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Noise-induced enhancement of signal transduction across voltage-dependent ion channels

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THE presence of noise in a signal transduction system usually interferes with its ability to transfer information reliably. But many nonlinear systems can use noise to enhance performance¹, and this phenomenon, called stochastic resonance, may underlie the extraordinary ability of some biological systems to detect and amplify small signals in noisy environments^{2–5}. Previous work has demonstrated the occurrence of stochastic resonance in a complex system of biological transducers and neural signal pathways⁶, but the possibility that it could occur at the sub-cellular level has remained open. Here we report the observation of stochastic resonance in a system of voltage-dependent ion channels formed by the peptide alamethicin. A hundred-fold increase in signal transduction induced by external noise is accompanied by a growth in the output signal-to-noise ratio. The system of ion channels considered here represents the simplest biological system yet known to exhibit stochastic resonance.

Voltage-sensitive ion channels in cell membranes are essential for primary biological signal transduction, playing important roles in the generation of nerve action potentials, synaptic transmission, and other critical cellular functions⁷. These channels switch randomly (in response to thermal fluctuations) between conductive and nonconductive states, but this switching is correlated with many external variables. These variables include external electric fields, the concentration of channel blocking or activating molecules and ionic strength. The possibility of a noise-induced improvement of weak signal detection in ion channels has been predicted^{1,3,8} but has not yet been observed. Studies intended to investigate the role of internal noise in ion channels⁹ and neuron behaviour¹⁰ have been inconclusive.

We have analysed the signal transduction properties of the alamethicin channel¹¹ in the absence and presence of an external electric noise source using the experiment schematically shown in Fig. 1. The polypeptide alamethicin (relative molecular mass ~2,000), promotes the formation of ion channels in lipid bilayers in a highly voltage-dependent manner. An average alamethicin-induced conductance increases by e times every 4 or 5 mV, depending on bilayer lipid composition¹². The molecular mechanism responsible for this voltage sensitivity is not fully established. One possible mechanism, shown in Fig. 1*a* inset, is the realignment of the dipoles of alamethicin helices in response to a change in the transmembrane potential. Application of an

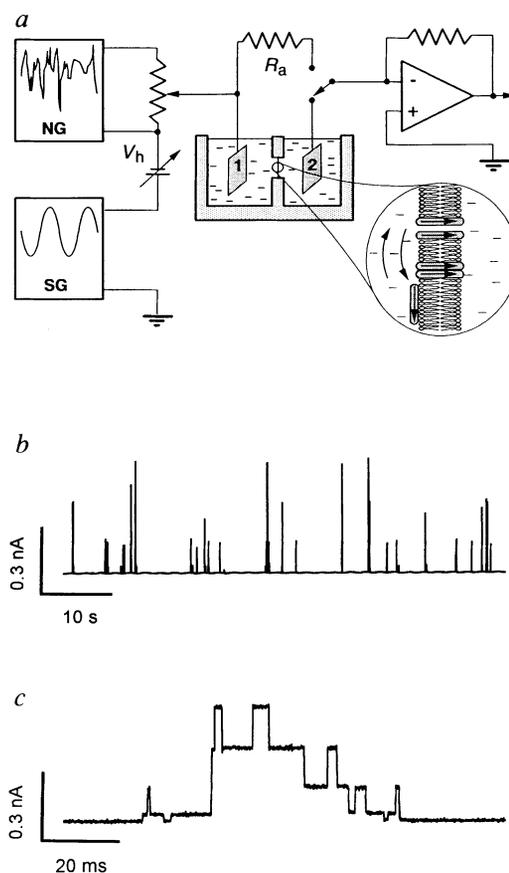


FIG. 1 Experimental apparatus (a) and output currents at a 5-mV (r.m.s.) 0.5-Hz sine-wave signal and zero external noise (b, c).

