

Conductance Fluctuations in Ranvier Nodes*

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Summary. Voltage fluctuations associated with the sodium system were measured upon elimination of the potassium current in the nodal membrane by internal application of cesium-ions. The intensity of this noise reaches a maximal value at a membrane potential in the vicinity of -40 mV. Here the power spectrum consists of two additive components: a $1/f$ component and a Lorentzian. The Lorentzian is associated with h-gate kinetics and is consistent with the binary state conduction model. On the basis of this model the sodium-channel conductance is calculated to be 2 to $5 \cdot 10^{12}$ S. The analysis is complicated by the existence of an incomplete slow sodium inactivation process.

Key words: Ranvier Node – Membrane Noise – Channel Conductance.

Siebenga *et al.* [9] presented evidence that relates part of the spontaneous voltage fluctuations of a depolarized Ranvier node to potassium currents. The sodium system was excluded since the power spectrum of the fluctuations did not change after addition of tetrodotoxin nor after changes in the external sodium concentration.

Internal application of both cesium- and tetraethylammoniumions blocks the potassium system [4, 5]. This procedure eliminates the noise of this source. The remaining noise has been investigated. The results show the existence of conductance fluctuations of the sodium system in a steady state depolarized Ranvier node and are described in this communication.

Methods

Motor fibres of the sciatic nerve of the brown frog *Rana temporaria* were used. Details of the experimental set-up and of the data-analyses were described before [9]. The central node was superfused in Ringer's solution, followed by either choline-Ringer or TTX-Ringer ($3 \cdot 10^{-7}$ M) at 20°C. In order to block the potassium system, the outside nodes (with internodal distances of about 1200 μ m) were cut in a solution of 100 mM CsCl and 20 mM TEA-Cl. A complete block of the potassium system was established after about 35 min. The noise voltage was recorded at

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different membrane potentials, each for a period of 30 s. Each track was started half a minute after the change to a new membrane potential.

Since variance *vs.* membrane potential curves are a convenient way to monitor the amount of blocking of spectral components (the variance is the integrated power spectrum over the frequencies investigated), these curves were measured during a continuous change of membrane potential at a rate of 2 mV per second and followed by detailed spectral measurements.

The variance of the noise voltage was measured in a bandwidth of 20 to 10^3 s^{-1} with a true rms-measuring device (2425 Brüel-Kjaer electronic voltmeter).

Results

A. Variance of the Noise Voltage

In nodes of Ranvier, with normal inside potassium concentration, the variance of the noise voltage is a monotonously increasing function of membrane potential upon depolarization from the resting potential (cf. [8]).

Intra-axonal Cs^+ and TEA^+ change this behaviour in the following way

1. The noise variance decreases during the diffusion of these ions. In the stationary state the noise variance *vs.* membrane potential curve is bell shaped (Fig. 1). The maximum of this curve lies at $38 \pm 3 \text{ mV}$ (6 nodes). Its value is small compared to the variance of axons with normal potassium concentration at the same potential (about 12%).

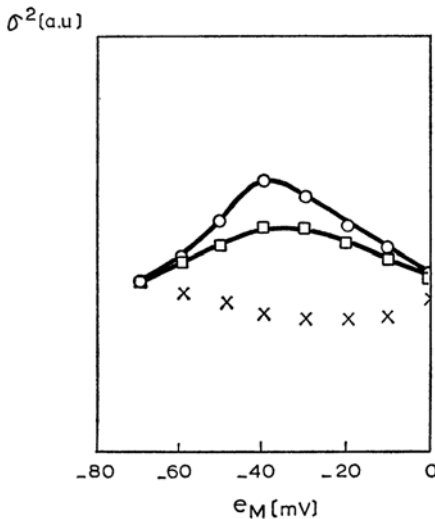


Fig. 1. Noise variance σ^2 in arbitrary units plotted linearly *vs.* membrane potential e_m (bandwidth $20 - 10^3 \text{ s}^{-1}$). (o) σ^2 , e_m changed 2 mV/s, ringer; (□) σ^2 , at mean e_m for 30 s (steady state), ringer; (x) σ^2 , at mean e_m for 30 s, ringer + TTX

2. After addition of TTX or replacement of sodium chloride by choline chloride the bell shape disappears. This indicates that this part is related to the sodium system.

It is to be noted that the magnitude of the noise variance in the steady state (30 s after change in membrane potential) is about 0.7 times smaller than the variance measured when the membrane potential is changed at a rate of 2 mV/s. This phenomenon is reversible and is absent when the sodium current is made zero by TTX.

