capacitance in squid giant axon, is thought to arise primarily in the narrow Schwann cell clefts that make up the anatomical correlate of the Frankenhaeuser-Hodgkin space. Because of the presence of this series resistance element, the true transmembrane potential, V_m , will differ from the value recorded between the voltage-measuring electrodes, V_c , in a voltage-clamp arrangement by an amount, $I_m R_{ser}$, that is proportional to the membrane current, I_m . Hodgkin et al. (1952) introduced the procedure known as compensated feedback (series resistance compensation), in which a voltage proportional to the membrane current is added at the summing junction of the control amplifier. The proportionality constant depends upon the value of the series resistance and correct compensation requires an accurate determination of its value. Experiments performed without series resistance compensation or with incomplete series resistance compensation will generate clamp currents that do

not represent the response of the membrane to step changes in voltage, and the inferred kinetics for the membrane conductance changes will be in error. Taylor et al. (1960) showed that a change of a few ohms per square centimeter in the series resistance will result in noticeable alterations in the current-voltage relations for both sodium and potassium. Similarly, AC impedance measurements will be distorted, and the values obtained for the membrane electrical parameters will be incorrect. The problem posed by the series resistance has been studied extensively since then (e.g., Binstock et al., 1975), but the methods have been of necessity somewhat indirect and susceptible to various criticisms.

The method that we have used has the advantage that it is not essentially an electrical measurement at all, and it provides a completely independent determination, depending only on Ohm's law and the linearity of the optical changes exhibited by certain potentiometric dyes (Ross et al., 1977; Cohen and Salzberg, 1978; Salzberg, 1983). These molecular probes bind to cell membranes and change their optical properties in microseconds in response to changes in transmembrane potential. At the same time, they are insensitive to changes in membrane conductance or current.

The possibility of an optical determination of the series resistance was recognized implicitly by Davila et al. (1974), and an optically derived estimate of this quantity in *Limulus* photoreceptors was reported by Brown et al. (1979).

Preliminary measurements have been communicated in abstract form (Salzberg et al., 1980, 1981).

METHODS

Segments of the hindmost giant axons from the Atlantic squid *Loligo pealei* were cleaned of small fibers and mounted in a horizontal voltage-clamp chamber designed for optical measurements. The floor, ceiling, and front of the chamber were made of glass, and the rear wall was of Delrin, into which were fitted rectangular platinized platinum electrodes for current passing and measurement (center electrode = 3.75×4.0 mm; guards = 6.0×4.0 mm). The voltage reference electrode was located in the rear wall, immediately under the central plate. The chamber was mounted on the mechanical stage of a Reichardt Zetopan (Buffalo, NY) compound microscope with a focusable head. The diameter of the axon was measured with an accuracy of better than $5 \mu\text{m}$ using a calibrated reticule eyepiece, and a cover glass was placed over the chamber to eliminate the meniscus.

Electrical Measurements

The voltage-clamp circuitry was an improved version of that described by Bezanilla et al. (1982). The voltage amplifier settled in $<1 \mu\text{s}$ and the clamp permitted compensation for at least the full value of the series resistance present. The internal electrode was of the piggy-back type (Chandler and Meves, 1965), with the aperture of the voltage-measuring pipette oriented away from the axial wire.

The electrical estimation of the series resistance was performed by passing short current pulses (Cole and Moore, 1960) and observing the voltage at the "pulse-off." The time course of the voltage at pulse-off exhibited a fast jump that decayed into a ramp. Two measurements, which should bracket the true value of the series resist-

ance, were taken from fast oscilloscope recordings. The first, a lower limit, corresponded to the fast jump itself. The second, an upper limit, was the value of the voltage at the time of the pulse-off, extrapolated from the ramp slope.

