*NERVE MEMBRANE CURRENT NOISE: DIRECT MEASUREMENTS UNDER VOLTAGE CLAMP**

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Abstract.—Fluctuations in the steady-state current of the membrane of the lobster giant axon have been measured under voltage-clamp conditions. These fluctuations have a power density spectrum that is inversely proportional to frequency. The magnitude of the fluctuations is determined largely by the potassium component of the membrane current.

Precise measurements of the excitable properties of nerve membranes have suggested that the processes involved in the generation of the action potential include stochastic components. It is known that myelinated and unmyelinated axons respond to identical electrical stimuli of threshold amplitude in a random fashion and fail to consistently generate action potentials. When action potentials occur, their latency varies.[1] Similarly, random components are reported in the pattern of the spontaneous discharges of neurons which appear to be free from synaptic input, such as the pacemaker cells of *Aplysia*.[2] Interest in these fluctuations has been focused mainly on their relevance to the coding and processing of neural information. Accordingly, numerous models of the input-output characteristics of isolated neurons include a stochastic element.[3] Less attention has been given, thus far, to the information that such fluctuation phenomena might provide about the physical-chemical membrane processes from which they presumably arise.[4]

Because of their small magnitude, electrical fluctuations have only recently been directly observed in the nerve membrane: Derksen and Verveen, using feedback isolation[5] and cross-correlation techniques,[6] have successfully detected random components in the resting potential of a frog node of Ranvier under current-clamp. It was found that the spectral density of these fluctuations was inversely related to frequency, over a frequency range extending from a few Hz to a few kHz. Their data suggested that the fluctuations were associated with the passive movement of potassium. More recently, additional fluctuating components that appear to be related to the sodium system have been reported for the same preparation.[7]

This report summarizes the results of experiments in which fluctuations have been observed in the form of a current noise recorded under voltage-clamp conditions. It has thus been possible to obtain additional evidence for the presence of fluctuations in another neural membrane and to further examine their relationship to known membrane processes.[8]

Measurements were made on the giant axon of *Homarus americanus*, using the sucrose-gap technique.[9] This preparation (diameter 80–130 μ) is particularly suitable: present technology, with amplifiers adapted for a range of source impedance (100 $k\Omega$ to 1 mΩ) typical of an artificial node simulated on the

lobster axon can yield significantly better noise figures than is possible with higher impedance preparations such as the node of Ranvier. Two independent voltage-clamp systems were used. The mean membrane current, I_m, and the random deviations, ΔI_m, in amperes, A, were recorded for a constant or linearly swept (rate 0–10 mV/sec) membrane potential, V_m, through a system which was optimized for low-noise performance (spot noise figure: 0.2 db at 5 kHz and 1 db at 50 Hz) which will be described elsewhere.[10] The nerve chamber was designed to minimize the lengths of axoplasm extending under the sucrose gaps and their corresponding components of Johnson noise. Thus, fluctuations of membrane current could be directly observed in "quasi steady-state" without resorting to cross-correlation techniques. The quantities ΔI_m, I_m and V_m were recorded on FM magnetic tape. The spectral density of ΔI_m was obtained off-line by using a set of second-order active band-pass filters ($Q = 10$) whose squared outputs were averaged over appropriate periods of time. The second voltage-clamp system was available to obtain step-clamp data. The preparation could be rapidly connected to either one of these two systems. The current fluctuations and the usual voltage-clamp data could then be compared on each node. The temperature of the preparation ranged between 6 and 9°C in these experiments.

A family of power density spectra $S(f)$ (dimensions: A^2/sec) corresponding to different values of V_m and obtained on a typical node (approximate area, 15,000 μ^2) bathed in a solution of artificial sea water (ASW: 465 mM KCl, 4 mM $MgCl_2$, 4 mM $MgSO_4$, 25 mM $CaCl_2$) is shown in Figure 1.[11] These data, displayed on log-log coordinates, can be represented by an expression of the form $S(f) = Nf^{-a}$ where a is close to unity, and N is independent of frequency, f. It is noteworthy that the $1/f$ character of $S(f)$ remains unaffected by changes in the potential of the clamped membrane as seen in Figure 1, where V_m ranges between -50 and $+50$ mV. Straight-line fits to 250 spectra measured on 22 nodes in

