Voltage Fluctuations and Collective Effects in Ion-Channel Protein Ensembles

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We study experimentally the noise in ensembles of voltage-gated ion channels. The coupling between the microscopic state of the channels and the macroscopic voltage fluctuations leads to collective effects which (i) induce damped oscillations in the voltage noise and (ii) reduce the voltage noise in absolute terms, with a stronger reduction for larger numbers of participating channels. These results are confirmed by numerical simulations and explained in the context of a simple model that presents the ensemble of channels as a damped harmonic oscillator. [S0031-9007(96)01604-3]

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Voltage-gated ion-channel proteins are macromolecules responsible for generating electrical signals in nerve and other excitable cells. They work by selectively conducting ionic currents through the otherwise impermeable cellular membrane [1]. Generally the channels can switch between different conformational states, which are conducting (open $-O$) or nonconducting (closed $-C$), with voltage-dependent rates of transition. One consequence of the voltage-dependent transition rates is the intrinsic noise an ensemble of ion-channel proteins generates: The stochastic switching between the C-O states feeds back, through the voltage-dependent rates, on the transition process and may lead to nontrivial dynamics. It is our aim to understand the signature of the intrinsic noise generated by conduction fluctuations of voltage-gated channels. To gain insight into this problem, ensembles of *single-type* channels must be studied using the voltage as a free, dynamical variable (rather than the current [2]). Recent advances in molecular biology and electrophysiological techniques enable us to construct and study such ensembles of ion channels in a patch of membrane *isolated from intracellular and other biochemical effects*. In this Letter we use this approach to study experimentally, for the first time, the spontaneous transmembrane voltage fluctuations due to the microscopic channels' fluctuations, in voltage-gated ion-channel ensembles. We show that the transmembrane voltage introduces nontrivial correlations among the fluctuating channels.

A simplified physical picture of this system models the ensemble of ion channels as particles occupying an asymmetric double potential well, with a voltage-dependent barrier [1]. The potential wells represent the closed and open states of the channels and the source of noise is the thermally activated, spontaneous fluctuations of channels across the barrier. Renewed interest in transport phenomena, induced by nonequilibrium fluctuations [3], is partially inspired by biology (e.g., motor proteins [4]). Much of the recent focus is on cases in which the potential itself is fluctuating [5], where surprisingly rich dynamics is found in the noisy systems; stochastic resonance [6] and resonant activation [7] are two important examples. In these studies, however, the potential in which the particles reside is *externally* modulated. In contrast, voltage-gated ion channels give us a unique opportunity to study fluctuations in a potential that is modulated by *internal* events. Our main results are that the coupling between the states of the channels and the voltage, via the voltage-dependent rates of transition, reduces the voltage noise in absolute terms, with stronger effects for larger numbers of channels, and induces damped oscillations in the noise. Furthermore, we show that under the experimental conditions a two-state model of the channel is sufficient to explain the collective dynamics. This is not *a priori* obvious, as the channel proteins are believed to exhibit complex kinetics (reflecting many nonconducting states).

The experimental setup consists of a small patch of membrane ($\sim 2\ \mu m^2$) containing one type of voltage-gated potassium channel [8,9]. The patch of membrane is studied, in isolation, using standard patch-clamp techniques [10]. The voltage noise is measured under a constant external holding current (current clamp). The number of channels embedded in the patch of membrane and the amplitude of the external holding current are the two control parameters used in our experiments. Figure 1 shows that the voltage-gated channels generate considerable voltage noise due to their conduction fluctuations (trace b), compared with a membrane containing no channels (trace a). Both voltage traces were measured with zero holding currents. The arrow in trace c of Fig. 1 marks the point at which a small positive (depolarizing) current (2.6 pA) was applied to the membrane. This constant current forces a small increase in the number of open channels (~ 7 open channels on average) relative to that at zero current (~ 3 open channels on average) and leads to a reduction in the voltage fluctuations [11]. In both cases the number of open channels is small relative to the total number of channels in the ensemble (~ 100). All the experiments presented in this Letter are restricted to this regime.

