Signal Transduction Across Alamethicin Ion Channels in the Presence of Noise

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ABSTRACT We have studied voltage-dependent ion channels of alamethicin reconstituted into an artificial planar lipid bilayer membrane from the point of view of electric signal transduction. Signal transduction properties of these channels are highly sensitive to the external electric noise. Specifically, addition of bandwidth-restricted "white" noise of 10–20 mV (r.m.s.) to a small sine wave input signal increases the output signal by ~20–40 dB conserving, and even slightly increasing, the signal-to-noise ratio at the system output. We have developed a small-signal adiabatic theory of stochastic resonance for a threshold-free system of voltage-dependent ion channels. This theory describes our main experimental findings giving good qualitative understanding of the underlying mechanism. It predicts the right value of the output signal-to-noise ratio and provides a reliable estimate for the noise intensity corresponding to its maximum. Our results suggest that the alamethicin channel in a lipid bilayer is a good model system for studies of mechanisms of primary electrical signal processing in biology showing an important feature of signal transduction improvement by a fluctuating environment.

INTRODUCTION

Electrical signal transduction and processing by living organisms are among most intriguing topics in modern sensory biology (Block, 1992). The principles of biological amplification are far from being understood; what is clear is that biological amplifiers are unique in their ability to detect small signals in noisy environments (Polk, 1995). Working in the "field" they successfully compete with man-made "laboratory" systems of signal detection and analysis. A remarkable sensitivity (~1 nV/cm) to a slow near-periodic electrical signal was found from behavioral experiments with *Elasmobranch* fish (Kalmijn, 1982). This sensitivity is generally attributed to electroreceptive organs called *ampullae* of Lorenzini (Clusin and Bennet, 1979). Careful "in vitro" measurements performed recently on isolated ampullary organs showed that changes in the spike firing rate of the organ's afferent nerve were evident for increments in holding potentials as small as 3 μV, and that the ampullary epithelium acts as a linear amplifier within the operation range of 0–100 μV (Lu and Fishman, 1994). It was concluded that ion channels in apical and basal membranes of receptor cells play a crucial role in the primary steps of signal amplification.

A wide range of different systems have been shown to exhibit "stochastic resonance" (Wiesenfeld and Moss, 1995), a phenomenon of enhancement of small periodic stimuli by large-scale environmental fluctuations. The concept of stochastic resonance was initially introduced to explain the suggested periodicity of Earth's ice ages, and a dynamical mechanism of small periodic perturbations amplification by environmental noise was described (Benzi et al., 1981). Since then, the phenomenon acquired an interdisciplinary nature—it was shown to exist in electronic, physical, and even sensory biological systems. Stochastic resonance theory predicts great importance of this phenomenon for information transfer and processing, especially in biology, where a sensory signal transduction could benefit from utilization of ever-present ambient noise (Bulsara et al., 1994). Still, a few biological examples that have been shown so far to manifest stochastic resonance features were complex, and involved elaborate organization of biological receptors, neural signal pathways, and motor functions (Douglass et al., 1993; Pei et al., 1996a).

To the present, theoretical studies have been mostly concentrated on dynamical systems subjected to an external force composed of random noise and usually sinusoidal (but see Neiman and Schimansky-Geier, 1994; Collins et al., 1995) signal (Benzi et al., 1981; Dykman et al., 1990; Jung and Hanggi, 1991; Jung, 1993; Bulsara and Gammaitoni, 1996). Not long ago, two theoretical studies of *non-dynamical* systems exhibiting stochastic resonance appeared in physics journals (Gingl et al., 1995; Jung, 1995). Both of them exploit threshold crossing dynamics in the systems of the type of a level-crossing detector. These systems generate a uniform output pulse when and only when the input voltage exceeds a given threshold; otherwise, output is silent.

We show, both experimentally and theoretically, the existence of stochastic resonance in a *non-dynamical* and *threshold-free* system (Bezrukov and Vodyanoy, 1995, 1997). The signal-to-noise ratio of the pulse train produced by spontaneously firing ion channels reaches a maximum value at a certain level of external noise. In contrast to threshold systems where sub-threshold forcing generates no output signal at all (Gingl et al., 1995; Jung, 1995), voltage-dependent ion channels are able to transduce small signals

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in the absence of any external noise. As we demonstrate below, the transduction coefficient approaches a finite value even for arbitrarily small input signals. This qualitatively different behavior stems from the fact that the probability for a channel to open, being exponentially voltage-dependent, is still finite for every given input voltage.

Experimentally, we have examined a simple system where ion channels formed by a well-studied polypeptide, alamethicin (Hall et al., 1984), control the transmembrane current by random switching between their open states and the closed one. The probability of the channel being open strongly depends on the applied membrane potential, while the relative probabilities of different conductance states are only weak functions of the voltage. In some sense, therefore, a membrane doped with alamethicin channels resembles a voltage driven two-state system with one state being degenerate.

Theoretically, we consider the statistical properties of a random pulse train produced by channel voltage-dependent openings and subsequent closings. We restrict ourselves to the adiabatic and small-signal regime only. That is, our treatment is valid for signals that are slow enough for all other processes in the system to equilibrate at a particular value of input voltage and small enough not to disturb the system significantly. We calculate the output signal originating from periodicity in the pulse train rate that is induced by the input signal. For the output noise we consider two components. The first one is the basic noise of a Poisson wave of identical pulses; the second component describes correlations between pulses introduced by the input noise. We show that this theory (which is exact for sufficiently small and low-frequency signals) adequately describes our main experimental findings. In particular, it gives a good estimate for the input noise intensity corresponding to maximum signal-to-noise ratio.

