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Membrane Shot-Noise in Electrically Depolarized Nodes of Ranvier[*][**]

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Summary. The power spectra of the spontaneous voltage fluctuations (membrane noise) of the node of Ranvier were measured in the frequency range from 0.3 to 1500 cycles per second at different levels of the membrane potential (-90 to $+30$ mV, inside negative). Up from about -30 mV the power spectrum shows a $\frac{1}{1+(2\pi f\tau)^2}$ component, which increases with depolarization. This shot like noise component is independent of and occurs in addition to the $1/f$ component. The source of this shot like noise component is probably given by fluctuations in the conductance for potassium ions. With the use of a minimum parameter model which consists of channels that switch randomly in time from the closed to the open state and vice versa, independent of each other, the number of active channels per μm^2 appears to be of the order of 1000. The elementary unit of the potassium system conductance is then of the order of 10^{-11} S per channel[1] and the mean frequency of switches per second per channels is about 160.

Key words: Membrane Voltage Noise — Shot Noise — Potassium Conductance — Minimum Parameter Model — Elementary Unit of Conductance.

Upon repeated stimulation of a nerve fibre its responses show fluctuations in excitability and latency [1,5,22]. To search for the processes that underlie these phenomena Verveen and Derksen started direct measurements of membrane voltage noise in 1964 [23].

Since that time three components have been detected in the electrical noise of neural membranes [cf. 24]. They are mentioned here with reference to the power frequency density spectrum $S(f)$—abbreviated to *power spectrum*—of the noise voltage, i.e. the average power per unit of bandwidth (in V^2s) as a function of frequency f.

The first component is frequency independent (the so-called white noise, $S(f) \simeq f^0$) and can be measured at the high frequency end of the power spectrum. For the second component the power spectrum varies

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[1] The S.I. unit for conductance is the Siemens, abbreviated to S, formerly Ω^{-1} or mho.

inversely with frequency (one over f noise: $S(f) \simeq f^{-1}$). Its amplitude distribution is gaussian, which is also the case for the white component. For the third component $S(f) \simeq f^{-2}$ (one over f squared noise). Its amplitude distribution is positively skewed.

For the $1/f$ component of membrane noise a relationship with the passive, i.e. non-metabolic flux of potassium ions through the membrane has been found (Derksen, 1965 and Derksen and Verveen, 1965 for the frog node of Ranvier; Poussart, 1969 for the crayfish giant axon; Fishman, 1972, for the giant axon of the squid and recently by Anderson and Stevens [personal communication] and by DeFelice and Adair [3] for frog muscle). Its intensity c_1 in $S(f) = c_1 f^{-1}$ is proportional to the mean power dissipated in the membrane by the current through it [4,20]. The $1/f^2$ component is visible as irregularly occurring depolarizing bursts of noise; sometimes in the resting state and, usually, upon hyperpolarization of the node of Ranvier [25]. This component was found to be related to the non-metabolic flux of sodium ions through the membrane.

The existence of a fourth component in the voltage noise of neural membrane was predicted by Stevens (1969, personal communication; [21]) and, recently, by Hill and Chen [11]. This component should show a power spectrum similar to shot noise [cf. 19] $S(f) \simeq \{1 + (2\pi f\tau)^2\}^{-1}$ which is white for frequencies $f \ll \frac{1}{\tau}$. This component was proposed to result from fluctuations of the potassium channel conductance. It should then become measurable upon depolarization of the membrane. In this paper the existence of such a component is reported.

Methods

Isolated nerve fibers from the sciatic nerve of the brown frog *Rana temporaria* were used. The membrane voltage noise of a single Ranvier node was measured at room temperature with the use of a three-terminal arrangement [5,25].

A length of nerve fibre containing three nodes was mounted in a tray with five pools. The middle node lay in the central pool which was filled with Ringer solution. Each of the other nodes lay in one of the outer pools filled with isotonic KCl. The second and fourth pools contained Ringer solution and were in contact with each of the two internodes. These pools were used for electronic feedback compensation of the external fluid shunts between adjacent nodes ([5], after Frankenhaeuser, [9]). The potential difference between each pair of adjacent nodes was amplified separately. A current clamp could be applied via one of the outer nodes to change the membrane potential of the central node.