The power spectral density (PSD) for the voltage fluctuations in the ensemble of channels drops as f^{-4} at high frequencies, and it is flat at low frequencies [Fig. 2(a)]. These characteristics are independent of the parameters.

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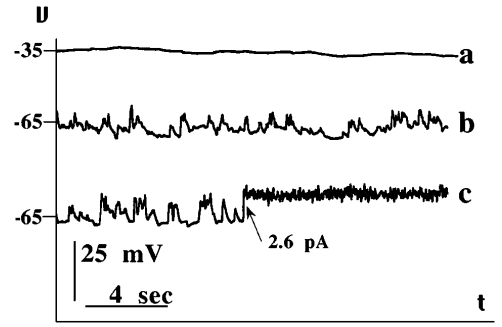

FIG. 1. Voltage traces for (*a*) a membrane with no voltage-gated channels; (*b*) a membrane with approximately 100 voltage-gated potassium channels, both at zero current; (*c*) a current of 2.6 pA applied at the point marked by the arrow to (*b*).

The crossover frequency depends on the number of channels, the capacitance of the system, and the holding current. The voltage fluctuations in a membrane with no voltage-gated channels exhibit a typical Lorentzian PSD (inset). For the same data, Fig. 2(b) summarizes the normalized voltage autocorrelation functions. The voltage in a membrane containing no channels exhibits decaying correlations on a time scale of a few hundred ms, irrespective of the applied current. The presence of voltage-gated ion channels introduces *decaying oscillations* on much faster time scales (of the order of 30 ms). A negative current closes the channels, destroying the fast decay of the autocorrelation and eliminating the oscillations. In this case the autocorrelation decays on a time scale of approximately 100 ms, which we take as the reference time scale for the numerical simulations below.

We performed Monte Carlo simulations of the two-state kinetics

$$C \underset{\beta(V)}{\overset{\alpha(V)}{\rightleftarrows}} O\,,$$

where the voltage-dependent rates α and β have been measured in separate experiments [12]. The transition probability $P = 1 - e^{-L\Delta t}$ (L is the transition rate) is computed at each time step (0.1 ms). A transition in the state of the channel is made if P is larger than a random number pulled from a uniform distribution. The transmembrane potential is updated at each step by the equation [1]

$$cdV/dt = -[g_k N_0(V - V_K) + g_L(V - V_L)] + I\,,$$

where c is the capacity; g_k is the single-channel conductivity; N_0 is the number of open channels updated according to the above kinetics; V_K is the potassium Nernst potential (-97 mV); g_L is the leak conductivity; V_L is the

