

## Letters to the Editor

### A Comment on the Sensitivity of Fish to Low Electric Fields

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The sensitivity of sharks, skates, rays, and similar animals to extremely low electric fields is a popular topic in the non-linear analysis community. It has been considered often in general editorial comments (Tsong, 1994; Glanz, 1996) and very recently in the “New and Notable” section of *Biophysical Journal* (Moss, 1997). Indeed, the sensitivity of some fish to electric fields appears to be astounding. Behavioral evaluation of the lowest field perceived by rays, found earlier to be 10 nV/cm in water (Kalmijn, 1982), has recently been reduced to 1–2 nV/cm (Kalmijn, 1997). Such a field, if applied directly to a sensory cell, produces a change in the transmembrane potential that is absolutely negligible in comparison with spontaneous fluctuations. On this premise, the usually accepted evaluation of  $10^{-7}$  for the signal-to-noise ratio (SNR) is obtained (Block, 1992)—a really astonishing value. It seems impossible to detect such a low signal by ordinary means, thus lending credence to the idea that detection requires methods qualitatively different from traditional techniques of linear analysis. It has been said that in order to reach the sensitivity of fish “artificial devices . . . should work at liquid nitrogen temperature” (Glanz, 1996).

Let us take a closer look at the actual biological situation to evaluate both the signal produced at the cellular level by the external field and the noise in the membrane potential in a receptor cell.

We start with the evaluation of the potential drop induced by the external field on the membrane of the electroreceptors. To make a straightforward comparison with the former evaluation, we will consider a field of 10 nV/cm, the value already used to calculate an  $\text{SNR} = 10^{-7}$ . This value was obtained by assuming that the potential drop through the membrane of a receptor cell was equal to that occurring over the cell length (10  $\mu\text{m}$ ). Actually, the weak field generated by the prey is measured over a much longer distance: the Lorenzini ampulla is a relatively insulated organ, connected to the external water through insulated canals filled with a conductive jelly (Waltman, 1966; Kalmijn, 1974; Murray, 1974). A simple way to enhance the electric signal before contamination with the noise in the membrane potential of the receptor is used; elasmobranchs

(fish like sharks and rays) take advantage of their extended body. The potential drop on the receptors in the sensory epithelium corresponds to that occurring between the canal pore and the ampulla (5 cm for the longest canals in medium-sized dogfish) and is therefore of the order of 0.05  $\mu\text{V}$  for a field of 10 nV/cm. A factor of  $5 \times 10^3$  is therefore introduced with respect to the former evaluation.

The noise in the membrane potential has been evaluated by applying basic physical principles (Weaver and Astumian, 1990; Block, 1992). Schematically, a cell membrane can be represented as a first approximation by an equivalent circuit in which membrane resistance (R) and capacitance (C) are combined in parallel. Considering the voltage drop across a cell membrane, it is known by the Johnson-Nyquist theorem that  $\langle V_n^2 \rangle = k_B T/C$ , where  $k_B$  and  $T$  have their usual meaning. For a spherical cell of radius  $r$  (10  $\mu\text{m}$ ), and assuming the specific capacitance of a lipid bilayer is 1  $\mu\text{F}/\text{cm}^2$ , C is about 3 pF; therefore,  $\langle V_n^2 \rangle^{1/2} = 30 \mu\text{V}$ . This is a lower limit for the noise that one might expect on the voltage across the membrane of an average-sized cell. This value cannot be lowered; however,  $\langle V_n^2 \rangle^{1/2}$  is inversely proportional to the square root of C and therefore decreases with increasing membrane surface. Moreover, it is important to evaluate the number of cells that connect the same area in the central nervous system. Figs. 1D and 1F in Murray (1974) show that electroreceptors in each ampulla at the bottom of a canal number about 10,000, divided among a small number of swellings; the number of fibers innervating each ampulla (five) is about the same as the number of the swellings and they project to the same brain area. Therefore it is conceivable that the output of  $10^4$  cells are averaged in certain areas of the brain, and in this case the voltage thermal fluctuations might be as low as 0.6  $\mu\text{V}$  root-mean-square.