The outputs of the amplifiers were recorded on tape (Ampex FR 1300) and were cross-correlated on-line with a Hewlett Packard 3721 A correlator. With this procedure the autocorrelation function of the voltage fluctuations of the central node was obtained. This function was displayed on a scope and processed by a computer (IBM 1800) to calculate the power spectrum. The power spectrum was on-line displayed on a memory scope (Tektronix 601). It was also recorded on film (both the graph and the numerical data). Each time two decades of frequency were measured for the power spectrum. When necessary a larger frequency range was determined with the use of overlapping power spectra.

Results

Power spectra were measured at the resting membrane potential and for different levels of depolarization. A set of spectra is shown in Fig. 1. For each spectrum the intensity per unit of bandwidth (V^2s) is plotted against the frequency for a range of two decades (from 10 to 1000 s^{-1}) on double-logarithmic paper. In such a plot a f^{α} spectrum has a slope of α.

When the node is in the resting state or slightly depolarized the spectra are of the $1/f$ type and its changes in intensity are in accordance with results reported before [5,23]. Upon stronger depolarization a change in shape of the spectrum becomes visible at about -30 mV membrane potential (inside negative). This effect becomes more pronounced with further depolarization. For the lower range of frequencies within this range the slope α changes from -1 ($1/f$ noise) to about zero (white noise). For the higher frequencies the slope changes from -1 into -2 ($1/f^2$ noise). This implies the occurrence of a noise component of the shot type $S(f) = c\,\{1 + (2\pi f\tau)^2\}^{-1}$ upon depolarization. In this expression c denotes the intensity of this component. The time constant τ determines the change of the spectrum into the $1/f^2$ part at the higher

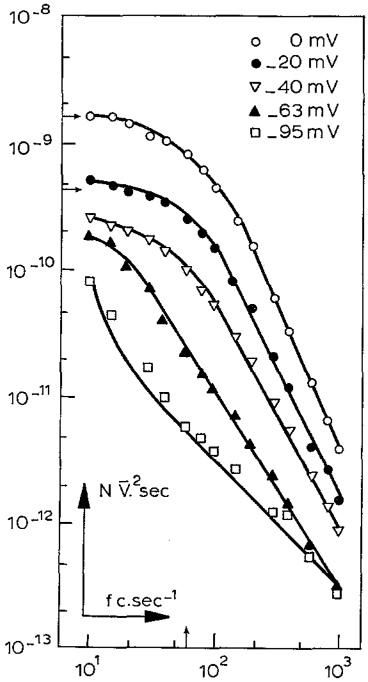

Fig. 1. Voltage noise spectra of a node of Ranvier between 10—1000 c/sec at different levels of membrane potential (as indicated)

frequencies. τ can be read from the half-power cutoff frequency f_0 since $f_0 = (2\pi\tau)^{-1}$.

Similar results were obtained for all 14 nodes of Ranvier investigated. The intensity c of this shot noise component (measured in V^2s, and indicated with horizontal arrows in the Figures) increases with further depolarization, which was measured for membrane potentials up to + 30 mV. The cutoff frequency f_0 (indicated with vertical arrows in these Figures) apparently does not change with membrane potential. The time-constant τ might, therefore, be independent of the membrane potential. Its value was found to be about 3 ms. In Fig. 2a set of power spectra is presented for a larger range of frequencies, from 0.3 to 1500 s^{-1}. At frequencies below about 2 s^{-1} the spectra are, again, of the $1/f$ type since the slope approaches -1 and above this frequency the slope first approaches 0 and than goes down to -2. For the power spectra measured at membrane potentials of -10, $+10$ and $+30$ mV the spectra indicate that the shot noise component is an additive component to the $1/f$ noise. For these spectra curves were calculated to fit the measured power-