MATERIALS AND METHODS

Lipid bilayers and ion channels

The alamethicin channels were reconstituted into planar lipid bilayer membranes. Solvent-free membranes were prepared from a pentane solution of diphytanoyl phosphatidylcholine (DPhPC; Avanti Polar Lipids, Inc., Alabaster, AL) on an ~80-μm diameter orifice in a 15-μm thick Teflon partition that separated two Teflon chambers (Bezrukov and Vodyanoy, 1993, after Montal and Mueller, 1972). Both chambers contained ~1 ml of 1 M NaCl aqueous solutions buffered at pH 6.2 by 5 mM MES (Calbiochem Corp., La Jolla, CA). The orifice was pretreated with a 5% (v/v) solution of hexadecane in pentane. The bilayer membrane capacitance was 30–50 pF.

Natural alamethicin (The Upjohn Co., Kalamazoo, MI), purified as described by Balasubramanian et al. (1981), was added after membrane formation to one membrane bathing solution only. The first single channels appeared 30–40 min after peptide addition, but the steady-state conditions necessary for acquiring accurate statistics were achieved only by equilibrating the bilayer and the peptide-containing aqueous bathing solution for 2–3 h at a constant holding potential of 100–150 mV. The membrane current was measured with Ag-AgCl electrodes with agarose bridges assembled within standard 200-μl pipette tips (Bezrukov and Vodyanoy, 1993). The applied potential difference is defined as positive when the potential is greater at the side of peptide addition. Measurements were done at $T = (24.0 \pm 1.5)$°C.

Measuring circuits

The block diagram of the electronic set-up is shown in Fig. 1. A sine wave voltage from a Hewlett-Packard 33120A function generator (SG) was added to a d.c. holding potential, V_h, that was adjusted within +100 to +150 mV range to maintain a desired number of channel "bursts" per second. This combined signal was applied to the left electrode through a low-resistance (<3 kOhm) output circuit of a laboratory-made noise generator (NG). The current through the lipid bilayer with ion channels was picked up with a "virtual ground" electrode using a Dagan 3900 patch-clamp amplifier (Minneapolis, MN) or a laboratory-made operational amplifier with 10 MOhm-1 GOhm feedback resistors. In all measurements reported here the total ion channel resistance was kept above 1.0 MOhm to render possible corrections from a finite resistance of input circuits negligible.

The laboratory-made noise generator provided a good quality "physical" noise. The basic element of the generator was a 1 GOhm resistor (K and M Electronics, Inc., West Springfield, MA) whose equilibrium Johnson noise was carefully amplified using a series array of OPA 111 integrated circuit operational amplifiers (Burr-Brown Corp., Tucson, AZ) and proper electromagnetic shielding (Amuneal Manufacturing Corp., Philadelphia, PA). Fig. 2 presents the generator output spectral density in comparison with the spectral density of a Hewlett-Packard 33120A function generator in its "noise" mode. The advantage of the laboratory-made noise generator is immediately seen—it produces signals without periodicity that is evident for the 33120A function generator output in this low frequency range. The signal was stationary, Gaussian, and with zero mean value (deviations from zero were within ±0.1 mV at the maximum output and 30 min averaging). Output voltages for the sine wave and noise generators are given as r.m.s. values.

RESULTS AND DISCUSSION

Signal transduction in the absence of external noise

Regarding electric signal transduction, the most important property of ion channels is their voltage sensitivity. Volt-

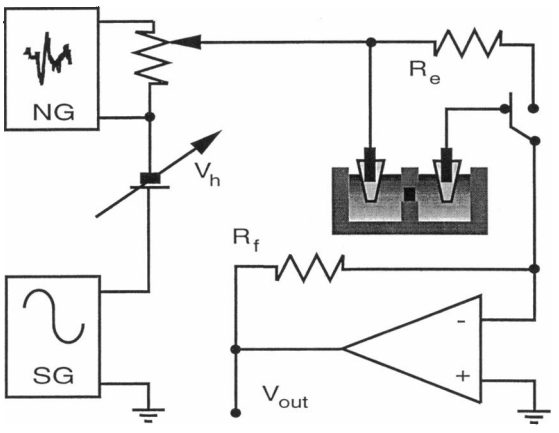

FIGURE 1 Schematic presentation of the circuit used in measurements. The sum of voltages from a sine wave generator (SG), a d.c. holding potential source (V_h), and a laboratory-constructed noise generator (NG) was applied to the membrane via the potential electrode and membrane-bathing solution on the left side of the cell. The "virtual ground" electrode on the right side of the cell was used to pick up currents through the membrane.

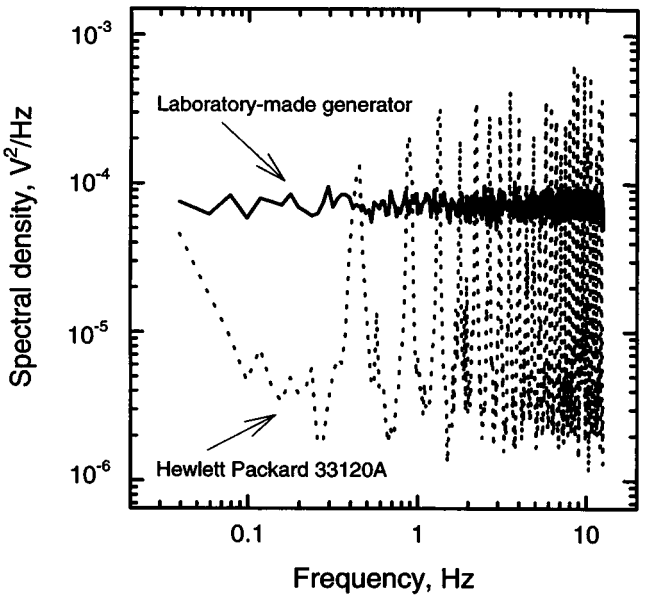

FIGURE 2 Comparison of voltage spectral densities at the outputs of Hewlett Packard 33120A generator in its "noise" mode and a laboratory-made noise generator. The output of the 33120A generator shows deterministic features. Periodicity is manifested by spikes in the spectral density. This periodicity is absent in the output of the laboratory-made generator that uses carefully amplified "physical" noise source (see text).