The measurement of total series resistance, r_{ser} (ohms)* includes contributions from the portion of the axoplasm between the internal voltage electrode and the axolemma, r_{ax} , the Schwann cell layer, r_{sch} , and the portion of the experimental chamber between the Schwann cell layer and the external voltage electrode, r_{ex} . It is possible to estimate r_{ex} by measuring the total chamber resistance, r_{ch} . This measurement was accomplished by positioning the electrode, without an axon, and measuring the voltage displacement resulting from passing a known current. Then,

$$r_{\text{ex}} = r_{\text{ch}} - r_{\text{ax}}^{\text{sw}}, \quad (1)$$

where $r_{\text{ax}}^{\text{sw}}$ is the resistance, in the external solution, between the internal voltage electrode and an imaginary cylindrical surface located at the position of the axolemma. This quantity was calculated from

$$r_{\text{ax}}^{\text{sw}} = \frac{\rho}{2\pi l} \ln\left(\frac{b}{a}\right), \quad (2)$$

where b is the measured radius of the axon, a is the radial distance between its longitudinal axis and the voltage-measuring pipette, ρ is the solution resistivity, and l is the length of the central current-measuring plate. The axonal series resistance, r_s , which is equal to the sum of r_{ax} plus r_{sch} , is given by:

$$r_s = r_{\text{ser}} - r_{\text{ex}}. \quad (3)$$

Further, the contribution of the Schwann cell layer alone can be evaluated from:

$$r_{\text{sch}} = r_s - r_{\text{ax}}, \quad (4)$$

and r_{ax} is calculated from Eq. 2, with a value of ρ equal to the resistivity of the internal perfusion solution. The values of ρ for the internal perfusion solution (45 $\Omega \cdot \text{cm}$) and the artificial seawater (ASW) (26.5 $\Omega \cdot \text{cm}$) were measured in a conductivity cell.

Optical Measurements

Extrinsic optical changes were recorded after external staining of the axon with seawater solutions containing low concentrations (10–50 $\mu\text{gm/ml}$) of potentiometric probes (NK 2367, a merocyanine-oxazolone; WW 781, an oxonol; or WW 375, a merocyanine-rhodanine) for a period of 10 min. The giant axon was imaged on an arrangement of four independently moveable knife edges in the objective image plane of the microscope. The resulting rectangular slit restricted the light reaching a silicon photodiode (PV-444; EG&G, Inc., Salem, MA) to that passing through a selected space-clamped region of the axon. The incident light was provided by a 100-W tungsten-halogen lamp. The light was focused on the giant axon by a large-aperture bright-field condenser, after being collimated, heat filtered, and rendered quasi-monochromatic by an interference filter. In one experiment, using the probe WW 781, extrinsic fluorescence was monitored at 0° by intercalating a cut-on filter (RG-645; Schott Optical Glass Inc., Duryea, PA) between the axon and the photodetector to block the excitation wavelengths. The photocurrent output of the

* We have followed the convention that r denotes resistance (in ohms) and that R denotes specific resistance (in ohms $\cdot \text{cm}^2$).

photodiode was injected at the summing junction of an operational amplifier in an I - V converter configuration, whose output was AC coupled (time constant = 1.25 s) to a second stage amplifier, which was connected to a digital signal averager. The time constant of the light-measuring system was 17.5 μ s for most of the experiments, and 7 μ s for some measurements.

RESULTS

The linearity of many potentiometric probes has been demonstrated previously (e.g., Cohen et al., 1974; Ross et al., 1977; Gupta et al., 1981) using squid giant axons under voltage-clamp conditions in which the series resist-