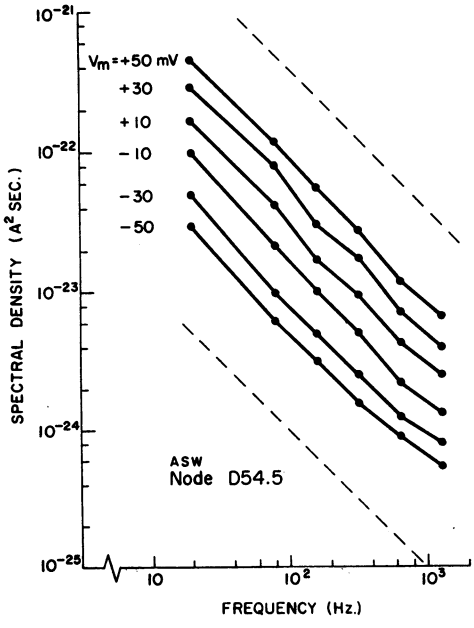

FIG. 1.—Power density spectra of the fluctuations from the mean membrane current of the lobster axon for different values V_m of the clamped membrane potential. Dashed lines have slope -1.

artificial water have yielded values of a ranging from 0.8 to 1.3, with a mean value of 1.002, and a standard deviation of 0.076. Indeed, no significant correlation between a and V_m was evident among these spectra, for which V_m was varied from -100 mV to $+100$ mV to cover the full physiological range. Consequently, the fluctuations of membrane current can be characterized by the single parameter, N.

The relation between N and membrane variables was systematically investigated. The membrane current recorded under step-clamp conditions was separated into its peak early (sodium) I_{Na}, steady-state (potassium) I_K, and unspecific (leakage) I_L components in the well-known manner.[12] In particular, plots of N and of I_K as a function of V_m were found to exhibit qualitative similarities shown in Figure 2a for two typical sets of data recorded from the same node in artificial sea water. N is nearly constant with a value of 3.4×10^{-22} A^2 for V_m lying between -100 and -60 mv, then begins to increase gradually as V_m is further increased, reaching 2×10^{-20} A^2 at $V_m = 60$ mV. It is difficult to ascertain precisely the values of I_K for V_m less than approximately -40 mV because of the relatively large contribution of I_L to the total membrane current. It is noteworthy, however, that N begins to increase in the same range of V_m where an appreciable value of I_K can first be detected, and that both N and I_K increase monotonically beyond that range.

More precisely, N is not constant between -100 and -60 mV. Plots of N vs. V_m frequently show a broad minimum, not visible on the scale used in Figure 2a, at a potential, $V_{m\,min}$, of approximately -80 mV. Thus N may be expressed in the form $N = A + B(I_K)$, where A is some constant and $B(0) = 0$. The data of Figure 2a are replotted as shown in Figure 2b. On these log-log coordinates, the data can be fit by an expression of the form $B = kI_K^m$, where k and m are

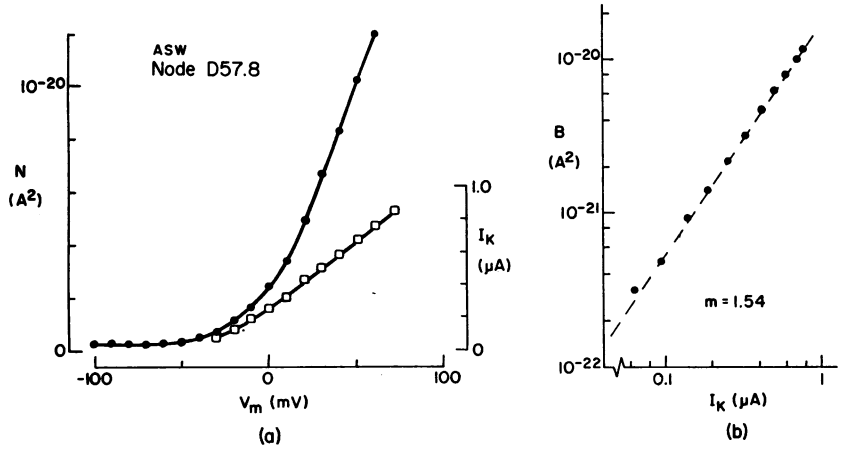

FIG. 2.—(a) Closed circles: intensity of the fluctuation N as a function of the potential V_m of the clamped membrane. Open squares: mean net potassium current I_K as a function of V_m, derived from step-clamp measurements. These two sets of data were obtained on the same preparation within approximately 1 min. (b) The quantity B in the expression $N = A + B(I_K)$ for the data shown in (a). Dotted line, with slope $m = 1.54$, corresponds to a straight-line fit to the empirical power-law relationship $B = kI_K^m$.