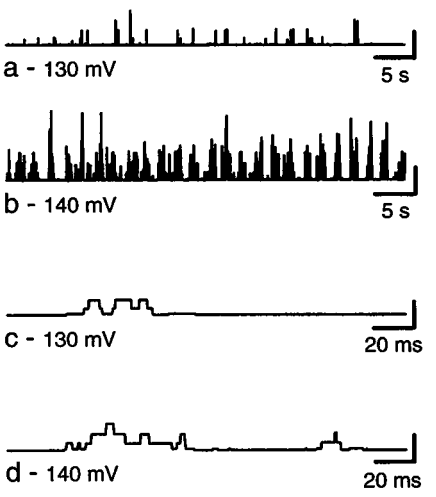

FIGURE 3 Ion currents through the membrane with alamethicin channels at two different holding potentials with a 5-mV (r.m.s.) sine wave signal added. Vertical bars correspond to 0.5 nA current. A 10-mV increase in holding potential increases the channel firing rate by an order of magnitude. Higher resolution recordings (*c* and *d*) show small fractions of *a* and *b* tracks to illustrate fine structure of a single-channel current "burst." It consists of several random reversible transitions between fixed levels whose probabilities are only slightly dependent on transmembrane voltage.

age-dependent channel gating translates into a nonlinear response to an external excitation that renders the system its interesting and non-trivial features. In the case of alamethicin, the probability of the channel to be open strongly depends on the applied membrane potential, whereas the relative probabilities of the different open states, their durations, and, therefore, overall open channel life-time, are only weak functions of the voltage.

Fig. 3 illustrates the phenomenology of alamethicin channel voltage dependence. Two recordings of spontaneous channel activity presented in Fig. 3, *a* and *b* are taken at the two different d.c. values of the holding potential, V_h, 130 mV and 140 mV with a 5-mV (r.m.s.) riding sine wave signal of 0.5 Hz frequency. Note that a 10-mV increment in membrane potential significantly increases the number of channels observed per unit time (approximately by an order of magnitude). At the same time, as demonstrated by higher resolution recordings in Fig. 3, *c* and *d* and statistically shown elsewhere (Boheim, 1974), the probabilistic structure and the mean channel life-time are not changed noticeably. For that reason, the average alamethicin-induced conductance at different membrane voltages mostly reflects the average number of simultaneously open channels, which is proportional to the rate of their spontaneous appearance at a given voltage. Our measurements show that for the particular parameters used in this study (lipid: DPhPC, salt: aqueous 1 M NaCl, transmembrane voltages: 100–150 mV, average number of simultaneously open channels at different holding potentials and noise amplitudes: 0.01–1000), the number of channels grows as $\exp[V_h/(4.1 \pm 0.6\ \text{mV})]$.

The mechanism of alamethicin channel voltage dependence is not fully established (Hall et al., 1984; Sansom, 1991; Woolley and Wallace, 1992; Cafiso, 1994). Most researchers agree that the crucial feature responsible for voltage sensitivity is the alamethicin molecule dipole moment of ~75 Debye that is related to alignment of the dipole moments of individual peptide bonds in its α-helical conformation. According to one of the oldest models (Baumann and Mueller, 1974), the electrical field applied across the membrane interacts with molecules via their dipoles, increasing the number of alamethicin monomers in the "transmembrane" orientation (Fig. 4). Then, these properly oriented monomers reversibly aggregate into ion-conducting clusters of different size, thus accounting for different conductance states of the alamethicin channel.

Alamethicin channels appear as "current bursts" (Fig. 3) rising from the background current of $<10^{-12}$ A to conducting states of 1 to 5 $\times$ 10^{-10} A. Channel opening and closing corresponding to the burst onset and consecutive disappearance, as well as transitions between different conductive states within a single burst, are random, *kT*-driven events. The presence of a small sine wave riding signal in the potential across the membrane modulates the rate of channel spontaneous generation, introducing correlations in the moments of current burst onsets. Though hardly perceptible by eye, especially in the case of a low firing rate in Fig. 3 *a*, these correlations are clearly demonstrated by Fig. 5, which presents power spectral density of the output signal by a prominent peak at the applied signal frequency.

It is easy to show that this peak reflects voltage-dependent "gating" of the channels that brings correlations in the current burst onsets, and not just their ohmic conductance.

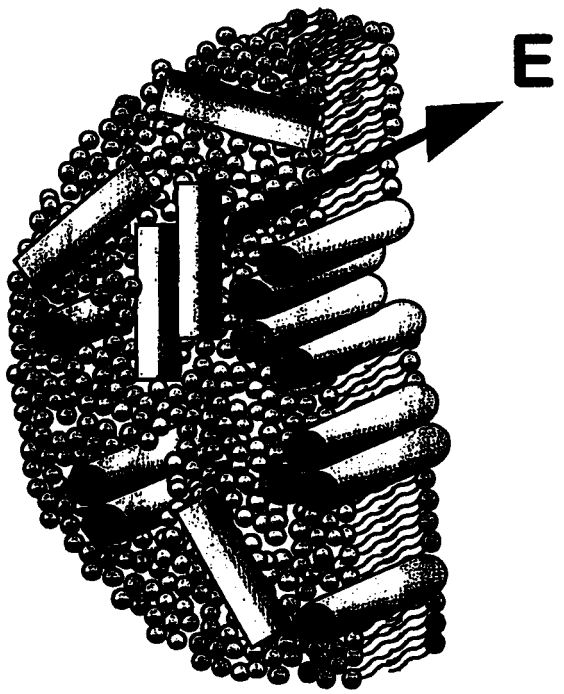

FIGURE 4 A model of voltage dependence for alamethicin-induced membrane conductance. The electric field interacts with alamethicin molecules (*cylinders*) via their dipole moments, so that a stronger field supports a larger alamethicin population in the transmembrane orientation. Trans-oriented monomers then assemble into conducting clusters seen as current bursts. At a given voltage, bigger clusters correspond to higher current levels (Fig. 3, *c* and *d* tracks).