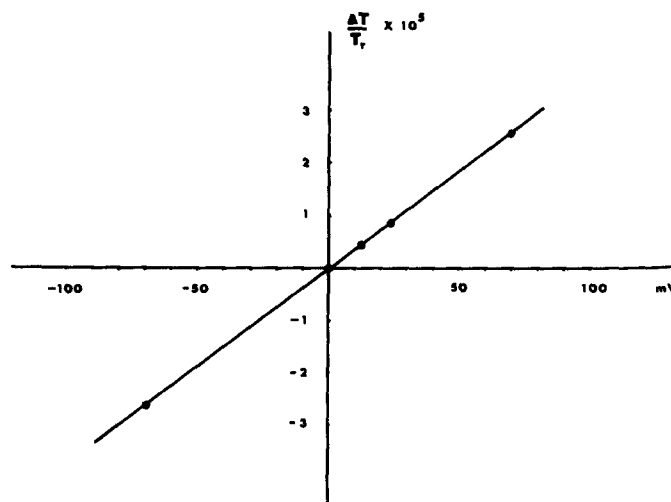


FIGURE 1. Linearity of the extrinsic absorption change with voltage-clamp steps in the absence of nonlinear membrane currents. The fractional change in transmitted intensity is plotted as a function of the size of the applied voltage step from a holding potential of -70 mV. The axon had been stained for 10 min in ASW containing 10 μ g/ml WW 375. Tetrodotoxin (300 nM) and 3,4-diaminopyridine (4 mM) were used to block most of the sodium and potassium currents, respectively. The potential steps were 5 ms in duration, and the light response was measured at the beginning of the step. Diameter = 638 μ m. The response time constant of the light measurement was 17.5 μ s. $\lambda = 722.4 \pm 20$ nm. $T = 22^\circ\text{C}$. (LP 309.)

ance is compensated approximately. We have confirmed the linearity of the optical response to voltage changes of the merocyanine-rhodanine dye WW 375 (dye XVII, Ross et al., 1977) by measuring the changes in extrinsic absorption as a function of membrane potential displacement when the nonlinear ionic currents have been minimized. Fig. 1 shows a plot of the initial transmitted intensity change as a function of the voltage step applied to an axon treated with tetrodotoxin and 3,4-diaminopyridine (Kirsh and Narahashi, 1978) to block most of the sodium and potassium currents. Under these conditions, the true membrane potential, V_m , is directly proportional to the imposed voltage, V_c :

$$V_m = V_c(1 - R_{ser}/R_m), \quad (5)$$

where we have assumed that the membrane resistance, R_m , is independent of V_m . In practice, the leakage ($1/R_m$) is slightly voltage dependent, but in a healthy axon, its value is small enough that V_m is nearly equal to V_c . The linearity of the optical signal with membrane potential, demonstrated in Fig. 1, allows us to use the extrinsic absorption change as a direct measure of the true transmembrane potential, V_m .

Fig. 2A shows a typical voltage-clamp experiment in which the imposed voltage (lower trace), the membrane current (middle trace), and the transmitted intensity (upper trace) were recorded from a stained axon in which the sodium and potassium currents were intact. During the two smaller depolarizations, the optical record closely resembled the imposed voltage. However, during the third clamp pulse, the optical signal exhibited an extra component having the shape of the inverted membrane current. This is the expected result if the control voltage is imposed across the membrane plus the series resistance and the dye is monitoring the true membrane potential, since a part of the clamp voltage proportional to the membrane current drops across the series resistance ($V_m = V_c - I_m R_{ser}$). Evidence for this interpretation is provided in Fig. 2B. In these records, partial series resistance compensation was introduced, as can be seen in the voltage record (lower trace). It is clear that although the membrane current (middle trace) is increased over that in Fig. 2A, the current component of the optical signal (upper record) is significantly reduced. (The setting of the series resistance compensation was $1.92 \Omega \cdot \text{cm}^2$, which was only 53% of the optically determined value; see below.) Further, the reduction in the membrane current-dependent component of the optical signal was governed monotonically by the setting of the potentiometer in the feedback circuit, until the point that the optical signal assumed a square shape despite the activation of large nonlinear currents (Davila et al., 1974). In most experiments a hyperpolarizing voltage-clamp step approximately matched to the depolarizing step was included in the pulse protocol. Since no nonlinear membrane currents were activated during this potential change, the true membrane potential was proportional to the control voltage, and the optical signal closely resembled the clamp step. An approximate value of the series resistance could then be obtained by varying the compensation potentiometer until the optical signals during symmetric hyperpolarizing and depolarizing clamp pulses had the same square shape. Conditions of undercompensation and slight overcompensation were obvious in the optical records and provided the lower and upper limit entries in Table I for axons 310 and 305, respectively.

A more accurate method for determining the value of the series resistance optically, and the one that we relied upon, is illustrated in Fig. 3B. The lower trace shows the depolarizing and hyperpolarizing potential steps of magnitudes V_d and V_h , respectively. The upper trace shows the resulting changes in extrinsic absorption, expressed as changes in transmitted intensities, T_d and T_h . Then, $T_d/T_h = (V_d + I_m R_{ser})/V_h$. Since I_m is measured independently, together with the voltages and transmitted intensities,

$$R_{ser} = [V_h T_d / T_h - V_d] / I_m \quad (6)$$

yields the value of the total series resistance directly (in ohms per square centimeter), without introducing any compensation.