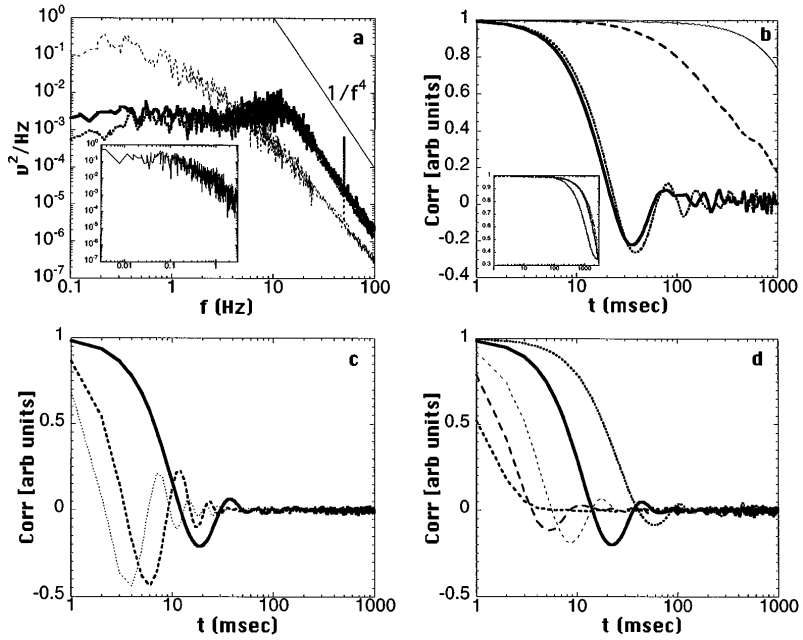

FIG. 2. (a) Power spectral densities (PSD) for the voltage noise measured for the same patch of membrane containing a few hundred channels: solid line — zero current, dotted — 2.6 pA, dashed — (−)2.1 pA (inset: a membrane containing no channels); (b) normalized voltage autocorrelation functions (averaged over 1 min), $\langle V_i V_{i+\varphi}\rangle = \sum_i (V_i - \overline{V})(V_{i+\varphi} - \overline{V})/\sum_i (V_i - \overline{V})^2$. V_i is the digitized voltage (1 ms resolution) at point i and $\overline{V}$ is the mean voltage, for the same experiment (thin solid — for a membrane with no voltage-gated channels at zero current, compared to a negative and positive holding currents in the inset). The same characteristic PSD and voltage autocorrelation were observed in at least 20 patches of membrane. The voltage autocorrelation from numerical simulations ($\tau_0 = 100$ ms and $\overline{g} = 0.5$) computed: (c) for 1000 channels: solid — zero current, dashed — 10, thin dashed — 30, in units of the leakage current at $V = -50$ mV; (d) for 100 channels at different τ_0 (in ms): dotted — 500, solid — 100, thin dashed — 10, dashed — 5, and short dashed — 1.5.

leakage reversal potential (~ -9 mV), and I is the holding current. The normalization parameters are $\tau_0 = c/g_L$ and $\overline{g} = g_k/g_L$. Figure 2(c) shows the normalized autocorrelation functions for 1000 channels at different holding currents. The main experimental features are reproduced by these simulations. The single channel's resident time (inverse sum of transition rates) has a sharp peak around 10 ms, of the order of the relaxation and oscillation times in the experiments.

The capacitance time τ_0 and the charging power of a single channel $\overline{g}$ determine the voltage response time. If, for example, τ_0 is significantly different from the resident time of the channels, the oscillations are eliminated [Fig. 2(d)]. This behavior demonstrates an interesting interplay between the time scales of barrier fluctuations and the channel's resident time. If the above time scales are well separated, the dynamics is merely relaxational. This behavior is reminiscent of other activation phenomena in modulated potentials [5–7].

Figure 3 summarizes the behavior of the voltage standard deviation as a function of the control parameters. Figure 3(a) shows that the voltage noise (absolute value) diminishes for larger positive holding currents (a decreasing function of the mean transmembrane voltage). The absolute noise is also a decreasing function of the total number of channels [Fig. 3(b)]. The behavior of the noise in the numerical simulations agrees fairly well with the experimental results [Figs. 3(c) and 3(d)]. Note that two independent parameters are needed to determine the voltage fluctuations. The average number of open channels is not enough; it is approximately constant in Fig. 3(b) [or 3(d)] but varies considerably in Fig. 3(a) [or 3(c)].

We model the system by a deterministic equation for the membrane potential and a Langevin-type equation for the number of open channels. In dimensionless form

$$d\nu/d\tau = -(\overline{N}p + 1)\nu + \overline{N}p\nu_k + g_I\,,$$
$$dp/d\tau = -\lambda p + \overline{\alpha} + \xi\,,$$

where $\nu = V/V_0$ ($V_0 = -50$ mV is a characteristic potential of the kinetics), $\tau = t/\tau_0$, $\overline{N} = \overline{g}N_t$, $\nu_k = V_k/V_0$, and $g_I = I/g_L V_0$. Here N_t is the total number of channels, p is the fraction of open channels, $\lambda = (\alpha + \beta)\tau_0$ (minimal at V_0), $\overline{\alpha} = \alpha\tau_0$ and ξ is the normalized noise per channel. We assume, for simplicity, that $V_L = 0$ and that ξ is a Gaussian white noise of independent channels.