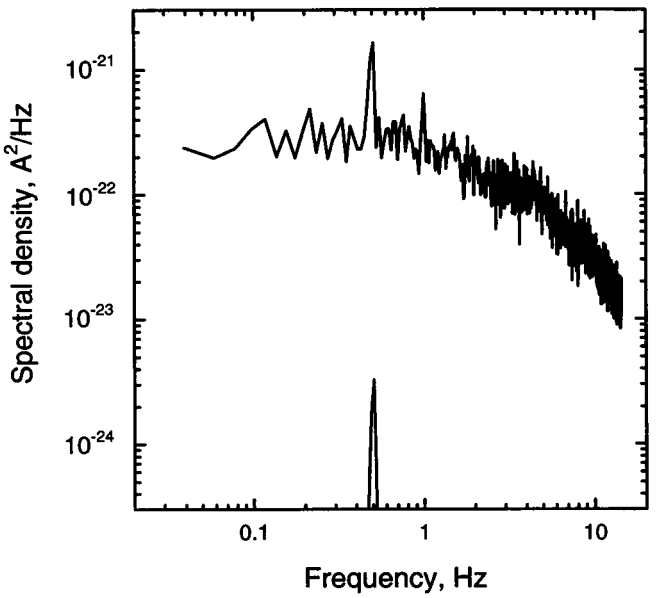

FIGURE 5 Spectral density of the ion current through the membrane with channels (*upper trace*) in comparison with spectral density of the current through a carbon resistor of the same conductance (*peak* at the bottom). No external noise was applied at this stage. Both measurements were done with a 5-mV 0.5-Hz sine wave signal added to a 130-mV holding potential; each spectrum is an average over 23 spectral estimates representing a 20-min sample.

For this purpose we substitute the real membrane by an equivalent circuit accounting for membrane capacitance and average channel-induced conductance. As a result, we obtain orders of magnitude smaller spectral component at the signal frequency. In the example shown in Fig. 5, the spectral peak at the bottom is measured using a linear carbon resistor representing the average membrane conductance (R_e in Fig. 1). The contribution from the membrane capacitance (40 pF) is very small and is ignored here.

Ion channels produce a higher output signal than would a passive linear circuit of equal conductance, even including contributions from membrane capacitance. This "amplification" can be characterized quantitatively by the ratio of the signal spectral peak obtained from ion channels—with background noise subtracted—to the signal peak measured from the equivalent resistor. For the particular case illustrated in Fig. 5 we obtain ~30 dB gain in signal transduction.

The nature of this gain can be understood if we consider a strong dependence of channel generation rate, $r[V(t)]$, on the transmembrane potential, $V(t)$. For potentials that are changing not too fast for the system to respond, we have:

$$r[V(t)] \propto \exp[neV(t)/kT], \tag{1}$$

where n is the effective "gating charge" in units of elementary charge e, k is the Boltzmann constant, and T is the absolute temperature. For the transmembrane potential composed of a d.c. holding potential and a slow sine wave signal, $V(t) = V_h + V_s \sin(2\pi f_s t)$, we have:

$$r[V(t)] = r(V_h)\exp\left(\frac{neV_s \sin(2\pi f_s t)}{kT}\right). \tag{2}$$

The ensemble-averaged current through the system is then proportional to $r[V(t)]V(t)$. [The complete expression for the current will include channel life-time and conductance as additional multipliers; however, in our model they are independent of applied voltage and can be omitted in consideration of relative effects.] To calculate the gain related to the channel voltage sensitivity, this expression should be compared to $r(V_h)V(t)$, describing the current through the channels with gating voltage sensitivity switched off, that is, with $n = 0$. Calculating the corresponding first harmonic for the case of small sine wave amplitudes, that is for $V_s \ll kT/ne$, and taking its ratio to $r(V_h)V(t)$, we have $\alpha = 1 + neV_h/kT$. Therefore, for slow, small-amplitude sine wave signals we obtain the spectral gain as

$$\alpha^2 = (1 + neV_h/kT)^2, \tag{3}$$

We studied the signal transduction properties of alamethicin ion channels at different holding potentials, signal amplitudes, and signal frequencies. The data presented in Fig. 6 show that for slow 3-mV signals the measured gain is in good agreement with Eq. 3 ($n = 6.3$). It is proportional to the square of the d.c. holding potential and does not depend appreciably on frequencies between 0.2 and 1 Hz. A 3-dB drop is reached at ~2 Hz and at higher frequencies the