Fig. 3A shows an experiment (axon 308) carried out in ASW. Again, the upper trace shows the transmitted intensity changes resulting from the imposition of the voltage-clamp pulses exhibited in the bottom trace, which were accompanied by the membrane currents recorded simultaneously in the middle trace. Note that the second depolarizing pulse, which barely activated

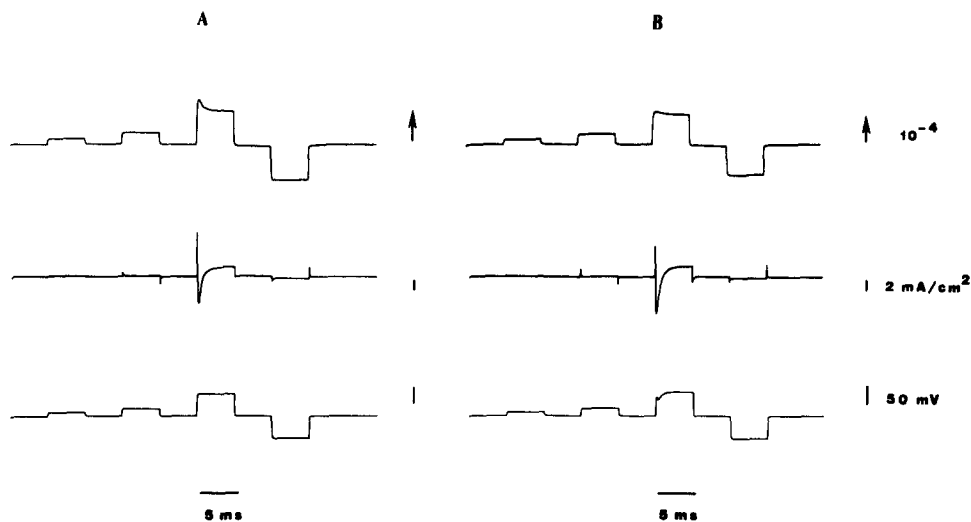


FIGURE 2. Series resistance compensation. (A) A voltage-clamp experiment in which the extrinsic absorption changes (top trace) and membrane currents (middle trace) were recorded during the voltage-clamp steps (bottom trace) applied to a stained axon in which the sodium and potassium currents were intact. In this experiment, no series resistance compensation was introduced. During the two smaller depolarizations and during the hyperpolarizing potential step, the optical record closely resembled the imposed voltage. However, during the third clamp pulse, the optical signal exhibited an extra component having the shape of the inverted membrane current, the expected result if the control voltage is imposed across the membrane plus the series resistance ($V_m = V_c - I_m R_{ser}$). Diameter = 431 μm . 22°C. (LP 310.) (B) The same experiment as in A, except that partial series resistance compensation was introduced, as can be seen in the voltage record (lower trace). The reduction in the membrane current component of the optical signal was governed monotonically by the level of feedback compensation, until the optical signal assumed a square shape (not shown) despite the activation of large nonlinear currents. Diameter = 431 μm . 22°C. (LP 310.) In this and Fig. 3, the vertical arrows to the right of the optical records (top traces) represent the fractional change in transmitted intensity during a single sweep; the staining was with WW 375 (10 $\mu\text{g}/\text{ml}$ for 10 min); the holding potential was -70 mV; the response time constant of the light measurement was 17.5 μs ; illumination was at 722.4 ± 20 nm; 128 sweeps were averaged.

a nonlinear current, resulted in a true transmembrane potential change (top trace), which was already contaminated by the effects of the uncompensated series resistance.