Biological excess noise is to be expected because ion channels switch continuously from the open to the closed conformation also because of thermal fluctuations. However, the current flowing through the open channel is due to the electrochemical gradient through the membrane, and the nonequilibrium situation is the actual source of the excess noise. The excess noise depends on the number and types of channels that are open in the resting condition. It is possible to retrieve experimental evaluations of  $\langle V_n^2 \rangle^{1/2}$  in receptor cells. For instance, the amplitude for the voltage fluctuations in retinal bipolar cells (cell membrane capacitance of 11 pF) of the larval amphibian axolotl, a kind of salamander, was evaluated to be 100–300  $\mu\text{V}$  (Tessier-Lavigne et al., 1988), whereas the voltage noise of an insect

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photoreceptor (cell membrane capacitance of 14 pF) is  $\sim 100 \mu\text{V}$  (Stephenson, 1988).

We made numerical simulations of the equivalent electric scheme of a cell membrane to obtain qualitative insight into the dependence of voltage noise on cell parameters. The obvious result was that voltage fluctuations due to the switching of ion channels decrease when the ion channel kinetics are fast compared to the membrane time constant, suggesting a possible way to minimize nonequilibrium noise in specialized structures. As for the fundamental thermal noise, a decrease of the excess voltage noise with the square root of the cell surface is to be expected.

A reasonable estimate of the voltage noise due to ion channel switching in a single electroreceptor is therefore  $200 \mu\text{V}$  (based on the data by Stephenson (1988), normalized for the difference in cell radii); by the same reasoning used for thermal noise, we consider that averaging this noise over  $10^4$  cells results in a final root-mean-square value of  $2 \mu\text{V}$ . However, it is worth recalling that the responses to voltage steps lasting 0.5 s in the excised ampullary organ have a dynamic range between  $-100$  and  $20 \mu\text{V}$  (Lu and Fishman, 1994a,b), and that a variation in the discharge of the nerve fibers has been measured for voltage steps of  $3 \mu\text{V}$ , which suggests that the noise at low frequencies is only a fraction of a microvolt (and each nerve fiber averages over just  $2 \times 10^3$  electroreceptor cells). Clearly, only direct experimental measurements can reveal how much noise originates in the electroreceptors and how its spectral distribution is shaped.

The evaluation of SNR using the estimates of voltage noise ( $2 \mu\text{V}$ ) and voltage drop ( $50 \text{ nV}$ ) reported above yields an SNR value of  $\approx 10^{-2}$  for a canal of 5 cm. This value, however, should be divided by 10 according to the lowest evaluation of the threshold field,  $1\text{--}2 \text{ nV/cm}$  (Kalmijn, 1997). This evaluation, very different from the usually accepted value of  $10^{-7}$  for the SNR, is in agreement with considerations reported by Weaver and Astumian (1990) (see their Table 1 and the explanation reported in their note 2).

Could ordinary techniques detect signals with such SNR? By "ordinary techniques" we mean not only averaging of different units with independent noise, but also Fourier analysis or similar processing. Fourier analysis is a powerful tool for the detection of small signals, sorting them out of the noise contributions at different frequencies. This sorting out is in principle only limited by the time of observation. In a cell Fourier analysis may be implemented if the transduction system acts as a selective amplifier around a resonance frequency, as seems to occur in the excised electric organ (Lu and Fishman, 1994a). As for further averaging, about 1000 canals of different length are present in a single animal

(A. J. Kalmijn, personal communication) and the longest ones (about 20) are nearly parallel; i.e., to a reasonable approximation 20 equivalent units can be averaged. This could also help reduce the noise originating in the ohmic resistance of the canals.

Thus with the present evaluation of SNR we propose that ordinary tools can work, whereas the former evaluation of  $10^{-7}$  seemed to rule out this possibility. Only the experimental study of the system under physiological conditions can give direct answers to questions about how electroreception operates. However, there are insufficient reasons to assume that traditional techniques such as linear analysis do not work. The hope that systems exhibiting stochastic resonance can improve SNR has recently been denied by a clear-cut note in *Nature* (Dykman and McClintock, 1998).