Linearization is performed around the steady state: $\nu = \nu_s + \upsilon$ and $p = p_s + \rho$, yielding the following second order differential equation for the voltage fluctuations.

$$\frac{d^2\upsilon}{d\tau^2} = -\gamma\frac{d\upsilon}{d\tau} - \omega_0^2\upsilon + R\,,$$

where $\gamma = \lambda_s + \overline{N}p_s + 1$ and $\omega_0^2 = \lambda_s(\overline{N}p_s + 1) + (\overline{\alpha}_{,\nu} - \lambda_{,\nu}p_s)\overline{N}(\nu_s - \nu_k)$ are the damping coefficient

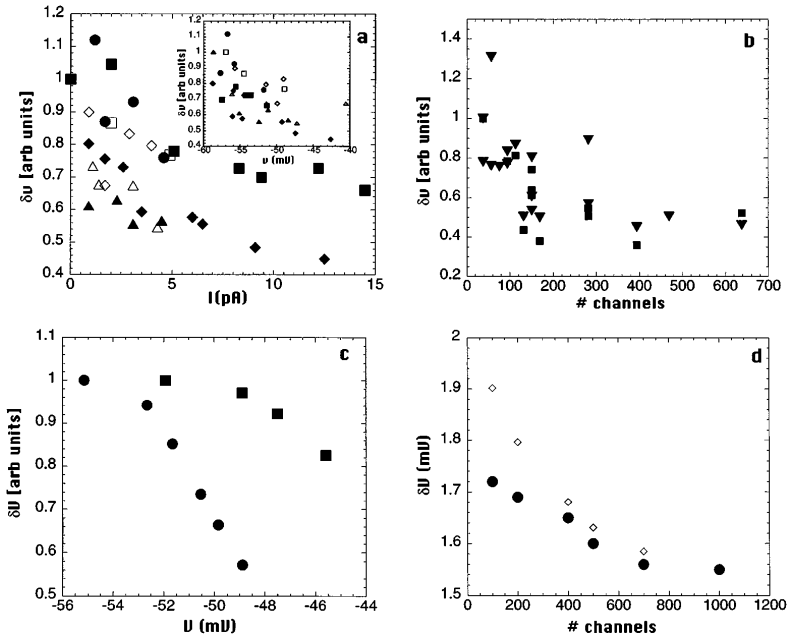

FIG. 3. The standard deviation of the transmembrane voltage. Experimental: (a) membranes with different total numbers of channels (filled squares — 640, empty squares — 282, filled circles — 150, filled triangles — 94, empty triangles, rhombus, and filled rhombus — 56), as a function of the holding current. The noise is normalized for each membrane by its value at zero current (inset: the same data versus the mean transmembrane voltage); (b) at zero current for different membranes with different total numbers of channels (triangles), and for the same experiments, the integrated noise (squares) in a limited bandwidth (0.1 — 7 Hz). Simulations: (c) for different holding currents as a function of the mean voltage for total number of 100 channels (squares) and 1000 channels (dots), (d) for different total numbers of channels. The empty symbols are the results of the linear model (see text), normalized at 1000 channels.

and the natural frequency of a damped harmonic oscillator, driven by a noise term $R = \xi\overline{N}(\overline{\nu}_k - \nu_s)$. Here $\overline{\alpha}_{,\nu}$ and $\lambda_{,\nu}$ are the derivatives of the kinetic parameters at the steady-state voltage ν_s, and λ_s is λ at the same voltage. The spectrum is given by

$$|(\nu_\omega)|^2 = \frac{|R_\omega|^2}{(\omega_0^2 - \omega^2)^2 + \gamma^2\omega^2},$$

where R_ω is the spectrum of the noise. The variance is $(\delta v)^2 \propto |R_\omega|^2/\gamma\omega_0^2$ [13], showing that the voltage noise is indeed a decreasing function of the total number of channels. In particular, it decreases as $1/\overline{N}$ for $\overline{N} \to \infty$.