The results of our series resistance determinations are summarized in Table I. R_s (optical) is the optical determination from Eqs. 6 and 3, normalized

TABLE I

Axon (Diameter)	External solution	R_s (optical)	R_{sch} (optical)	R_{sch} (electrical)	R_{sch} (comp)	Dye
		$\Omega \cdot cm^2$	$\Omega \cdot cm^2$	$\Omega \cdot cm^2$	$\Omega \cdot cm^2$	
298	ASW	4.12	2.86			NK 2367
(523 μm)	ASW	4.74	3.48			NK 2367
	ASW	5.05	3.79			NK 2367
	ASW	3.31	2.09			NK 2367
299	ASW	2.97	1.75	1.03–4.02		NK 2367
(515 μm)	ASW	4.13	2.72			WW 781
	ASW	4.15	2.74		2.49	WW 375
304	ASW	2.21	1.11			WW 375
(554 μm)	ASW	2.46	1.36	0.51–2.97	<2.25	WW 375
	715 Na		(0.61*)			WW 375
	ASW	(6.21)	(5.11)			WW 375
306	ASW	4.22	3.17	0.47–2.27		WW 375
(477 μm)						
307	ASW	2.48	1.28	0.63–2.19		WW 375
(511 μm)						
308	ASW	4.31	3.26	1.09–3.81		WW 375
(477 μm)						
309	ASW	4.98	3.16	1.29–3.90		WW 375
(638 μm)						
310	ASW	4.45	3.60	1.49–3.12	>1.92	WW 375
(431 μm)						
181	ASW	3.07	2.23			NK 2367
(429 μm)	Acetate	(6.12)	(4.64)			NK 2367

Mean R_s (optical) in ASW \pm SD = $3.78 \pm 0.95 \Omega \cdot cm^2$ ($n = 15$).

Mean R_{sch} (optical) in ASW \pm SD = $2.57 \pm 0.89 \Omega \cdot cm^2$.

ASW: 440 mM NaCl; 50 mM MgCl₂; 10 mM CaCl₂; 10 mM Tris chloride, pH = 7.7. 715 Na = ASW + 275 mM NaCl. Acetate = ASW with all chloride replaced by acetate.

Values of R_s (optical) and R_{sch} (optical) in parentheses were not included in the mean.

* In the case of 715 Na, the final resistivity of the internal solution was unknown, so that it is not possible to obtain R_s (optical) or R_{sch} (optical). Instead, we have subtracted the total chamber resistance (in 715 Na) from the measured value of R_{sch} (optical). This should result in slight overestimates of the true values.

to the membrane area. The column labeled R_{sch} (optical) contains the values of the series resistance (multiplied by the membrane area, ohms per square centimeter) contributed by the Schwann cell layer, after subtraction of the chamber and axoplasmic resistances, $R_{sch} = (r_s - r_{ax}) \times \text{area}$; cf. Eq. 4. The column labeled R_{sch} (electrical) contains the estimated upper and lower bounds for the value of the series resistance obtained using the current-step procedure, as described in the Methods section. The column headed R_{sch} (comp) corresponds to estimates of R_{sch} determined optically by introducing

series resistance compensation. Table I includes two experiments in which we changed the external solution in an effort to vary the conductivity of the medium and hence the series resistance. In the experiment on axon 305, the normal ASW was replaced with one containing 715 mM sodium. Two previous optical determinations of the series resistance on this axon had yielded a mean value of $1.24 \Omega \cdot \text{cm}^2$, within the bounds ($0.51\text{--}2.97 \Omega \cdot \text{cm}^2$)