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## Reconstitution of Phospholipid Bilayer by an Atomic Force Microscope Tip

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In a recent paper, Carlson et al. (1997) reported the use of the atomic force microscope (AFM) to initiate bilayer fusion in a controlled manner. In their study of reconstituted high-density lipoproteins (HDL) they obtained discoidal bilayers of dipalmitoyl phosphatidylcholine (DPPC) and apolipoprotein A-I (apoA-I), the primary protein component of HDL. Under certain experimental conditions the fusion of the discoidal bilayers into a planar bilayer was induced by the scanning process in the AFM. In other cases, scanning of the HDL particles resulted in digging a monolayer-thick hole. The difference between fusion and digging did not appear to depend on force or scan rate. It was not clear whether protein played a role in the fusion process.

Independently, we observed a similar effect on a phospholipid bilayer in the absence of protein. The results were first obtained on a control sample used in our study of the interaction of the polyene antibiotic filipin with planar bilayers (Santos et al., 1998). The bilayer of 7:3 (w:w) dipalmitoyl phosphatidylethanolamine (DPPE):cholesterol was prepared by Langmuir-Blodgett deposition on freshly cleaved mica at a surface pressure of 25 mN/m. The sample was then imaged by contact-mode AFM under water. Silicon nitride tips with a nominal spring constant of 0.12 N/m (Digital Instruments, Santa Barbara, CA) were used. The bilayer was unintentionally exposed to air for a moment during transfer to the fluid cell of the AFM. The short exposure to air probably led to the disruption of the bilayer into monolayer and trilayer domains; subsequent resreading led to a highly defective bilayer.

Fig. 1 *a* shows the AFM image of the resread bilayer. The bilayer contains numerous holes with a depth of about 2.5 nm, which is consistent with the thickness of a monolayer. A  $5 \times 5 \mu\text{m}^2$  square region in the central area of the image was generated by scanning at a force of 20 nN for 2 minutes, then imaged by increasing the scan area and immediately recording the image at a force of 5 nN. The scanning process heals the defective bilayer and produces a flat hole-free area. Fig. 1 *b* displays the AFM image of another DPPE/cholesterol sample prepared under the same conditions as the previous sample with an air exposure of

2 s. Monolayer patches are clearly seen within holes, which are also one layer thick. We could not produce a hole-free area like in Fig. 1 *a* by applying a higher force, although fusion of patches could be induced. Fig. 1 *c* shows the same area as in Fig. 1 *b* after 10 min of scanning with a force of 25 nN and a scan rate of 10 Hz. Some patches fused with their nearest neighbors. Applying a higher force did not accelerate fusion and eventually led to the rupture and removal of the bilayer.

Exposure to air was not necessary to induce the healing of holes. Fig. 2 *a* shows a pure DPPE bilayer deposited at a surface pressure of 25 mN/m. The sample was kept under water during transfer to the AFM. A flat hole-free square region in the center of the image was generated by scanning for 2 min with a force of 100 nN, then imaged by increasing the scan size and recording the image at a force of 5 nN. Note that such healing could be induced only for samples with monolayer-thick holes. It is not clear why Langmuir-Blodgett deposition sometimes produces monolayer-thick holes in DPPE bilayers. It is much more common to have bilayer-thick holes in bilayers (Mou et al., 1995, Shao et al., 1996) although monolayer-thick holes have also been observed (Hui et al., 1995). In the samples with bilayer-thick holes we did not observe any healing. Applying a higher force led to the coalescence of nearby holes and then to removal of the bilayer.

The mechanism of the tip-induced reconstitution is not clear although it is logical that energy brought by the AFM tip helps to convert unstable holes or disks into a lower-energy bilayer. One possible explanation is that the AFM tip drags lipid molecules that are loosely attached or extracted from bilayer until they fill in holes in bilayer. These molecules might also adsorb on the AFM tip during this process, forming a layer on it. Fig. 2 *b* shows an AFM image of a hole in a DPPE/cholesterol bilayer. The hole appears to be one layer thick and to contain another monolayer-thick hole inside. However, it is clearly seen that the lower edges of both holes have exactly the same shape, which is a very unlikely event. Such an image might be generated if the hole were two layers thick and the AFM tip had an adsorbed monolayer on it. As the substrate step is deeper than the step on the AFM tip, the tip cannot reach the bottom of the hole and the hole edge is imaged twice, once with the tip itself and once with monolayer on it (double tip effect). Thus, the image in Fig. 2 *b* suggests that lipids can adsorb to the AFM tip during the scanning of bilayers in water.