B. Power Spectra

Cs^+ - TEA^+ treated nodes with the largest signal-to-noise ratio were selected for spectral analysis under steady-state conditions. The power spectra were measured within the frequency range $10 - 10^3 \text{ s}^{-1}$ at the

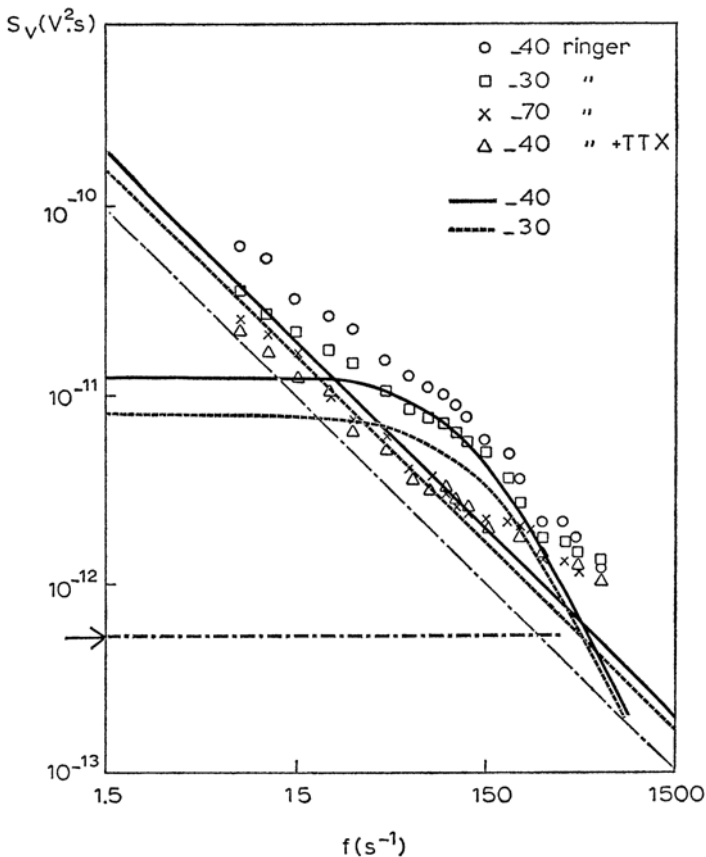


Fig.2. Powerspectra of voltage fluctuations of a node of Ranvier with internal Cs^+ and TEA^+ . Lines represent the spectral components, arrow indicates thermal noise level

following membrane potentials: -70 , -50 , -40 , -30 and -20 mV. At all these membrane potentials a $1/f$ component is present. Its intensity depends on membrane potential, with a peak in the vicinity of -40 mV.

At -40 and -30 mV (in the bell-shaped part) an additional Lorentzian component $\rightarrow S(f) = \frac{S(+o)}{1 + (2\pi f\tau)^2}$ was measured (Fig.2). Its zero frequency intensity $S(+o)$ also has a maximum in the neighbourhood of -40 mV. It decreases with a factor of about 2 at -30 mV and it drowns in the $1/f$ noise at other depolarizing potentials.

Upon addition of TTX and or after replacement of sodium by choline the Lorentzian component disappears, while the $1/f$ component is also reduced. A residual $1/f$ component remains, with unclear voltage dependence.

Discussion

The possibility to specifically block two of the three ionic currents (I_{Na} , I_K , I_L) in the nodal membrane and consequently to eliminate conductance noise from these sources, makes it possible to identify the following excess noises within the frequency range of about $1 \cdot 10^3$ s $^{-1}$.

For the potassium system a C_K/f and a $\frac{S(+o)_K}{1 + (2\pi f\tau)^2}$ component (cf. [9]), for the sodium system a C_{Na}/f and a $\frac{S(+o)_{Na}}{1 + (2\pi f\tau_n)^2}$ component and for the leakage system a C_L/f component¹.

A. The Potassium System

In the presence of internal Cs^+ and TEA^+ the steady-state $I-V$ -curve is a straight line over the whole range of membrane potentials investigated (from -80 up to $+30$ mV). A potassium system current, if present, must be less than the accuracy of our measurement (10^{-10} A). Within this range the strong increase of both the $1/f$ noise and the Lorentzian upon further depolarization has disappeared and no residual Lorentzian is detected upon strong depolarization [9].

It follows that the strong membrane voltage dependent $1/f$ and Lorentzian noise components reported in [9] are generated by the potassium system and that this system is completely blocked by Cs^+ + TEA^+ . With regard to the experiments with TTX (Fig.2) this conclusion applies to the whole range of membrane potentials investigated.

It is of considerable interest to note that the binary state conduction model, which follows from the simplest statistical interpretation

¹ It is of interest that upon hyperpolarization burst noise is still present when both the potassium and the sodium system are blocked (cf. [11], p. 243).

of the Hodgkin-Huxley equations [2,10], is in conflict with the experimentally measured behaviour of the Lorentzian component of the potassium system, since the measured corner frequency is not detectably dependent on membrane potential and since its low frequency intensity $S(+0)_K$ increases monotonously with depolarization. The graded conduction model of Stevens [10], based upon another statistical interpretation of the H-H-equations, also predicts a membrane potential dependent corner frequency.

