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MEMBRANE NOISE AND ION TRANSPORT IN THE NODE OF RANVIER

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Fluctuations in the excitability of nerve fibers were discovered and studied between the years 1932 and 1939 (Blair and Erlanger, 1932, 1935-36a and 1935-36b; Pecher, 1936, 1937 and 1939). After these initial studies little attention was paid to this phenomenon for a long time. Since 1958 they have again been studied by Verveen (Verveen 1960, 1961 and 1962, Verveen and Derksen 1965), and later, by DeBecker (1964) and by Poussart (1966).

It appeared not to be possible to understand the phenomenon from what was known about the nerve membrane at that time. Model studies suggested that spontaneous voltage fluctuations of the membrane (membrane voltage noise) could be a cause. In 1962 experiments were started with the aim to detect, to measure and to analyse membrane voltage noise. The studies were made on isolated nodes of Ranvier from the sciatic nerve of the green frog (Rana esculenta) or the brown frog (Rana temporaria). The very first experiments showed membrane voltage noise to be present (Verveen and Derksen, 1965). Its intensity was larger than expected on the basis of thermally generated fluctuations across the nerve membrane (so called Nyquist or Johnson noise) and the noise spectra, measured at the resting membrane potential, showed an intensity inversely proportional to frequency (so called 1/f noise, cf. fig. 1), while Nyquist noise should have a constant (or 'white') power spectrum. Similar noise spectra were later found in nerve fiber preparations from the lobster Homarus (Poussart, 1969) and for the giant axon

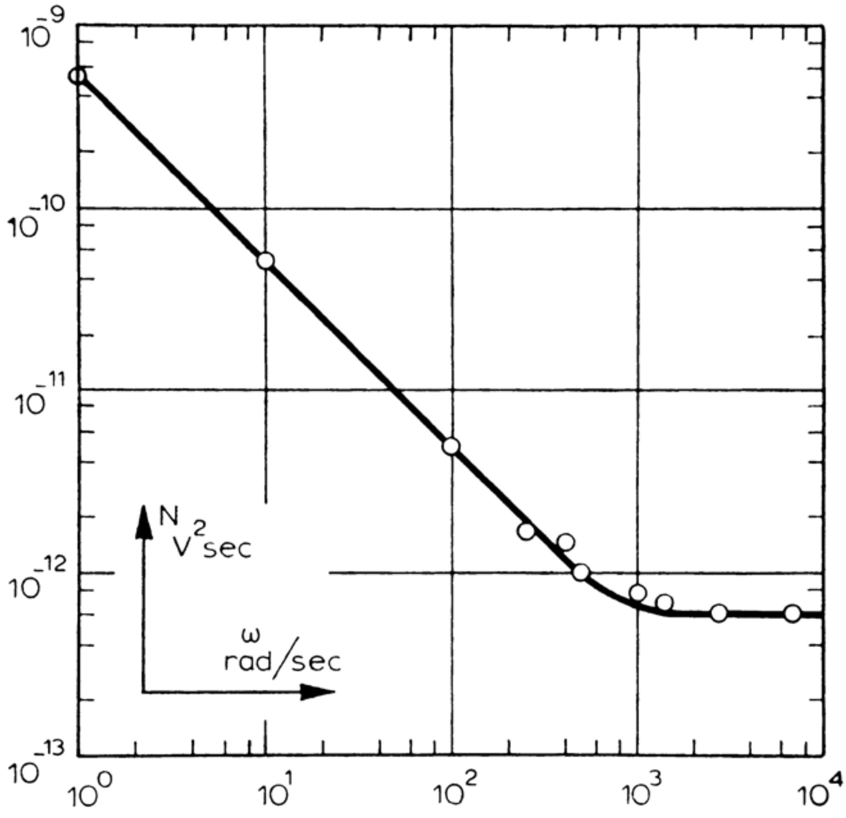

Figure 1. Noise power spectrum at resting membrane potential (from Derksen, Acta Physiol. Pharmacol. Neerl., 1965, 13, 373).

of the squid loligo (Fishman).

Subsequent experiments were carried out to investigate possible relationships between membrane 1/f noise and ion transport. Neither the interference with active ion transport nor that with passive sodium ion or chloride ion transport influenced this type of membrane noise. Interference with passive potassium ion transport did change the intensity of 1/f noise, both when the membrane was set on another level of membrane potential or when the outside potassium ion concentration was changed (Derksen, 1965; Derksen and Verveen, 1966). The intensity was minimal for the potassium equilibrium potential (Verveen and Derksen, 1969). It was concluded that the membrane 1/f noise is related to the nonmetabolic flux of potassium ions through the membrane. Since tetra-ethylammonium (TEA) ions block the flux of potassium ions through the membrane, then treatment with TEA should reduce the 1/f noise intensity. This prediction was confirmed in later experiments (Verveen, et al., 1967; Siebenga and Verveen, 1970).