METHODS. A sine-wave signal from a Hewlett-Packard 3310B function generator (SG) was added to a d.c. holding potential of 130–150 mV and applied to electrode 1 through low-ohmic circuitry (output resistance <3 k Ω). Currents through the lipid bilayer with the incorporated voltage-dependent ion channels (inset), with total resistance always >10 M Ω , were recorded via the 'virtual ground' electrode 2 using an operational amplifier with 10 M Ω –1 G Ω feedback resistors. A passive resistor R_a had a value to the current through the match ion channels at a particular holding potential. A white noise generator (NG) of our own design was used to study the influence of external noise on the signal transduction in the system. The alamethicin channels were reconstituted in $L\text{-}\alpha$ -diphytanoyl lecithin bilayer membranes. Membrane-leakage d.c. current was <10⁻¹² A, membrane capacitance was ~50 pF. The steady-state conditions of measurement were achieved by equilibrating the bilayer and the aqueous salt solution (1 M NaCl) for 2–3 h after alamethicin addition. Other experimental details are as described previously¹⁴. The inset in a shows a lipid bilayer doped with alamethicin molecules. Arrows drawn on the 'molecules' represent molecular dipole moments responsible for the alamethicin channel voltage dependence^{11,12}. Molecules adsorbed on the membrane surface (molecules lying parallel to the bilayer) change their orientation when they interact with applied electric field. Channel formation thus involves the effective 'gating charge' derived from considering molecular dipole moment, number of molecules in the channel, and lipid bilayer thickness and dielectric constant. The transition between conductive (open) and non-conductive states (molecular clusters) of the channel is shown by reaction arrows.

electric field increases the number of molecules oriented across the membrane to facilitate channel opening. Ion channels are expressed in a stochastic manner as 'current bursts' rising from the background (Fig. 1*b, c*). Though seemingly random, the occurrences of current burst onsets in the presence of a 5-mV sine wave signal are correlated in time.

The power spectral density in Fig. 2*a* displays these correlations. There is a prominent peak at the applied signal frequency.

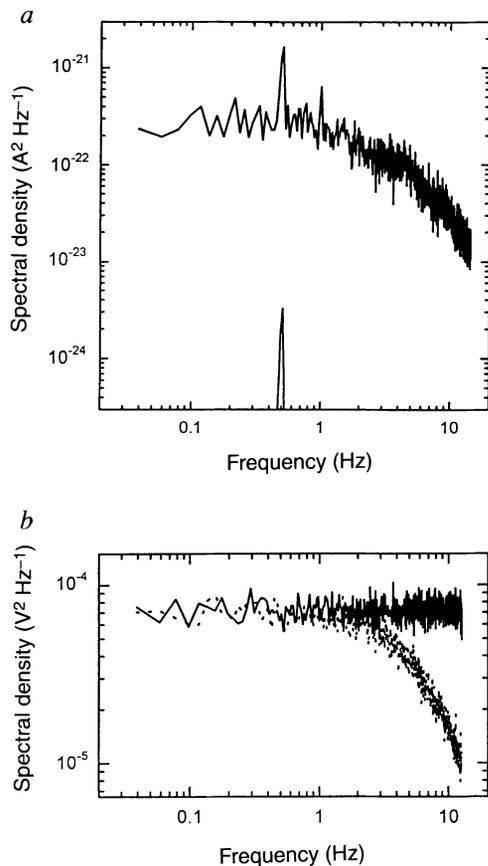


FIG. 2 *a*, Spectrum of the output current in the frequency range 0.02–20 Hz, showing correlations between moments of current burst onset. The signal component is much stronger than that expected from a passive linear circuit of the same average conductance (the peak at the bottom was measured for a $1.7 \times 10^{10} \Omega$ resistor). A 5 mV (r.m.s.) signal of 0.5 Hz was added to a 130 mV (d.c.) holding potential. No external noise was introduced at this stage. The spectrum is an average over a 20-min sample. *b*, Output voltage spectral density of our noise generator at the maximum output used in our experiments (solid line). Before application to the membrane, the output signal was filtered by an RC circuit to limit the noise spectral width to 5.3 Hz (dotted line), thus giving a value of 20 mV (r.m.s.). The basic element of the generator was a 1 G Ω resistor whose equilibrium Johnson noise was amplified. 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).

The bottom spectral peak is measured using the circuit illustrated in Fig. 1*a* where the bilayer is replaced by a resistor, R_a , representing an average conductance of the system.

Ion channels produce a higher output signal than would a passive linear system of equal conductance, even including contributions from membrane capacitance. This gain can be characterized quantitatively by computing output power spectral density at the signal frequency, and subtracting the average noise background measured in the immediate vicinity of the signal peak (Fig. 2*a*). By formally introducing an empirical amplification coefficient α^2 as the ratio of signal intensities at the outputs of these two systems, we obtain a 27 dB gain for these particular experimental parameters.