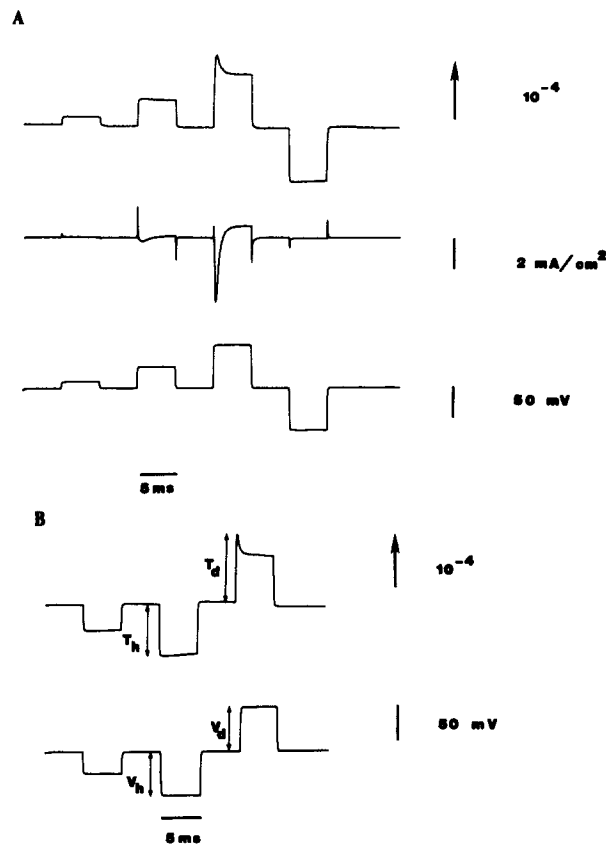


FIGURE 3. Optical determination of the series resistance. (A) An experiment carried out in ASW. The upper trace shows the transmitted intensity changes resulting from the imposition of the voltage-clamp pulses exhibited in the bottom trace, which were accompanied by the membrane currents recorded simultaneously in the middle trace. Note that the second depolarizing pulse, in which a nonlinear current was barely activated, resulted in a true transmembrane potential change (top trace), which was already contaminated by the uncompensated series resistance. Diameter = $477 \mu\text{m}$. $T = 23^\circ\text{C}$. (LP 308.) (B) Illustration of the method used to determine the value of the series resistance optically. The lower trace shows the depolarizing and hyperpolarizing potential steps of magnitude V_d and V_h , respectively. The upper trace shows the resulting changes in extrinsic absorption, recorded simultaneously, and expressed as transmitted intensities, T_d and T_h . $R_{\text{ser}} = [V_h T_d / T_h - V_d] / I_m$. Series resistance uncompensated. Diameter = $511 \mu\text{m}$. (LP 307.)

established by the electrical measurement, and are consistent with the upper limit suggested by the optical results of compensating $2.25 \Omega \cdot \text{cm}^2$. In ASW made hypertonic with NaCl, the optical measurement indicated that the series resistance had decreased to $\sim 0.61 \Omega \cdot \text{cm}^2$. Presumably, this result followed both from the decreased resistivity of the external solution and the shrinkage of the Schwann cells and consequent expansion of the Frankenhaeuser-Hodgkin space. However, the return to normal ASW was followed by a large increase in series resistance and deterioration of the axon. This may be explained by the entry of sodium into the Schwann cells, in hypertonic seawater, followed by swelling on return to normal ASW.

In the experiment on axon 181, we examined the effect of replacing all of the chloride in the seawater with acetate, which, having a lower mobility, is expected to increase substantially the resistivity of the external medium. The expected result was obtained, viz., a large increase in the series resistance contributed by the Schwann cell layer, R_{sch} , from 2.23 to $4.64 \Omega \cdot \text{cm}^2$.

DISCUSSION

As J. C. Maxwell clearly implied (Maxwell, 1865, pp. 493–497), it is impossible, in principle, to determine the value of a resistance in series with a lossy dielectric by electrical measurements made on the system as a whole. The difficulty arises from the fact that the series resistance cannot be separated unambiguously from the dissipative part of the complex capacitance. The separation can be made, however, if a direct measurement is performed across the dielectric itself. In the case of the squid axon, this would require that one voltage-measuring electrode be located in the axoplasm, immediately against the axolemma, with the second electrode in the 150-\AA wide periaxonal space. This seems an unlikely prospect. Alternatively, potentiometric dyes, which are small molecules that bind to the membrane, can be used to measure changes in the membrane potential without sensing voltage drops elsewhere. The use of such molecular probes is facilitated if their optical response (change in absorption or fluorescence) is linear with the voltage change across the membrane. We have verified that this assumption holds in the case of WW 375 (Fig. 1; see also Ross et al., 1977), and similar experiments suggest that NK 2367 and WW 781 behave similarly (e.g., Gupta et al., 1981). It is of interest to note that the optical determination of the value of the series resistance does not depend either upon the identity of the probe or on the mechanism by which it monitors transmembrane voltage. In one axon (Table I, axon 304), successive measurements of the series resistance gave identical values when the extrinsic fluorescence change exhibited by WW 781 and the extrinsic absorption change provided by WW 375 were used.