At the high frequency end of the power spectrum the 1/f noise changes into white noise (fig. 1). The intensity of this component is larger than the intensity that can be calculated from the measured resistance of the membrane with the use of Nyquist's formula, $\overline{e^2} = 4\ kTR$ Volt^2 per cycle of bandwidth. The cause of this phenomenon is still obscure (Derksen, 1965).

At the lower frequency end of the spectrum a change from 1/f noise into $1/f^2$ noise was found in some experiments. Further experiments showed that this change is due to another noise component: the irregular occurrence of smaller and larger depolarizing deflections of the membrane potential. The amplitude of the smallest deflections is of the order of 1 mV and the duration is about 1 msec, and these 'miniature depolarizing potentials' (Derksen, 1965; Verveen et al., 1967) are believed to be unitary phenomena, which build up the larger irregular depolarizing fluctuations shown in fig. 2. This type of noise, which was called "burst" noise, is sometimes present at the resting membrane potential, but in other cases the bursts occur when the membrane is hyperpolarized. These bursts were found to be related to the non-metabolic and probably batch-wise influx of sodium ions through the membrane, with a batch size of a few thousand ions per membrane site (Verveen et al., 1967, Verveen and Derksen, 1969). Tetrodotoxin, which blocks the sodium current during the action potential and in voltage clamp experiments, was expected to block the bursts also, but it did not do so. Why this does not happen is still an unsolved problem (Siebenga and Verveen, 1970).

The amplitude distribution of the noise at the resting membrane potential is Gaussian, in the absence of bursts. When burst noise is present the amplitude distribution is positively skewed, which is due to the asymmetry of the bursts. When the membrane is strongly depolarized the amplitude distributions become slightly negatively skewed from about zero mV membrane potential upwards (fig. 3).

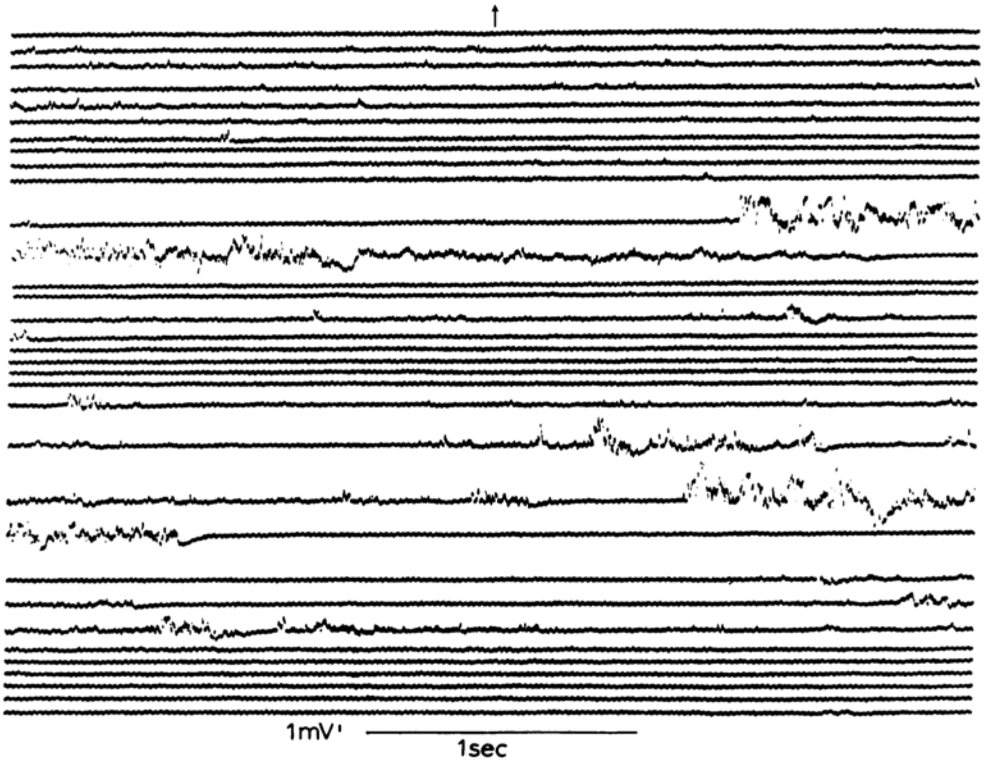

Figure 2. Membrane noise at a membrane potential of -90 mV (2-10.000 c/sec) (from Verveen and Derksen, Acta Physiol. Pharmacol. Neerl., 1969, 15-353).