B. The Sodium System

Measurable sodium system noise is present within the range of membrane potentials around -40 mV. From the application of the H-H-equation for the sodium system by Hille (standard node, 2) it follows that the steady state sodium current reaches its maximal value within this range. The size of the current, $60-70$ pA, is below the accuracy of our measurements. Nevertheless the presence of measurable sodium noise makes it possible to study the properties of the intact sodium system.

The binary state conduction model predicts seven Lorentzians associated with the sodium system (in case of m^3h cf. 10, pp. 240 - 242). With the standard node parameters [3] it can be shown that the intensities of the six Lorentzians associated with the m -process are small with respect to the Lorentzian related to the h -process. The corner frequency of the measured Lorentzian is membrane potential dependent and its value lies within 30 % of the standard $1/(2\pi\tau_h)$. Its intensity in the currentclamp is given by

$$S_h(f) = 2 N \left\{ \gamma \left(1 - \frac{E}{183} \right) \right\}^2 m_\infty^6 h_\infty \cdot (1 - h_\infty) \tau_h (E - E_{Na})^2 |Z|^2 \frac{1}{1 + (2\pi f \tau_h)^2} \quad [V^2 \cdot s].$$

Here N is the number of sodium channels per node, γ is the sodium channel conductance at $E = 0$ mV [4] and Z is the impedance of the membrane. With the same standard node parameters $S_h(f)$ has been calculated. The results of these calculations are given in Table 1, together with the experimental data.

The values for γ and N calculated with the use of Hille's value for $N\gamma$ at $E = 0$ mV are given in Table 2.

The number of channels for this system is then about 2 to $5 \cdot 10^5$. This amounts to about 1 to $3 \cdot 10^4$ channels per μm^2 . The single sodium channel conductance is found to be of the order of 1 to $3 \cdot 10^{-12}$ S. The

Table 1

Membrane potential	- 50	- 40	- 30	- 20	Units mV
Cut-off frequency					
Model	62	109	185	498	s^{-1}
Experiment 1	— ^a	104 ± 6	135 ± 10	—	s^{-1}
Experiment 2	—	113 ± 10	150 ± 10	—	s^{-1}
Low frequency intensity					
Model	1.9	8.5	7.3	1.2	$\bullet 10^6 N \gamma^2 \bullet V^2 \bullet s$
Experiment 1	—	1.2	0.7	—	$\bullet 10^{-11} \bullet V^2 \bullet s$
Experiment 2	—	2.0	1.4	—	$\bullet 10^{-11} \bullet V^2 \bullet s$

a Not measurable.

Table 2

Membrane potential		- 40	- 30	Units mV
γ	Experiment 1	1.9	2.0	$10^{-12} S$
γ	Experiment 2	3.1	2.5	$10^{-12} S$
N	Experiment 1	38	50	$\bullet 10^4$
N	Experiment 2	24	30	$\bullet 10^4$
$N/\mu m^2$ a	Experiment 1	3	4	$\bullet 10^4$
	Experiment 2	2	2	$\bullet 10^4$

* With the assumption of a nodal membrane surface $15 \mu m^2$. $N\gamma = g_{Na,o} = 0.75 \bullet 10^{-6} S$.

values for the single channel conductance obtained in these analyses are probably too small for the following reasons:

1. Rundown of the sodium system with a rate constant of $1.3 \bullet 10^{-4} s^{-1}$ [5] leads to a peak sodium current loss of 20% after 30 min.

2. A slow process (Results A; 2), probably an additional inactivation process with a time constant of the order of 10 sec. A rough estimate of this effect can be obtained by assuming that no appreciable slow inactivation has taken place during the initial period of the variance measurement. This gives a correction factor of 1.4.

Corrected for the above mentioned effects the single channel conductance may become as high as 2 to $5 \bullet 10^{-12} S$ and the corresponding total number of channels per node 15 to $38 \bullet 10^4$.

It is to be noted that the value for γ obtained from noise measurements (under the mentioned assumptions) is of the same order of magnitude as the value estimated from gating current experiments [7].

From the value of $N\gamma^2$ found, the intensities of the other six Lorentzians predicted by the two-state model can now be calculated. For the whole range of membrane potentials these components drown in the other noises.

In a recently published paper Dubois and Bergman [1] point at the existence of a late sodium current, possibly generated by sodium channels devoid of inactivation gates.

These channels form a fraction of about 2% of the sodium channels in sensory fibres of the frog node of Ranvier. In motor fibres they report this component to be very small or absent. Although our measurements are restricted to motor fibres this possibility should be kept in mind, but cannot be evaluated as yet.

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