WHITE NOISE MEASUREMENT OF SQUID AXON MEMBRANE IMPEDANCE

Rita Guttman and Lance Feldman

Department of Biology, Brooklyn College of the City University of New York, Brooklyn, New York and Marine Biological Laboratory, Woods Hole, Massachusetts.

Received September 12,1975

Summary

The use of white noise techniques for system identification is illustrated by the following characterization of the subthreshold membrane impedance of the squid giant axon, space-clamped in a double sucrose gap. Power spectra were also computed. Depolarization increases the resonance, shifts the resonant frequently upward and decreases the membrane's inductive reactance. Reduced external Ca^{++} increases the resonance, shifts the resonant frequency downward and increases the inductive reactance.

Engineers have found the input-output relationship or transfer function to be very useful. The transfer function of a system can be readily found when pseudo-random white noise is applied to the system, as all frequencies are thereby simultaneously applied: Recently this method has been used to analyze biological systems, Halpern and Alpert (1), Marmarelis and Naka (2,3), and Fishman (4,5).

White noise measurements permit a rapid and accurate determination of the impulse response without the undesirable effects upon the biological preparation of actual impulse testing. It is preferable to bridge measurements because of the great speed implicit in applying a waveform with uniform probability of power at all frequencies and because it circumvents the problem of electrode polarization common to bridge measurements. From the impulse response it is possible to obtain the impedance function by the application of the appropriate Fourier transform.

In the present work, the cross-correlation of white noise current input and membrane voltage response is used to characterize the subthreshold membrane impedance, in terms of magnitude and phase functions, of the squid giant axon, space-clamped by means of a double sucrose gap. Cole's pioneering efforts, Cole (6), in measuring membrane impedance have demonstrated that impedance measurements are a significant and important way in which to characterize the state of the living membrane.

Methods

The method of mounting the axon in the chamber, the input and recording circuits and the analysis circuits are described in a previous paper, Guttman, Feldman and Lecar (7). The input consisted of fifteen second applications of subthreshold white noise current with a bandwidth of 7 Hz to 10 Khz. Spectral analysis of the data was carried out using a Saicor Model 52 spectrum analyzer. Correlation functions were calculated using a Saicor Model 43A correlation function and probability analyzer. Impedance functions were produced with a Hewlett Packard 5451B Fourier analyzer system.

Specifically the data were analyzed as follows. The recorded data were played back and processed by a low pass filter, in order to eliminate aliasing error. The data were then digitized and multiplied by a special weighting function (called the Hanning function). This was done in order to reduce spurious frequency components introduced into the Fourier transform and power spectra, by taking a sample of finite length. These spurious frequencies are seen as side lobes in the power spectra. Following this, a discrete fast Fourier transform was calculated. Then the auto and cross power spectra were computed as was the cross-correlation function. Finally the coherence and transfer functions were found. For our data the transfer function reduces to the impedance function. The calculated functions were then variously displayed and recorded by means of an X-Y plotter, oscilloscope and a digital memory driven color television monitor.

Results

In Figs. 1, 2 and 3, the effects of a) polarization and b) variation of Ca concentration in the outer environment are illustrated.

Fig. 1 shows the effect of polarization on the membrane. In all cases subthreshold inputs were used. In Fig. 1a, the power spectra are displayed. It is seen that the resonance increases