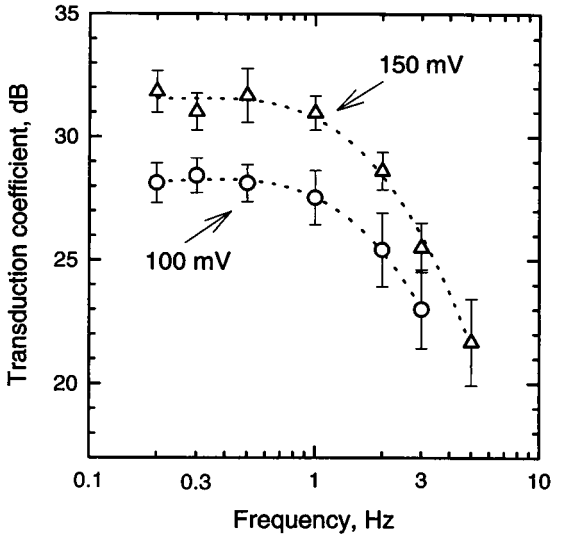

FIGURE 6 Signal transduction across alamethicin channels at different holding potentials as a function of signal frequency. It is defined as a ratio of the signal peak amplitude (Fig. 5) measured with alamethicin channels to the peak amplitude expected from the same number of channels with their voltage sensitivity switched off (zero gating charge). The "gain" is proportional to the square of holding potential and quickly decreases as signal frequency exceeds 2 Hz. This suggests an existence of a voltage-independent rate-limiting reaction with a characteristic time of ~0.1 s in the sequence of channel assembly.

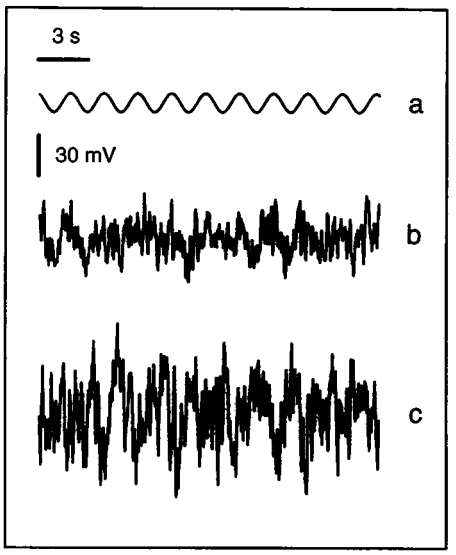

FIGURE 7 Input signal (5-mV 0.5-Hz sine wave) in the presence of different amounts of external noise ("white" noise processed by a 3.2 mHz to 5.3 Hz bandpass filter). *Trace a*, zero noise; *trace b*, 8 mV (r.m.s.) noise; *trace c*, 20 mV noise. A sufficiently long-lived 40-mV positive deviation of transmembrane voltage from the stationary value (holding potential) can increase channel generation rate by a factor of up to 10^5 in comparison to the "equilibrium rate" $r(V_h)$.

transduction coefficient quickly decreases. It is interesting to note that the shape of the gain/frequency curve is close to the shape of the spectral density in Fig. 5. Taking into account that the recording electronics bandwidth is at least three orders of magnitude wider than the frequency range in Figs. 5 and 6, this observation suggests that the characteristic average time of the current burst determining spectrum offset is also responsible for the cutoff frequency in system response (see also Kolb and Boheim, 1978).

Noise-facilitated signal transduction

To study the signal transduction properties of alamethicin channels in the presence of external noise, we measured the amplitude of the output signal, and the signal-to-noise ratio at the system output, as a function of input noise intensity. We used "physical" white noise with the bandwidth restricted between 3.2 mHz and 5.3 Hz (see Methods). Fig. 7 shows input signals with different amounts of external noise added. For the largest noise intensity used in our experiments, the input voltage fluctuates around its mean value, V_h, exceeding, for a short time, ±40 mV deviations in a random manner.

The output spectral density in the presence of 8 mV (r.m.s.) external input noise is shown in Fig. 8. The upper curve corresponds to typical conditions when a positive holding potential of 140 mV is applied to the membrane. An average initial rate of ~0.3 ion channels per second was supported by this voltage. Due to the nonlinear voltage dependence in the channel gating, the external noise increases this number by ~10-fold, increasing the output signal and noise. The lower curve describes the output signal when the channels are removed from the bilayer by switching to a negative holding potential of −140 mV. The input spectral density of the combined signal and noise is filtered by the membrane capacitance (~35 pF), introducing a frequency-dependent correction proportional to f^2. To reduce a possible overload of the recording electronics by noise at higher frequencies, we used an additional RC filtering at the noise generator output that is seen as the spectrum cutoff at $f > 5$ Hz.

Fig. 9 shows that the addition of external noise to the system input significantly increases the output signal (*triangles*) at an approximately constant signal-to-noise ratio (*circles*). Introduction of 20-mV band-limited white noise causes ~3×10^3-fold or 35 dB increase in the output signal, preserving the initial signal-to-noise ratio. At some intermediate values of noise intensities, a small but statistically significant increase in the signal-to-noise ratio is observed. This is a distinguishing feature of systems showing stochastic resonance. Small amplitudes of external noise increase both the signal and the noise at the system output, but the signal grows faster.

Fig. 9 (*inset*) demonstrates the statistics of signal-to-noise measurements on a finer scale over the same noise range of 0–20 mV r.m.s. Each point here represents an average over a 20-min recording. The signal-to-noise ratio is calculated according to SNR = $[S(f_s) - N(f_s \pm \Delta f)]/N(f_s \pm \Delta f)]$, where $S(f_s)$ is the spectral density component measured at the signal frequency and $N(f_s \pm \Delta f)$ is the background noise