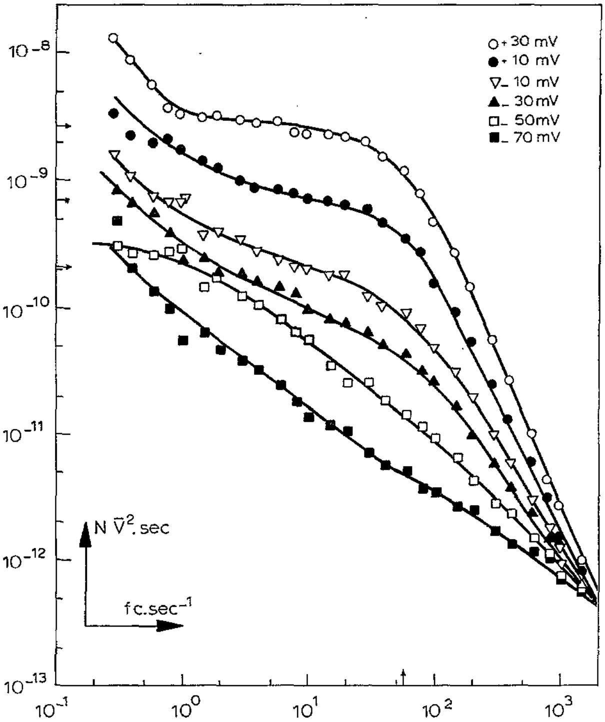

Fig. 2. Voltage noise spectra of a node of Ranvier between 0.3—1500 c/sec at different levels of membrane potential (as indicated)

spectra under the hypothesis that the measured spectra consisted of the sum of the $1/f$ noise and the shot noise components, i.e. $S(f) = c_1/f + c_2/\{1 + (2\pi f\tau)^2\}$. The white noise component was not taken into consideration since its intensity, compared to the other sources, is negligible [24].

The fits are reasonable, as shown, and the estimates for c_2 (the shot noise intensity) and f_0 are indicated with the arrows.

Discussion

In recent papers Stevens [21] and Hill and Chen [11] showed that fluctuations in the conductances for ions through membranes should be measurable by the presence of a spectrum of the shot noise type when the fluctuations in the conductance for each site in the membrane occur independently of each other and at random. They derived these results on the basis of the overall conductances for sodium and/or potassium ions as expressed in the Hodgkin-Huxley equations. For the potassium system this noise spectrum should then become present upon depolarization of the membrane [21]. Since this is found it is attractive to assume that our measured voltage noise spectra reflect these fluctuations in the potassium conductance as predicted. To test this hypothesis further experiments with TEA and with changes in the temperature of the node are also necessary. For squid axon membrane a similar component has been found to be present and to be associated with potassium current flow [8]. It is easy to see, however, that voltage fluctuations of the observed nature can be expected under constant current conditions when fluctuations δg in the membrane conductance g exist.

To consider the voltage fluctuations resulting from the passage of a constant current I through a fluctuating conductance, write $V = I/g$, where V is the mean voltage generated as I flows through the conductor with a mean conductance g. Then

$$\delta V = -\frac{I}{g^2}\,\delta g + \frac{1}{g}\,\delta I$$

and if $\delta I = 0$ (constant current), then

$$< \delta V^2 > = \frac{I^2}{g^4} < \delta g^2 > = \frac{V^2}{g^2} < \delta g^2 > .$$

If we assume that this source of noise is uncorrelated with the other components of membrane noise—notably $1/f$ and white noise—then we may simply add this conductance fluctuation term to the others. This result implies that the shape of the voltage spectrum is not changed by the change of the shot noise component from conductance to voltage, since the RC value of the membrane is between 0.03 and 0.1 ms [2] which gives a half-power cutoff frequency between 1300 and 5000 s^{-1} well outside the range for these phenomena.

The assumption that the noise processes are uncorrelated has been tested in a model experiment for $1/f$ noise and shot noise.