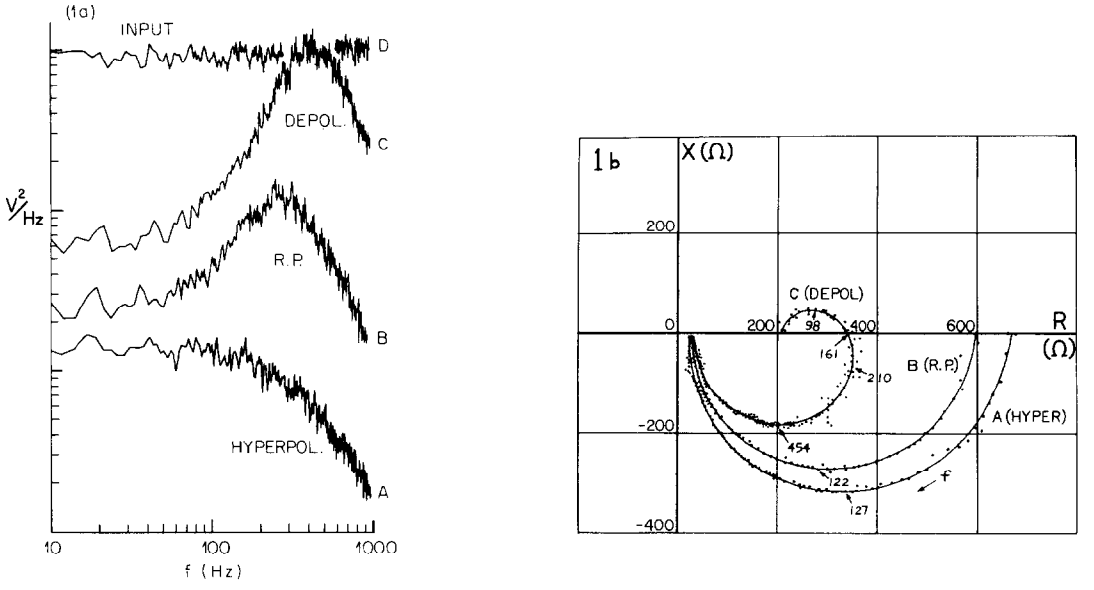

Fig. 1.
a) Power spectra of squid axon membrane voltage response to white noise current input for various membrane polarizations. A- hyperpolarized by 20 mv; B- resting potential; C- depolarized by 20 mv; D- white noise input. In these and the following power spectra, the traces have been slightly displaced vertically for clarity and the power units are arbitrary.
b) Complex plane impedance function (Z(f)) of the squid axon membrane for various membrane polarizations. A-hyperpolarized by 20 mv; B- resting potential; C- depolarized by 20 mv. Frequencies of interest are indicated on the impedance function by arrows.
Membrane area for a) and b) equals 1.08 mm^2; temperature is 22° C.

---

when the membrane is depolarized and decreases when the membrane is hyperpolarized. In addition there is an upward shift in the resonant frequency when the membrane is depolarized. This implies that there is a decrease in the membrane's inductive reactance with depolarization. Similar effects of polarization were found previously when sinusoidal currents were applied, Guttman and Hachmeister (8). In Fig. 1b, it is seen from the complex impedance function that there is an increase in the size of the inductive lobe with depolarization. This implies a decrease in the damping coefficient, i.e. an increase in the sodium conductance.

In Figs. 2 and 3, the effects of external Ca^{++} concentration

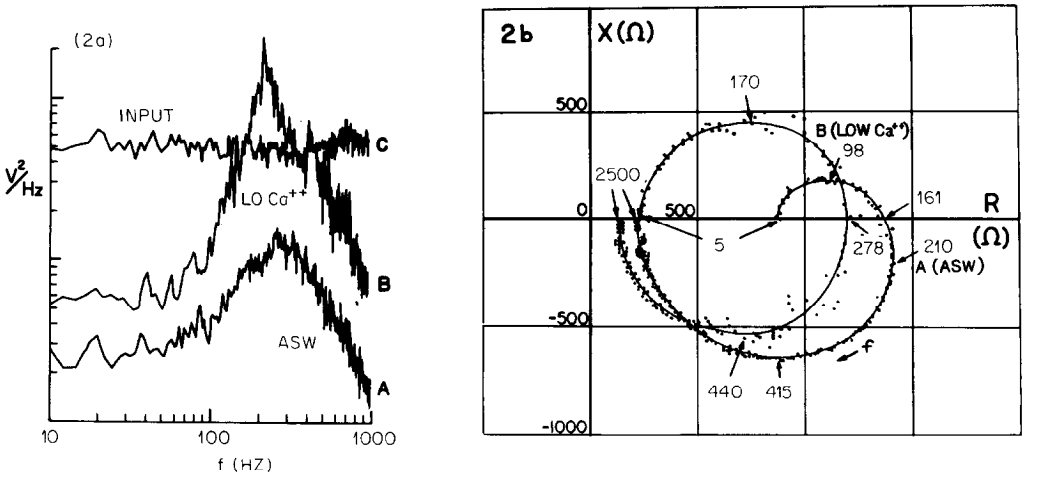

Fig. 2.
a) Power spectra of squid axon membrane voltage response to white noise current input for two levels of external Ca^{++} concentration. A- normal Ca^{++} concentration (9.27 mM); B- low Ca^{++} concentration (10^{-6} mM); C- white noise input.
b) Complex plane impedance function (Z(f)) of the squid axon membrane for two levels of external Ca^{++} concentration. A- normal Ca^{++} concentration (9.27 mM); B- low Ca^{++} concentration (10^{-6} mM).
Membrane area for a) and b) equals 0.96 mm^2; temperature is 22° C.

---

on the membrane are shown. In Fig. 2a, we see from the spectrum analysis that low Ca^{++} increases the sharpness of the tuning. Also the resonant frequency is shifted downward slightly. This implies an increase in the inductive reactance of the membrane with reduced external Ca^{++} concentration. This effect is also shown in Fig. 2b, where the inductive lobe of the impedance function is greatly increased when external Ca^{++} concentration is decreased. Neurophysiologists have long known that decreasing the Ca^{++} concentration will increase the oscillatoriness of the axon membrane and cause repetitive firing. High Ca^{++} produces no such effect and indeed seems to eliminate the inductive reactance (Fig. 3). The use of noise measurements, then, confirms the results of Cole (6) which have shown that the inductive reactance of the membrane can account for its oscillatory nature and the phenomenom of repetitive firing.