The nature of this signal amplification can be understood if we consider a strong dependence of the probability of channel formation, $P(V)$, on the transmembrane potential V (ref. 12). For $P(V) \ll 1$ we have

$$P(V) \propto \exp(neV/kT) \quad (1)$$

where n is the effective 'gating charge' in units of elementary charge e . Condition $P(V) \ll 1$ holds for all our experiments. In particular, it is immediately seen for the recordings of ion current in Fig. 1*b*, where the combined life-time of the channel is much smaller than the record overall time.

If alamethicin channels are in 1- α -diphytanoyl lecithin bilayers, then n is close to 5. Now, taking into consideration the periodic nature of the signal, it is easy to show that for small signals in the absence of external noise the ratio can be expressed as

$$\alpha^2 = (1 + neV_h/kT)^2 \quad (2)$$

where V_h is a holding potential across the membrane, k is the Boltzmann constant, and T is the absolute temperature. We studied signal transduction properties of ion channels at different holding potentials, signal frequencies and signal amplitudes. Our measurements show that the gain increases monotonically with signal amplitude, does not depend appreciably on frequencies ranging from 0 to 2 Hz, and quickly decreases at higher frequencies. For slow small signals (2 or 3 mV r.m.s.), the empirical amplification coefficient approaches the theoretically predicted value. For example, for the particular conditions as specified in Fig. 2*a* legend, we expect α^2 to be $\sim 7 \times 10^2$ which is in a good agreement with the measured ratio.

To study the interaction between external noise and signal transduction, we measured the amplitude of the output signal, and the signal-to-noise ratio at the system output, as a function of external noise intensity. Our results (Fig. 3) show that the addition of external noise ranging from 0 to 20 mV (r.m.s.) to the input significantly increases the output signal (up to 35 dB), at an approximately constant signal-to-noise ratio (circles). Five-millivolt (r.m.s.) sine-wave signals at 0.2 Hz (filled symbols) and 0.5 Hz (open symbols) were used. It should be noted that at some intermediate values of noise intensities, a small increase in the signal-to-noise ratio is observed (Fig. 3 inset)—a characteristic feature of systems showing stochastic resonance¹. Small

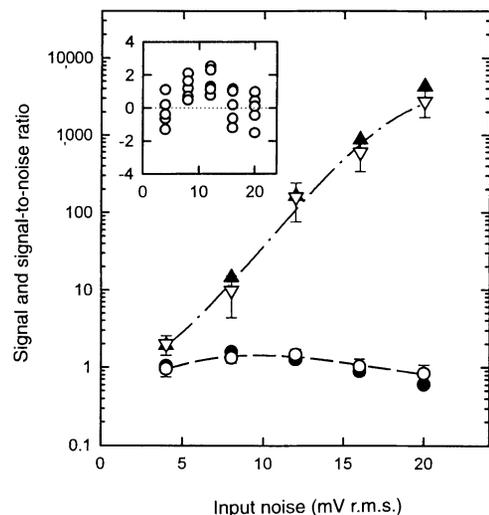


FIG. 3 A major noise-induced increase in signal output is achieved at an essentially constant, or even slightly increased, signal-to-noise ratio. The output signal (triangles) and the signal-to-noise ratio (circles) are given in units of their values at zero input noise. Sine waves of 5 mV (r.m.s.) and 0.2 Hz (filled symbols) and 0.5 Hz (open symbols) served as the input signal. The output signal-to-noise ratio was defined as the ratio of the signal peak maximum (Fig. 2*a*) to the background noise calculated as an average over spectral components in the immediate peak vicinity. Inset, the statistics of signal-to-noise ratio measurements on a finer noise voltage range. The vertical axis is labelled in dB units. Each point represents an average over a 20-min signal recording.