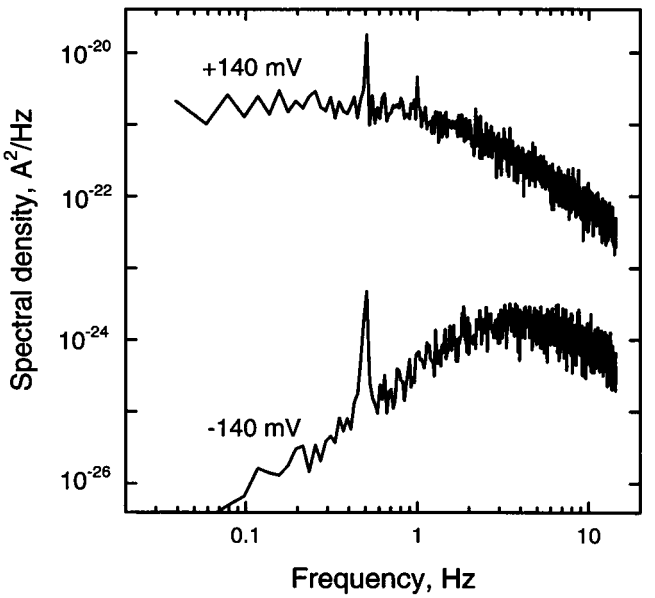

FIGURE 8 Spectral density of transmembrane current in the presence of input noise (8 mV) and signal (5-mV 0.5-Hz sine wave) at a positive holding potential (+140 mV) in comparison with spectral density of the membrane capacitive response. Capacitive response data were taken from the same membrane by changing polarity of the holding potential to the negative one that switched off all channels. It is seen that the capacitive contribution to the noise spectrum at the positive holding potential is negligible and stays below 1% in the whole range of frequencies used in measurements.

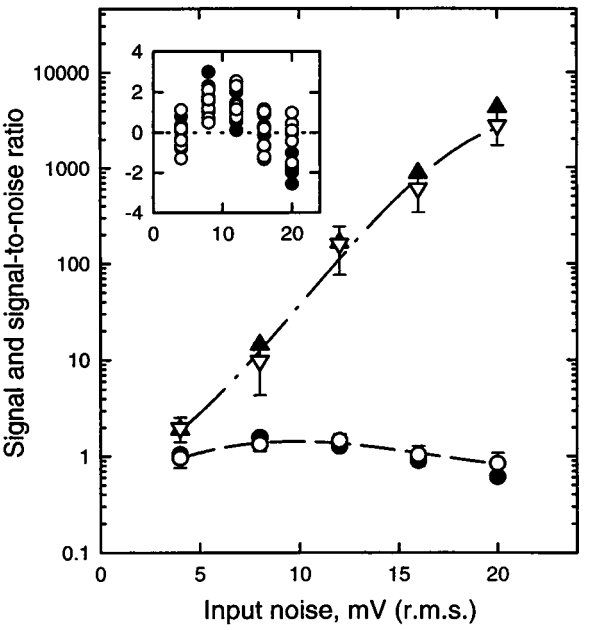

FIGURE 9 Signal (*triangles*) and signal-to-noise ratio (*circles*) in the membrane current as functions of input noise intensity. Sine wave signals of constant amplitude (5 mV r.m.s.) and frequencies (0.2 Hz, *filled symbols*; 0.5 Hz, *open symbols*) were mixed with different amounts of noise (Fig. 7) and applied to the potential electrode (Fig. 1). The SNR was defined as the ratio of the signal peak maximum to the background noise calculated as an average over spectral components in the immediate peak vicinity. The inset shows the statistics of SNR measurements in dB units at a finer scale.

represented by an average over spectral components in the immediate vicinity of the signal peak. [The procedure of noise subtraction is justified by statistical independence between the signal and noise. Power spectral densities of superimposed independent processes add linearly (DeFelice, 1981)]. Sine wave signals (5 mV) of 0.2 Hz (*filled symbols*) and 0.5 Hz (*open symbols*) were used. The inset shows that from the point of view of the output signal-to-noise ratio, there is an optimal amount of noise that should be admixed to the input signal to achieve the best quality in signal transduction.

Small-signal adiabatic theory of stochastic resonance in a threshold-free non-dynamical system

Until only two years ago it was generally believed that stochastic resonance features could be exhibited only by dynamical systems. Two recent papers describing stochastic resonance in non-dynamical systems (Gingl et al., 1995; Jung, 1995) showed otherwise. Both studies characterize noise-facilitated signal transduction within systems of the type of a threshold detector. As Wiesenfeld and Moss (1995) summarize in their short review, the simplest possible system capable of stochastic resonance consists only of a threshold, a sub-threshold signal, and added noise.

We demonstrated above that voltage-dependent ion channels are able to utilize external noise in the process of signal transduction. Alamethicin ion channels represent a *threshold-free non-dynamical* system showing stochastic resonance. Fig. 10 compares signal transduction through alamethicin channels and a typical threshold system in the absence of external noise. The difference in the systems' behavior is clear—the output of a threshold system in the absence of external noise is "silent" for sub-threshold signals, whereas ion channels have a finite transduction for however small signals (see Eq. 3).

The existence of stochastic resonance in voltage-dependent ion channels can be shown theoretically. An adiabatic small signal approach (Bezrukov and Vodyanoy, 1997) does not contain any adjustable parameters and predicts main features of stochastic resonance (such as optimum external noise intensity, maximum sharpness, etc.) surprisingly well. For simplicity we assume ion channel bursts to be identical pulses and consider the statistical properties of the resulting pulse train. We also neglect ohmic contributions that can be shown to be small in the case of large holding potentials, $V_h \gg kT/ne$. According to Eq. 1, the time-dependent channel generation rate at a holding potential V_h, external noise $V_n(t)$, and a slow sine wave signal $V_s \sin(2\pi f_s t)$, may be written as