Our results show that patterning similar to that shown by Carlson et al. (1997) can be obtained on samples without

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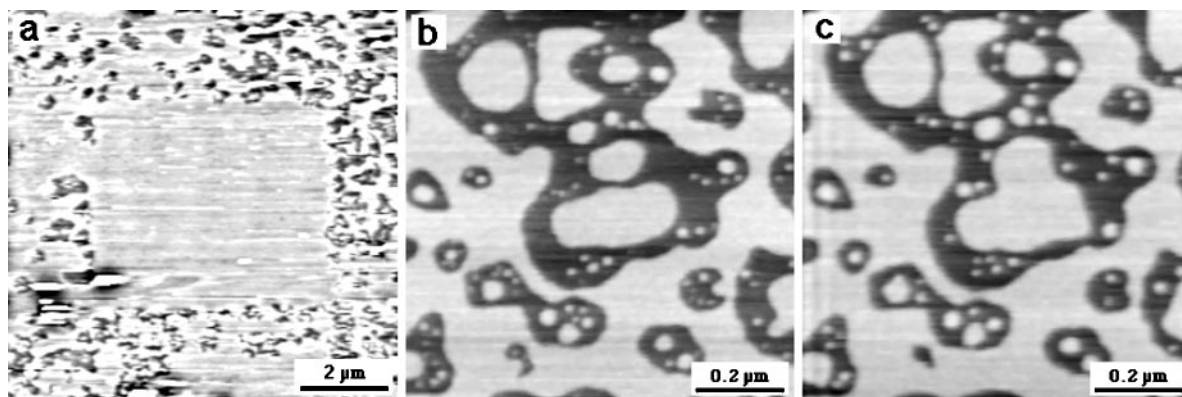


FIGURE 1 AFM height images of a DPPE/cholesterol bilayer which was exposed to air for a short time. All images are taken in a contact mode under water. (a) Area:  $9 \times 9 \mu\text{m}^2$ . A square defect-free region in the central part of the image was reconstituted by the AFM tip during several scanings at a force of 20 nN. (b) Area:  $900 \times 900 \text{ nm}^2$ . Monolayer-thick patches within holes are clearly seen. (c) Area:  $900 \times 900 \text{ nm}^2$ , the same region as in (b) after scanning at 25 nN for 10 minutes. Some patches fused with their nearest neighbors.

protein. We could not establish the precise conditions necessary for patterning but have shown that it is crucial to have a bilayer with monolayer-thick holes. While additional work will be necessary before the mechanism of patterning

is fully understood, the effect opens new possibilities for manipulation of phospholipid bilayers and formation of nanoscopic defect-free regions with a desired size and shape.

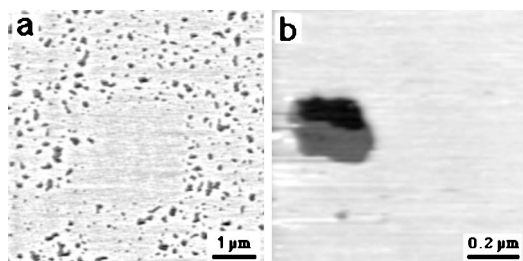


FIGURE 2 (a) AFM height image of a DPPE bilayer under water. A square defect-free region in the central part of the image was reconstituted by the AFM tip after scanning for 2 minutes at a force of 200 nN. Area:  $6 \times 6 \mu\text{m}^2$ . (b) AFM image of a hole in a DPPE/cholesterol bilayer. Note that the lower edges of the hole have the same shape (double tip effect). Area:  $1 \times 1 \mu\text{m}^2$ .

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