Fig. 3 shows the result of spectral measurements for the sum of these two noise components, one case for uncorrelated components and the other for 100% correlation. In the latter case both components were derived from the same white noise source with the use of adequate filters. When these spectra are compared with those obtained for neural membrane (Fig. 1) it then follows that our assumption for neural membrane holds true, since the spectra for the neural membrane have the shape which follows from the addition of two uncorrelated components. The component spectra were calculated: In Fig. 2 the intensity c of the shot noise component is indicated by the horizontal arrows.

Although measurements with a voltage clamp are necessary to compare our results on τ with those following from the Hodgkin-Huxley equation, its size (about 3 ms) and its independence of membrane potential may be at variance with data obtained by Dodge (1963) in voltage clamp experiments. He found smaller values for τ_m of the sodium conductance (between 0.05 and 0.1 ms) and for τ_n of the potassium conductance (between 0.5 and 3 msec at room temperature), and a decrease of τ_n to about 30% of its original value upon depolarization to + 30 mV. The difference may, perhaps, be due to the circumstance that voltage

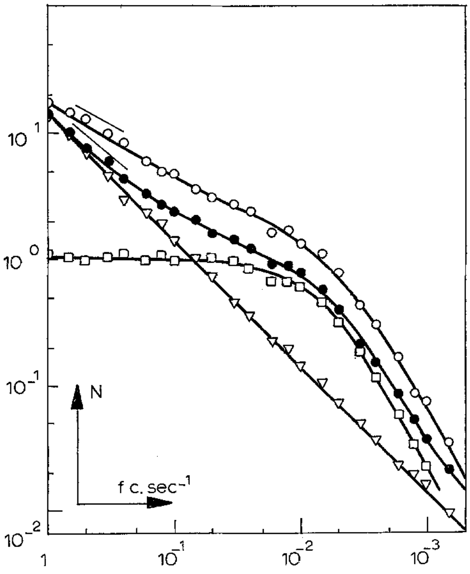

Fig. 3. Artificial voltage noise spectra, with intensity N in arbitrary units. Triangles: $1/f$ noise, squares: shot noise, filled circles: uncorrelated combination of $1/f$ and shot noise, open circles: correlated combination

clamp measurements are fast jumps from one steady state to another in a non-linear system while our measurements are long-term and for small deviations from a steady state for which the system behaves linearly. To clarify this point voltage clamp experiments on our nodes are planned.

Random Switch Model for Channel Conductance

A preliminary inference can be made from the data presented in Fig. 1 and 2 for a simple model. No *a priori* adjustment to the Hodgkin-Huxley equations need be made. Since many different models on that basis have been published [11, 21] among which it is as yet not possible to distinguish on the basis of experimental results, it is useful to examine the properties of the channels themselves with the use of a minimum-parameter model, which we will call *the random switch.*

The shot noise component is assumed to result from a two-state channel system for a total number of n active channels. Each channel switches randomly in time between the open state (with conductance δg) and the closed state (with zero conductance) and independent of the switches of every other channel. The average number of switches per channel per second is k. Since for this model each channel is in the open state on the average for half the time, the average conductance for all n channels is, therefore, under this assumption $\frac{n}{2}\,\delta g$. The conductance spectrum $S_g(f)$ and the conductance autocorrelation function $c_g(\tau)$ can be derived from the equations for the so-called random telegraph signal after Lee [15]:

$$S_g(f) = \frac{n\,\delta g^2}{2\lambda} \cdot \frac{1}{\lambda^{-2} + (2\pi f)^2} + 2\pi\left(\frac{n\,\delta g}{2}\right)^2 \delta(f)$$

and

$$c_g(\tau) = \frac{n\,\delta g^2}{4}\, e^{-|\tau|/\lambda} + \left(\frac{n\,\delta g}{2}\right)^2$$

with $\lambda = \frac{1}{2k}$.