Our determination of the series resistance from the optical data is in good agreement with the value measured by Taylor and Chandler (Taylor, 1965). The calculation assumes that the series impedance is a pure resistance, and consequently, has no frequency dependence ($V_m = V_c - I_m R_s$). Cole (1976) has shown that the capacitance of the Schwann cell layer starts contributing

to the series impedance only at frequencies above ~ 70 kHz. As we have used the peak inward current, which does not contain frequency components above a few kilohertz, in our determination of the series resistance, the contribution of the imaginary part of the series impedance will be negligible.

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REFERENCES

- Bezanilla, F., J. Vergara, and R. E. Taylor. 1982. Voltage clamping of excitable membranes. In *Methods in Experimental Physics*. G. Ehrenstein and H. Lecar, editors. Academic Press, Inc., New York. 20:445–511.
- Binstock, L., W. J. Adelman, Jr., J. P. Senft, and H. Lecar. 1975. Determination of the resistance in series with the membranes of giant axons. *J. Membr. Biol.* 21:25–47.
- Brown, J. E., H. H. Harary, and A. Waggoner. 1979. Isopotentiality and an optical determination of series resistance in *Limulus* ventral photoreceptors. *J. Physiol. (Lond.)*. 296:357–372.
- Chandler, W. K., and H. Meves. 1965. Voltage clamp experiments on internally perfused giant axons. *J. Physiol. (Lond.)*. 180:788–820.
- Cohen, L. B., and B. M. Salzberg. 1978. Optical measurement of membrane potential. *Rev. Physiol. Biochem. Pharmacol.* 83:35–88.
- Cohen, L. B., B. M. Salzberg, H. V. Davila, W. M. Ross, D. Landowne, A. S. Waggoner, and C.-H. Wang. 1974. Changes in axon fluorescence during activity, molecular probes of membrane potential. *J. Membr. Biol.* 19:1–36.
- Cole, K. S. 1976. Electrical properties of squid axon sheath. *Biophys. J.* 16:137–142.
- Cole, K. S., and J. W. Moore. 1960. Ionic current measurements in the squid giant axon membrane. *J. Gen. Physiol.* 44:123–167.
- Davila, H. V., L. B. Cohen, B. M. Salzberg, and B. B. Shrivastav. 1974. Changes in ANS and TN fluorescence in giant axons from *Loligo*. *J. Membr. Biol.* 15:29–46.
- Gupta, R. K., B. M. Salzberg, A. Grinvald, L. B. Cohen, K. Kamino, S. Leshner, M. B. Boyle, A. S. Waggoner, and C. H. Wang. 1981. Improvements in optical methods for measuring rapid changes in membrane potential. *J. Membr. Biol.* 58:123–137.
- 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.
- Kirsh, G. E., and T. Narahashi. 1978. 3, 4 Diaminopyridine. A potent new potassium channel blocker. *Biophys. J.* 22:507–512.
- Maxwell, J. C. 1865. A dynamical theory of the electromagnetic field. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 155:459–512.
- Ross, W. N., B. M. Salzberg, L. B. Cohen, A. Grinvald, H. V. Davila, A. S. Waggoner, and C.-H. Wang. 1977. Changes in absorption, fluorescence, dichroism, and birefringence in stained giant axon: optical measurement of membrane potential. *J. Membr. Biol.* 33:141–183.
- Salzberg, B. M. 1983. Optical recording of electrical activity in neurons using molecular probes. *Curr. Methods Cell. Neurobiol.* In press.

- Salzberg, B. M., F. Bezanilla, and H. V. Davila. 1980. An optical determination of the resistance in series with the axolemma of *Loligo pealei*. *Biol. Bull.* 159:491. (Abstr.)
- Salzberg, B. M., F. Bezanilla, and H. V. Davila. 1981. An optical determination of the series resistance in giant axons of *Loligo pealei*. *Biophys. J.* 33:90a. (Abstr.)
- Taylor, R. E. 1965. Impedance of the squid axon membrane. *J. Cell. Comp. Physiol.* 66(Suppl. II):21–25.
- Taylor, R. E., J. W. Moore, and K. S. Cole. 1960. Analysis of certain errors in squid axon voltage clamp measurements. *Biophys. J.* 1:161–202.