The mechanism, which causes this negative skewness, is still unknown (Verveen and Derksen, 1969).

In the meantime Poussart started his investigations on the spontaneous current fluctuations of the membrane of the giant axon of the lobster. He measured the current fluctuations under voltage clamp conditions. For all membrane potentials a 1/f power spectrum was found, for which the intensity is dependent on membrane potential. In his experiments burst activity was not noticed, which may be due to the relatively large membrane area (250 $\mu^{2)}$ from which the noise was measured, as compared with the area of the node of Ranvier (15 μ^2).

For the intensity of the 1/f noise Poussart found the following experimental relation $N = A + B\ (\overline{I_K})$.

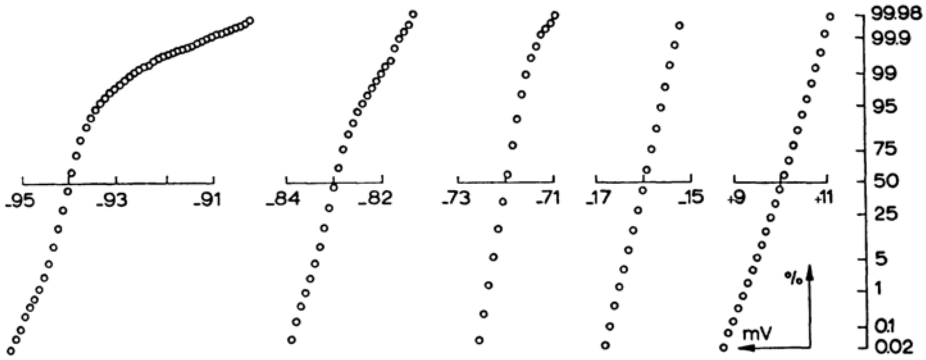

Figure 3. Gaussian plot of the cumulative distributions of membrane noise for different membrane potentials; ordinate: percentage of total number of samples (from Verveen and Derksen, Acta Physiol. Pharmacol. Neerl., 1969, 15, 353).

N is the noise intensity, A is a constant and B ($\overline{I}_K$) is a function of the potassium current $\overline{I}_K$ that can be expressed as B($\overline{I}_K$) = $h\overline{I}_K^m$, with $1.1 < m < 2.1$ and with a constant h (Poussart 1969 and 1971). This result is in agreement with our measurements on the node of Ranvier. At the potassium equilibrium potential $\overline{I}_K = 0$, and the 1/f noise intensity is minimal, but it is still present. This is possibly due to other ions, such as chloride. If this is true, then we can write $A = h' \, I_{Cl}^m$, but with $h' \ll h$. It is, therefore, nearly impossible to separate A from B and to find a relation between A and the transport of a particular ion through the membrane. This is like the excess noise in KCl filled microelectrodes, where at the equilibrium potentials of both potassium and chloride excess noise is still present. This is due to the fact that the excess is the sum of two components, one caused by the potassium ion flux and the other by the chloride ion flux through the tip of the electrode (DeFelice and Firth, 1971).

Recently the noise power spectra for the node of Ranvier were measured at membrane potentials up to + 40 mV (inside positive). At depolarizations from -20 mV membrane potential into the range of positive membrane potentials another noise component appears on top of the 1/f noise, a $1/f^2$ kind of noise with a power spectrum $S = c / (1 + (2\pi f)^2 \tau^2)$, where c is