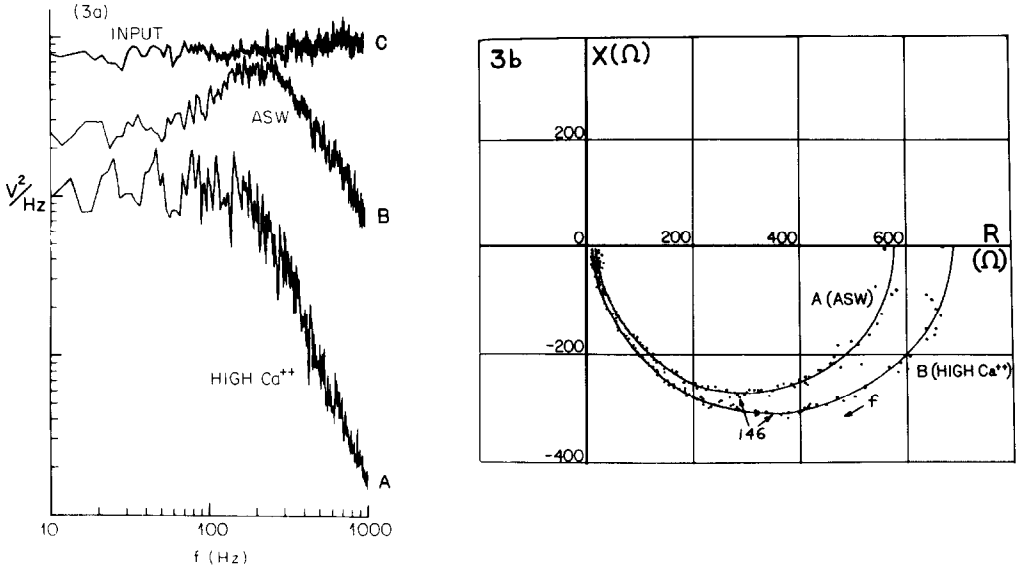

Fig. 3.
a) Power spectra of squid axon membrane voltage response to white noise current input for two levels of external Ca^{++} concentration. A- high Ca^{++} concentration (100 mM); B- normal Ca^{++} concentration (9.27 mM); C- white noise input.

b) Complex plane impedance function (Z(f)) of the squid axon membrane for two levels of external Ca^{++} concentration. A- normal Ca^{++} concentration (9.27 mM); B- high Ca^{++} concentration (100 mM). Membrane area for a) and b) equals 1.02 mm^2; temperature is 20° C.

It is obvious that these impedance loci are at least approximations to the pedal curves of ellipses produced by the biquadratic impedances of circuits with two reactances, Cole (6), which are in turn approximations to the Hodgkin-Huxley (HH) (9) equations for the squid axon membrane. However, convenient methods for locating frequencies on the complex impedance locus or for deriving equivalent circuits for comparison with the HH equations have not been developed. One complication is that the dielectric loss characteristic of the plasma membrane, Curtis and Cole (10), and often confirmed, is not obvious on the loci presented. It seems probable that a more complete representation of the impedance data, such as by the Bode plots of log $|Z|$ and ϕ vs. log frequency, may

be necessary for detailed presentation and analysis.

In conclusion, noise measurements yield a wide variety of significant information about the living membrane (i.e. power spectra, impulse response and impedance function), without substantially disturbing its physiological condition. This is especially important in view of the direct relationship between the membrane's electrical impedance and its ion permeability.

Acknowledgements

We wish to thank Dr. K.S. Cole and Dr. H.M. Fishman for valuable discussions and suggestions. This work has been aided by NSF grant 28257 and NIH grant 1R01NS12272 awarded to RG. In addition, some of the equipment for analysis was made available through Dr. Fishman from his NIH grant NS-11764.

References

1. Halpern, W. and Alpert, N.R. (1971) J. Appl. Physiol., 31, 913-925.
2. Marmarelis, P.Z. and Naka, K-I. (1972) Science (Wash., D.C.), 175, 1276-1278.
3. Marmarelis, P.Z. and Naka, K-I. (1974) I.E.E.E. B.M.E., 21:2, 88-101.
4. Fishman, H.M. (1975) Fed. Proc., 34, 1330-1337.
5. Fishman, H.M. Rapid complex impedance measurements of squid axor membrane via input-output cross-correlation function. Chapter in proceedings of the Conference on Testing and Identification of Nonlinear Systems; California Institute of Technology, in press.
6. Cole, K.S. (1968, 1972) Membranes, Ions and Impulses, University of California Press, Berkeley.
7. Guttman, R., Feldman, L. and Lecar, H. (1974) Biophys. J., 14, 941-955.
8. Guttman, R. and Hachmeister, L. (1971) J. Gen. Physiol., 58, 304-321.
9. Hodgkin, A.L. and Huxley, A.F. (1952) J. Physiol., 116, 500-544.
10. Curtis, H.J. and Cole, K.S. (1938) J. Gen. Physiol., 21, 757-765.