The coupling between the state of the channels and the transmembrane voltage leads to a collective effect: A phase lag between the response of the channels, determined by their kinetics, and the dynamics of the voltage (determined by the capacitance time τ_0 which normalizes ω_0) is the physical cause of the relaxed oscillations. When the response times of the voltage and the channels are well separated, the oscillations are eliminated due to the loss of coupling. This phenomenon is reminiscent of a mechanism that leads to relaxation oscillations in other physical systems (e.g., spiking lasers [14]). The noise in the linear model decreases faster with the number of channels than the one from the numerical simulations [Fig. 3(d)]. In the experimental system, nonlinear effects are important.

In summary, within a limited range of parameters, channel fluctuations exhibit collective dynamics resulting in damped, oscillatory voltage noise. This result is general since the ion-channel protein used here is a typical representative of voltage-gated ion channels that work to relax excitable membranes.

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[1] B. Hille, *Ionic Channels of Excitable Membranes* (Sinauer Associates Inc., Sunderland, Massachusetts, 1992), 2nd ed.

[2] L.J. DeFelice, *Introduction to Membrane Noise* (Plenum Press, New York, 1981); M.M. Millonas and D.R. Chialvo, Phys. Rev. Lett. **76**, 550 (1996); D. Petracchi *et al.,* Biophys. J **66**, 1844 (1994).

[3] P. Hanggi, P. Talkner, and M. Borkovec, Rev. Mod. Phys. **62**, 251 (1990).

[4] S. Leibler, Nature (London) **370**, 412 (1994).

[5] D.L. Stein *et al.,* Phys. Lett. A **136**, 353 (1989); M. Bier and D. Astumian, Phys. Rev. Lett. **71**, 1649 (1993); M.M. Millonas and D.R. Chialvo, Phys. Rev. E **53**, 2239 (1996).

[6] K. Wiesenfeld and F. Moss, Nature (London) **373**, 33 (1995); E. Pantazelou, F. Moss, and D. Chiavlo, in *Noise in Physical Systems and I /f Fluctuations,* edited by P. Handel and A. Chung, AIP Conf. Proc. No. 285 (AIP, New York, 1993), p. 549; F. Moss and X. Pei, Nature (London) **376**, 211 (1995); J.J. Collins, C.C. Chow, and T.T. Imhoff, Nature (London) **376**, 236 (1995); S.M. Bezrukov and I. Vodynoy, Nature (London) **378**, 362 (1995); P. Jung and G. Mayer-Kress, Phys. Rev. Lett. **74**, 2130 (1995).

[7] C.R. Doering and J.C. Gadoua, Phys. Rev. Lett. **69**, 2318 (1992); P. Reimann, Phys. Rev. Lett. **74**, 4576 (1995).

[8] S. Marom *et al*. (to be published).

[9] The potassium channel Shaker-*IR* is expressed in the membrane of a Xenopus oocyte. The experiments were performed in the inside-out configuration at room temperature. The pipette solution contained (in mM) 96 NaCl, 2 KCl, 1 $CaCl_2$, 1 $MgCl_2$, and 10 Hepes. Both at *p*H 7.5. An Axopatch 200A amplifier (Axon Instruments) was used (filter 5 kHz, gain = 100, no capacity correction). The capacitance of the system is largely determined by the miropipette ($\sim$1 pF). The number of voltage-gated channels was determined by using the single channel conductivity $g_k = 0.013$ pA/mV [8]. The range of lead conduction was $g_L = 0.01-0.03$ pA/mV.

[10] *Single-Channel Recording,* edited by B. Sakmann and E. Neher (Plenum Press, New York, 1995), 2nd ed.

[11] The mean number of open channels is estimated by equating the leakage current plus the external holding current to the channels' current and using the single channel's conductivity [9].

[12] $\alpha = 0.03(V + 46)/(1 - \exp[-0.8(V + 46)])$ and $\beta = -0.02V \exp[-0.023(V + 148)]$ in ms^{-1} (V in mV) (measured in [8]).

[13] R. Kubo, M. Toda, and N. Hashitsume, *Statistical Physics II, Nonequilibrium Statistical Mechanics* (Springer-Verlag, New York, 1985).

[14] A.E. Siegman, *Lasers* (University Science Books, Mill Valley, California, 1986), p. 958.