The voltage spectrum $S(f)$ and the voltage autocorrelation function $c(\tau)$ can be derived with the use of the before mentioned equation $< \delta V^2 > = \frac{V^2}{g^2} < \delta g^2 >$, i.e.

$$S(f) = \frac{V^2}{g^2}\, S_g(f) \text{ and } c(\tau) = \frac{V^2}{g^2}\, c_g(\tau)\,,$$

in which V is the voltage difference between the depolarised state (with constant current I) and the resting membrane potential. Since $g = \frac{n\,\delta g}{2}$ substitution gives

$$S(f) = \frac{2V^2\lambda}{n} \cdot \frac{1}{1 + (2\pi f\lambda)^2} + 2\pi V^2\,\delta(f)$$

and

$$c(\tau) = \frac{V^2}{n}\, e^{-|\tau|/\lambda} + V^2\,.$$

Table 1. Data for three nodes of Ranvier

											Units
Resting potential	— 40				— 60			— 55			mV
Membrane potential	— 20	— 10	0	+ 10	— 40	— 20	— 5	— 40	— 20	0	mV
Shot noise intensity for low f: $S(o)$	2.7	6.5	13.6	25.5	3.2	11.2	23.8	1.7	3.7	10.2	$10^{-10} V^2 s$
$\sigma^2 = S(o)/2\lambda$	4.6	10.7	23.5	42.5	5.1	18.7	39.1	2.9	6.3	17	$10^{-8} V^2$
$\mu^2 = V^2$	4	9	16	25	4	16	30.3	2.3	12.3	30.3	$10^{-4} V^2$
$n = \mu^2/\sigma^2$	0.9	0.8	0.7	0.6	0.7	0.8	0.7	0.7	2.0	1.8	10^4
n per[a] μm^2	0.6	0.6	0.5	0.4	0.5	0.5	0.4	0.5	1.3	1.2	10^3
g_K	7	8	9	10	8	11	12	8	10	17	$10^{-8} S$
$\delta g = g_K \Big/ \frac{n}{2}$	1.5	1.9	2.6	3.4	2.4	2.7	3.7	1.9	1.0	1.9	$10^{-11} S$

[a] With the assumption of a nodal surface of 15 μm^2.

The model is not extended to different average opening and closing times τ_0 and τ_c $\left(\text{which gives } \frac{1}{\lambda} = \frac{1}{\tau_o} + \frac{1}{\tau_c}\right)$, since this requires an additional measurement which cannot be made as yet.

For 14 nodes, each investigated for 3 or more levels of depolarization a time constant λ was found of about 3 msec. From the model presented it follows that these channels each change their state with a mean rate k of about 160 switches per second. Under the mentioned assumptions the number of channels in the node can then be estimated from the variance σ^2 and the mean squared voltage V^2 of this process. The variance $\sigma^2 = c(o) - c(\infty) = \frac{V^2}{n} = \frac{1}{2\lambda} S(o)$ in which $S(o)$ is the intensity of the noise spectrum for frequencies $f \ll \frac{1}{2\lambda}$, while $n = \frac{V^2}{\sigma^2}$.

The single channel conductance δg then follows from the measured conductance g for this system after division by $\frac{n}{2}$. For three nodes n was calculated for at least three different levels of depolarization between -40 and $+10$ mV (Table 1). No clear relationship between the size of n and the level of depolarization was found within this range. The number of channels for this system is then about 10^4. For nodal surfaces of about 15 μm^2 this amounts to between 500 and 1000 channels per μm^2 under the given assumptions. This number is about one order of magnitude larger than that given for the sodium system (cf. Keynes [13]) which under these assumptions is also an argument in favour of the potassium system. The shot noise is due to the potassium system, since the sodium system is inactivated at these membrane potentials and because TEA reduces this component (unpublished observations). This allows the calculation of the unitary channel conductance δg from the g_k (which was also measured for these nodes), under the assumption that the channels each are open half the time. After division of g_k by $\frac{n}{2}$, δg is then found to be of the order of 10^{-11} S. Both the larger number of channels per μm^2 and the smaller conductance with respect to the sodium system are in accordance with the expectations expressed in the literature (cf. Keynes [13]).

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