$$r[V(t)] = r(V_h)\exp\{ne[V_N(t) + V_s \sin(2\pi f_s t)]/kT\}. \quad (4)$$

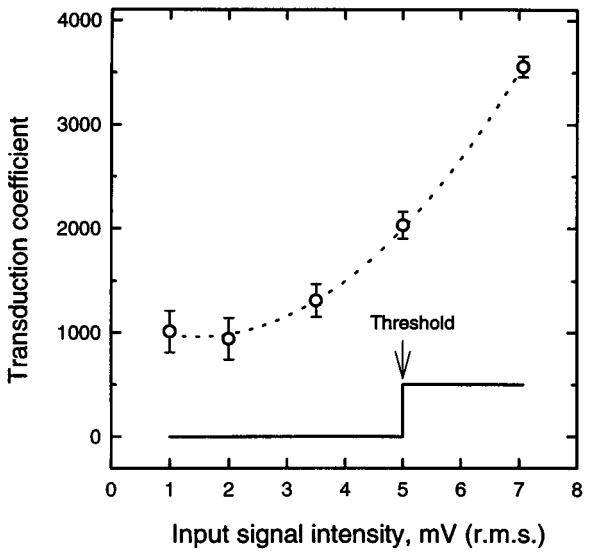

FIGURE 10 Transduction coefficient for alamethicin channels (V_h = 110 mV) as a function of the input signal amplitude in comparison to the idealized threshold system behavior. It is seen that in contrast to threshold devices that are "silent" at signals of sub-threshold amplitudes, voltage-dependent ion channels respond to arbitrary small signals. The transduction coefficient found in the limit of small signals (1–2 mV) agrees well with the theoretical prediction (see text).

If the signal frequency, f_s, is much lower than any other characteristic frequency in the system, the low-frequency part of the power spectral density of this time-dependent Poisson process (Cox and Lewis, 1966) can be written in the form

$$S_i(f) = 2Q^2\langle r[V(t)]\rangle + 4[Qr(V_h)]^2 \int_0^\infty \langle \exp[neV(t)/kT]\exp[neV(t+\tau)/kT]\rangle \cos(2\pi f\tau)d\tau, \quad (5)$$

where Q is the total charge transported during a single channel "burst."

For the input noise having a Lorentzian spectrum with a corner frequency f_c (single pole filtering) and small amplitude signals only, that is $V_s \ll kT/ne$, and $V_s \ll \sigma$, where σ is the r.m.s. noise amplitude, we have (neglecting a d.c. component in the output signal)

$$S_i(f) = 2Q^2 r(V_h)\exp\left(\frac{1}{2}\left(\frac{ne\sigma}{kT}\right)^2\right) + \frac{2}{\pi f_c}\left(\frac{Qr(V_h)ne\sigma}{kT}\right)^2 \exp\left(\left(\frac{ne\sigma}{kT}\right)^2\right)\sum_1^\infty \frac{1}{m!\,m}\left(\frac{ne\sigma}{kT}\right)^{2m-2} + \frac{1}{2}\left(\frac{Qr(V_h)neV_s}{kT}\right)^2 \exp\left(\left(\frac{ne\sigma}{kT}\right)^2\right)\delta(f-f_s). \quad (6)$$

The first term on the right-hand side of this expression accounts for the noise expected from a time-independent Poisson wave of pulses with the area Q and the rate of their appearance exceeding the "equilibrium rate" $r(V_h)$ by a factor $\exp[(ne\sigma/kT)^2/2]$. The second term represents the external input voltage noise, $V_n(t)$, transduced to the output. It includes not only a small-signal part of the noise transduction, but also contributions from the "cross-talk" between different spectral noise components. The last term describes signal transduction at $f = f_s$. It shows that the power of the output signal grows exponentially with the noise variance and is finite at zero input noise.

To obtain the signal spectral component measured by a spectrum analyzer (dimensionality A^2/Hz), we substitute the delta-function in the last term of Eq. 6 with the inverse of the analyzer unit spectral window, Δf_a (DeFelice, 1981). The signal-to-noise ratio is given then by the ratio of the last term to the first two:

$$SNR = \frac{\left(\frac{neV_s}{kT}\right)^2 \frac{r(V_h)}{2\Delta f_a}\exp\left(\frac{1}{2}\left(\frac{ne\sigma}{kT}\right)^2\right)}{2 + \frac{2r(V_h)}{\pi f_c}\exp\left(\frac{1}{2}\left(\frac{ne\sigma}{kT}\right)^2\right)\sum_1^\infty \frac{1}{m!\,m}\left(\frac{ne\sigma}{kT}\right)^{2m}}. \quad (7)$$

Analysis of Eq. 7 shows that, except for the trivial dependence on the input signal amplitude (V_s) and the details of measuring technique (Δf_a), the signal-to-noise ratio can be controlled by the noise intensity (σ) and its frequency bandwidth (f_c). It also depends on the equilibrium rate [$r(V_h)$] of the Poisson wave undisturbed by noise or signal. One of the most important conclusions is that even when the initial statistics are good [i.e., $r(V_h)$ is large], the output signal quality can be further improved if the noise bandwidth is high enough for the condition $f_c > 2r(V_h)/\pi$ to hold. For the input noise with a sharp spectral cutoff (e.g., multipole Butterworth filtering), the corresponding condition reads $f_c > r(V_h)$.

Fig. 11 illustrates Eq. 7 for several values of the ratio between noise bandwidth, f_c, and the initial channel generation rate, $r(V_h)$. The SNR is plotted as a function of dimensionless noise amplitude, expressed in kT/ne units. It could be seen that at certain parameters Eq. 7 predicts a maximum in the signal-to-noise ratio. The decrease in the channel generation rate at the constant noise bandwidth shifts the optimal noise intensity to higher values.