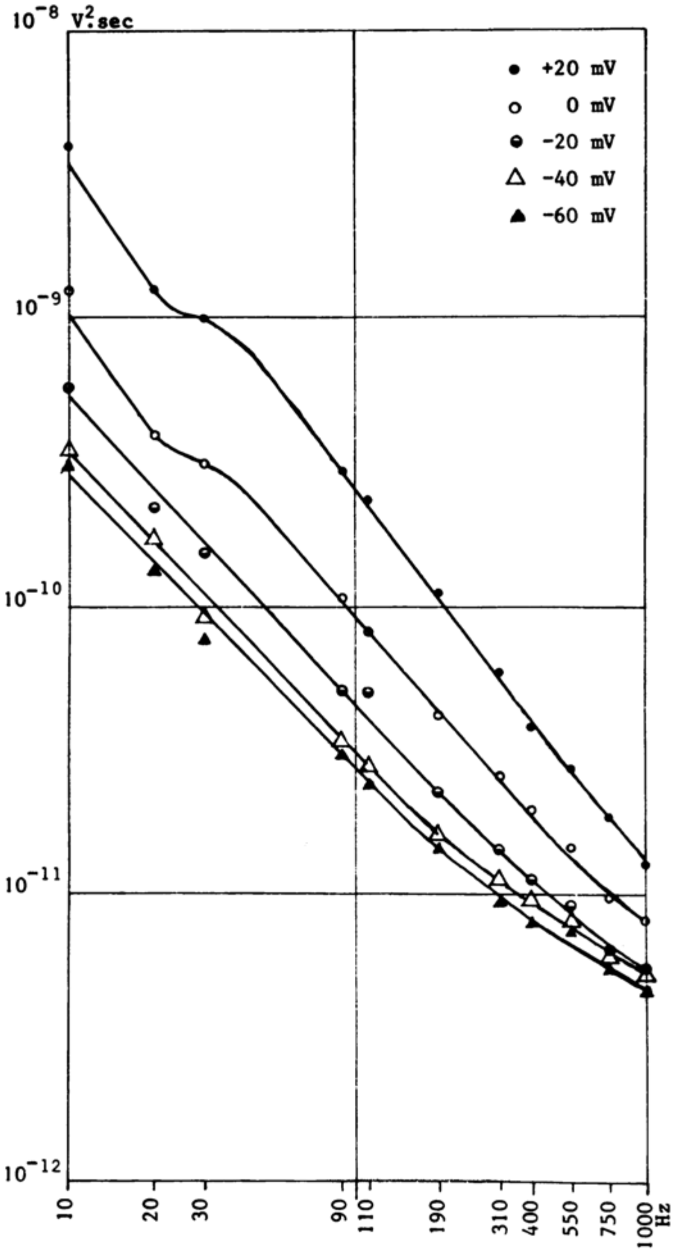

Figure 4. Noise intensity in power/cycle of bandwidth plotted against frequency (log-log plot) for different levels of membrane potential, indicated in the graph, at a temperature of 17°C (from the Proceedings of the First European Biophysics Congress, Vol. V, p. 221, Verlag der Wiener Medizinischen Akademie, A-1090 Vienna, Alserstrasse 4, 1971).

the intensity and $1/\tau$ the cut¯off frequency. The time constant τ was found to depend on membrane potential (fig. 4) (Siebenga and Verveen 1971).

The theoretical power spectrum for potassium conductance fluctuations can be calculated from the Hodgkin-Huxley equations (Stevens). This predicted power spectrum has a $1/(1 + (2\pi f)^2 \tau_n^2)$ character, where τ_n^2 is the time constant of the potassium conductance in the H.H.-equations. Since τ_n depends on membrane potential and temperature (Cole, 1968) our next step was to investigate the influence of temperature on this $1/f^2$ kind of noise. From these experiments a decrease of the cut-off frequency and, therefore, an increase of τ was found with a decrease of the temperature.

Our experiments, therefore, confirmed qualitatively the existence of the predicted fluctuations in the potassium conductance. There is, however, a difference between τ and τ_n : τ is about five times larger than τ_n. This may be due to the difference in the measurements of τ and of τ_n. A measurement of τ from membrane noise is comparable to measurements with very small steps in membrane potential, while voltage clamp experiments use very large steps in membrane potential. When the object behaves nonlinearly, like the axon membrane, differences between these measurements may be expected.

To summarize the results obtained so far, a single equation for the power spectrum of axonal membrane voltage noise can be written down. This equation describes the power spectrum preliminarily as a sum of at least four components:

$$S = a + \frac{b}{F} + \frac{c}{1 + (2\pi f)^2} + \frac{d}{f^2}$$

a is the intensity of the white noise component, measurable at the high frequency end.

b is the intensity of the $1/f$ noise component and depends on the flux of potassium ions through the membrane. As yet no acceptable theory exist for the generation of $1/f$ noise. The search is for such a theory since we can then expect that this component gives us information about the mechanism of the movement of potassium ions (and some other ions) through the membrane.

c is the intensity of a component that is possibly caused by potassium conductance fluctuations. In that case this

component should give us more information on details of the potassium conductance mechanism.
d is the intensity of the burst noise. Further study of this burst noise might give us more information about the sodium conductance mechanisms in the membrane.

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