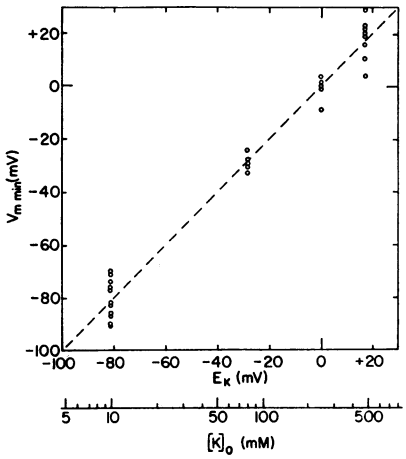

FIG. 3.—Scattergram of $V_{m\min}$ versus the external potassium concentration, $[K]_o$, and the corresponding equilibrium potential E_K. Data gathered on 18 nodes simulated on 7 axons.

constants. In this case, a value of m of approximately 1.5 is appropriate. Data obtained on 17 nodes in artificial sea water have yielded similar power-law expressions, with values of m ranging between 1.1 and 2.1 (mean 1.5).

On the basis of the available measurements of the internal concentration of potassium in the lobster axon,[13] it seems reasonable to equate $V_{m\,\min}$ with E_K, the Nernst equilibrium potential of the potassium ion, and hence to the value of V_m for which the mean net potassium current I_K goes through zero. The results of other experiments, which will be described in full elsewhere,[10] were also consistent with the conclusion that the intensity of the fluctuations of membrane current depends on the mean net potassium current. For instance, it was found that the value of $V_{m\,\min}$ could be systematically modified by changes in the external concentration $[K]_o$ of potassium. Figure 3 shows data collected on 18 nodes simulated on 7 axons, with $[K]_o$ of 10, 81, 244, and 478 mM (KCl substituted for NaCl). In this scattergram, $V_{m\,\min}$ is plotted against $[K]_o$ and the corresponding E_K. It is apparent that over this wide range of $[K]_o$, a minimum intensity of the fluctuation occurs for values of V_m where I_K is zero.

These measurements are consistent with the observations of Derksen and Verveen and do not support the hypothesis, commonly used in model studies of the excitability of the neural membrane, of the existence of fluctuations whose spectral density is both uniform and independent of membrane variables. It is possible, however, that the observed fluctuations of excitability reflect fluctuations associated with the sodium system, rather that those related to the potassium system. This point has not been examined in this study, since the sodium system was essentially inactivated throughout the present noise measurements.

Thus far, models of diffusion processes have dealt with experimental data corresponding to the average transport of ions across nerve membrane (for example, mean unidirectional flow of radioactive tracers, mean net flow of electric charges). The data obtained in the present experiments and those of Derksen and Verveen place significant additional constraints upon models by characterizing the second order statistical behavior which they should also exhibit.

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[1] Pècher, C., *Compt. Rend. Soc. Biol*, **124**, 839 (1937). Verveen, A. A., *Fluctuations in Excitability* (Netherlands Institute for Brain Research, 1961). Verveen, A. A., *Acta Physiol. Pharmacol. Neerl.*, **11**, 268, (1962). Poussart, D., *Quarterly Progress Report. No. 81, Research Laboratory of Electronics* (Cambridge: Massachusetts Institute of Technology, 1966), p. 213.

[2] Junge, D., and G. P. Moore, *Biophys. J.*, **6**, 411 (1966).

[3] Moore, G. P., D. H. Perkel, and J. P. Segundo, *Ann. Rev. Physiol.*, **28**, 493 (1966).

[4] Cole, K. S., *J. Gen. Physiol.*, **51**, 1 (1968).

[5] Frankenhaeuser, B., *J. Physiol.*, **137**, 245 (1957).

[6] Derksen, H. E., *Acta Physiol. Pharmacol. Neerl.*, **13**, 373 (1965). Verveen, A. A., and H. E. Derksen, *Kybernetick*, **2**, 152 (1965). Derksen, H. E., and A. A. Verveen, *Science*, **151**, 1388 (1966).

[7] Verveen, A. A., H. E. Derksen, and K. L. Schick, *Nature*, **216**, 588 (1967). Verveen, A. A., and H. E. Derksen, *Proc. IEEE*, **56**, 906 (1968).

[8] Poussart, D., Ph.D. thesis, *"Current-noise in the Nerve Membrane: Measurements under Voltage-Clamp,"* Massachusetts Institute of Technology (1968).

[9] Julian, F. J., J. W. Moore, and D. E. Goldman, *J. Gen. Physiol.*, **45**, 1195 (1962).

[10] Poussart, D., manuscript in preparation.

[11] Measurements of $S(f)$ were not extended below 10 Hz because of the difficulties of maintaining such a preparation in "steady state" over the correspondingly longer required integration periods. Above 2–5 kHz, the measurements were contaminated by an artifact component which could be assigned[8] to the Johnson noise of the axoplasm extending under the sucrose gaps.

[12] Blaustein, M. P., and D. E. Goldman, *J. Gen. Physiol.*, **49**, 1043 (1966).

[13] Brinley, F. J., *J. Neurophysiol.*, **28**, 742 (1965).