Equation 7 can be used to estimate the input noise r.m.s. value that optimizes signal transduction in voltage-dependent ion channels. Analysis shows that for values of $f_c/r(V_h)$ smaller than 10^5, the maximum in the signal-to-noise ratio is achieved at the input noise close to

$$\sigma_{opt} \cong \frac{kT}{ne}\sqrt{\ln\frac{\pi f_c}{2r(V_h)}}. \quad (8)$$

For the external noise with a sharp spectral cutoff, the factor $\pi/2$ under the logarithm sign should be omitted.

To apply Eqs. 7 and 8 to our experiments with alamethicin channels, we should note that the theory outlined above is formulated for idealized non-dynamical systems without

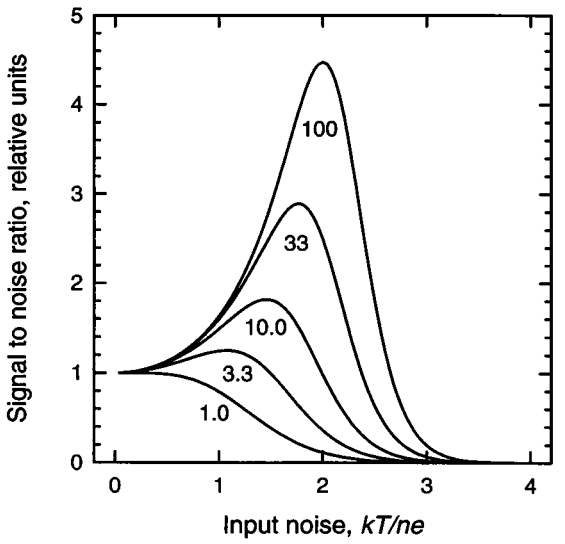

FIGURE 11 Theoretical prediction for a signal-to-noise ratio versus input noise with a Lorentzian spectrum. Numbers at the curves show different $\pi f_c/2r(V_h)$ ratios used in calculations. It is seen that the optimal value of input noise depends on the ratio of the cutoff noise frequency, f_c, and the initial pulse generation rate, $r(V_h)$. To obtain an improvement in the output signal, the condition $f_c > 2r(V_h)/\pi$ [or $f_c > r(V_h)$ for the input noise with a sharp spectral cutoff] must hold; otherwise, addition of noise to the system input only deteriorates the output signal.

inertia, that is, for systems without a delay between the change in potential and the change in generation rate. For the real system of alamethicin channels this is not true. Fig. 6 shows a cutoff frequency of ~2 Hz for the channel response. This cutoff frequency should be used as the noise bandwidth, since the channels are not sensitive to the higher noise harmonics. Taking $r(V_h)$ and f_c to be equal to 0.3 s^{-1} and 2.0 Hz, correspondingly, and n ranging from 5 to 7, we have $\sigma_{opt} \approx 6 - 8$ mV r.m.s. This is in excellent accord with our data (Fig. 9). Using Eq. 7 we obtain a good estimate for the output SNR, also. The equation (see also Fig. 11) predicts a 3-dB improvement in the output signal-to-noise ratio, which is very close to the observed value (Fig. 9).

Thus, the agreement between experiments and the theory is surprisingly good, even though at least two theory assumptions hardly hold in our experiments. First, the theory is formulated for identical events. In the real system of alamethicin channels both the time and the amplitude of pulses are highly variable (Fig. 3). Second, the SNR derivation was performed for small signals, that is, $V_s \ll kT/ne$ and $V_s \ll \sigma$. For technical reasons, in this study we used signal that is comparable to both the characteristic gating potential (3.5–4.7 mV) and the noise amplitudes (4–20 mV). Nevertheless, the theoretical predictions are accurate enough to assert that the theory based on a time-dependent Poisson process does not only provide a good qualitative picture, but also serves as a robust quantitative description of the observed phenomenon.

CONCLUSIONS

External noise can regulate signal transduction in the threshold-free non-dynamical system represented by voltage-sensitive alamethicin ion channels in a planar lipid bilayer.

Ion channels with voltage-dependent gating exhibit stochastic resonance features including improved output signal-to-noise ratio at an optimum level of input noise.

A small-signal adiabatic theory describes stochastic resonance in such systems. Without adjustable parameters the theory's predictions are surprisingly close to the results obtained with alamethicin channels.

Signal processing in biology often outperforms modern electronic devices. Examples of extraordinary biological signal processing include the response of electric fishes to electrical fields of several nV/cm (Kalmijn, 1982), and the sensitivity of the ear to auditory signals inducing hair cell displacements in a nm scale (Hudspeth, 1989). In electronic instruments, the first amplification stage is usually designed to produce high gain with minimal noise distortion of the signal. Special noise-reduction algorithms applied to recover small signals unfortunately always limit performance of signal processing. Perhaps nature devises different, still unknown, amplification mechanisms that incorporate ambient noise to improve signal transduction and to enable biological systems to operate successfully in highly fluctuating environments.

To understand the extraordinary sensitivity of sensory transduction in living organisms will require research at different levels of complexity. Our results show that ambient noise utilization is possible already at the level of voltage-dependent ion channels. We hope that this study, together with work done by others (Bialek, 1987; Chiabrera et al., 1989; Douglass et al., 1993; Bulsara et al., 1994; Lu and Fishman, 1994; Maddox, 1994; Tsong, 1994; Hong, 1995; Polk, 1995; Chiou-Tan et al., 1996; Collins et al., 1996; Cordo et al., 1996; Gluckman et al., 1996; Levin and Miller, 1996; Moss et al., 1996; Pei et al., 1996b; Chialvo et al., 1997; Gailey, 1997; Jung and Wiesenfeld, 1997; Longtin, 1997; Plesser and Tanaka, 1997), will shine light on the mechanisms of biological signal transduction and clarify the role of stochastic resonance in these mechanisms.

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