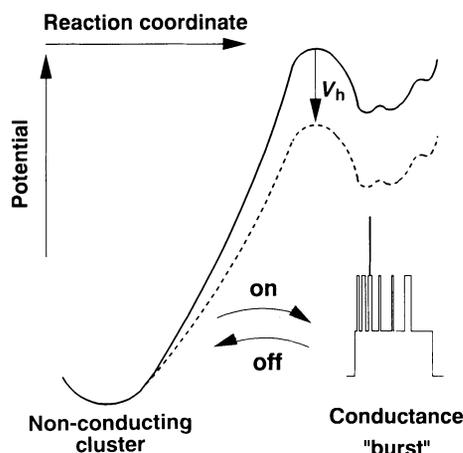


FIG. 4 The energy diagram of a quasi-bistable profile describing transitions of an alamethicin channel between non-conducting and conducting aggregates. The probability distribution along a reaction coordinate is sensitive to the transmembrane voltage mostly at the level of the transition between two main energy wells. In a conducting state corresponding to the higher-energy well, the channel voltage sensitivity is virtually lost. Transitions within this well describe flickering of the channel between different sub-levels during the conductance 'burst' shown in Fig. 1c. A small sine-wave signal modulates the probability of channel opening, introducing correlations in the moments of current burst (Figs 1 and 2). These correlations are the basis of the mechanism of electrical signal transduction through the system of voltage-dependent ion channels. Figure 3 shows that this transduction can be enhanced by application of random noise to the system input.

amplitudes of external noise increase both the signal and the noise at the system output, but the signal grows faster.

In our simple system, up to 100 parallel ion channels (formed by alamethicin) control the current by switching in a stochastic manner between the closed state and one of their open states. The probability of the channel being open strongly depends on the applied membrane voltage, whereas the relative probabilities of different conductive states are only weak functions of the voltage. A schematic diagram of the 'quasi-bistable' potential modulated by transmembrane voltage (Fig. 4) can explain such behaviour. The energy difference between the main potential wells is governed by the transmembrane voltage; the energy profile within 'burst' substates does not depend significantly on the voltage. A membrane with the alamethicin channel can therefore be viewed as a voltage-driven two-state dynamic system. A time-periodic signal applied to this system modulates the probability distribution between the two main states of the channel. Adding white noise to the signal increases the probability of conducting states. This happens at moments when the instantaneous value of the noise adds in phase with the positive half-waves of the applied signal.

Our results for a parallel array of voltage-dependent ion channels (representing a system of low biological complexity) suggest a novel mechanism of channel function regulation by a fluctuating environment. We have shown that addition of external noise to the input of this system induces a significant enhancement of signal transduction at constant (or, for some intermediate noise values, even increasing) signal-to-noise ratio at the output.

Biological signal processing often outperforms modern electronic devices⁴. Sensory transduction and neural signal processing are good examples of biological systems that function in highly fluctuating environments. In electronic instruments, the first amplification stage is designed to produce a high gain with minimal noise passed into the processing system. Special noise-rejection algorithms were invented that unfortunately always limited performance of signal processing devices. Perhaps nature devises amplification methods that can use an ambient noise to

improve signal transduction. Some of these amplifiers could be utilizing voltage-dependent ion channels in the way our system does.

Understanding signal processing and amplification by living organisms will require research at different levels of complexity. Our studies demonstrate that even the system of this simplicity (parallel voltage-dependent ion channels reconstituted in a lipid bilayer) is capable of ambient noise utilization. This is in accord with a recent model of stochastic resonance in parallel systems¹³ which showed that stochastic resonance might be a much more general phenomenon than previously thought and might apply to diverse systems not previously considered. □

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Effect of high salt concentrations on water structure

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THE characteristic tetrahedral structure of water is known to be disrupted by changes in pressure and temperature^{1–3}. It has been suggested that ions in solution may have a similar perturbing effect^{4,5}. Here we use neutron diffraction to compare the effects of applied pressure and high salt concentrations on the hydrogen-bonded network of water. We find that the ions induce a change in structure equivalent to the application of high pressures, and that the size of the effect is ion-specific. Ionic concentrations of a few moles per litre have equivalent pressures that can exceed a thousand atmospheres. We propose that these changes may be understood in terms of the partial molar volume of the ions, relative to those of water molecules. The equivalent induced pressure of a particular ion species is correlated with its efficacy in precipitating, or salting-out, proteins from solution⁶.

Salting-out and salting-in are processes whereby concentrated aqueous salt solutions fractionate, concentrate and crystallize proteins and other biological macromolecules from solution. One of the first studies of the phenomenon of salting-out was performed by Hofmeister⁶, who showed that different salts have different efficiencies at salting-out egg-white protein, and that some salts do not cause salting-out at all. The phenomenon of salting-out or -in is not yet understood but the conventional view⁷ is that competition between dissolved salt and dissolved protein for water of hydration results in a loss or gain in solubility