Possible Mechanism for Detection of Weak Low-Frequency Electric Fields.

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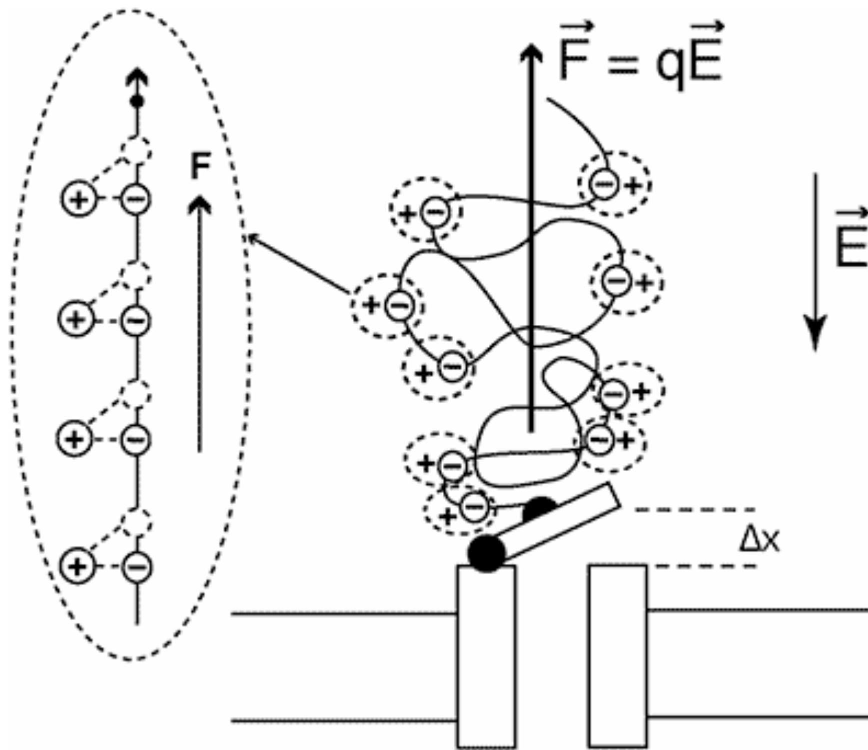
Abstract

The mechanism by which animals detect weak electric and magnetic fields has not yet been elucidated. We propose that transduction of an electric field (E) occurs at the apical membrane of a specialized cell as a consequence of an interaction between the field and glycoproteins bound to the gates of ion channels. According to the model, a glycoprotein mass (M) could control the gates of ion channels, where $M > 1.4 \times 10^{-6}/\mathbf{E}$, resulting in a signal of sufficient strength to overcome thermal noise. Using the electroreceptor organ of *Kryptopterus* as a mathematical and experimental model, we showed that fields as low as 2 μV/m (at 10 Hz) could be detected, and that the observation could be explained if a glycoprotein mass of 0.7×10^{-12} kg (a sphere 11 μm in diameter) were bound to channel gates. Antibodies against apical membrane structures in *Kryptopterus* blocked field transduction, which was consistent with the proposal that it occurred at the membrane surface. Although the target of the field was hypothesized to be an ion channel, the proposed mechanism can easily be extended to include other kinds of membrane proteins.

Proposed Model for Electroreception



An applied electric field exerts a force **F** on a negatively charged gel molecule thereby mechanically opening the gate of an ion channel. The molecule may be covalently bound to the gate, or may be interleaved with glyco-groups covalently attached to the gate. The displacement of the channel gate (Δx) is assumed to be 6 nm. The model assumes that the negative glyco-groups rotate slightly with respect to the counter-ions.

We postulate that electric fields are detected by means of a process in which the field exerts a force on glycoprotein molecules that are connected to the gate of an ion channel. Formally, $|q\mathbf{E}\Delta x| > U > kT/2$, where q is the net negative charge on the molecules, **E** is the electric field, Δx is the displacement of the channel gate (~6 nm), U is the potential-energy barrier between closed and opened channel states, k is Boltzman's constant, T is temperature, and kT/2 is the thermal energy associated with one degree of freedom. Each glycoprotein molecule contains many negative charges ($q = -eZ$, where e is the elementary charge and Z is the number of charges per molecule); the molecules could be connected to each other by covalent or non-covalent bonds. Thus, $neZ\mathbf{E}\Delta x > kT/2$, where n is the number of molecules that control a channel.

To estimate the mass (M) of the clycoproteins that could control a channel gate, we assume that the molecules are polymers and that each monomer has one negative elementary charge, and the same mass, m (assumed to be that of a hyaluronan disaccharide, 6.697 x 10-25 kg). Then, M = nZm, and from the inequality above, M > kT m/2 e**E**Δx = 1.4 x 10^{-6}/**E**.

Purpose

We propose that field transduction occurs at the apical membrane as a consequence of an interaction between the field and glycoproteins bound to the gates of ion channels. Our objective was to calculate the strength of the threshold field detected by *Kryptopterus*, an electrosensitive fish, to permit an evaluation of the reasonableness of the model. Our second objective was to test the model by showing that transduction occurred at the apical membrane, as proposed; this was accomplished by means of blocking antibodies.

Materials and Methods

Electrical Measurements

Glass catfish (*Kryptopterus bicirrhis*, approximately 5 cm long) were studied. Current was applied by means of parallel silver wires located on either side of the anal fin. The spike frequency of the electroreceptor nerve was measured using a glass Ag-AgCl microelectrode (10 MΩ) inserted into the opening of an electroreceptor organ.

For determination of the effect of antibodies (see below) on spike frequency, a micropipette was inserted into the same organ whose activity had been studied in the absence and presence of the field, and antibodies or control antibodies were injected at the surface of the electroreceptor cells using low positive pressure, after which the measurement of spike frequency in the absence and presence of the field was repeated. The effect of the antibodies on the field-induced change in spike frequency was evaluated using the Mann-Whitney U test.

Antibodies

Anal and tail fins, which contain numerous electroreceptor organs were dissected from approximately 70 fish and pooled, and antibodies against plasma-membrane fragments were produced using standard methods.

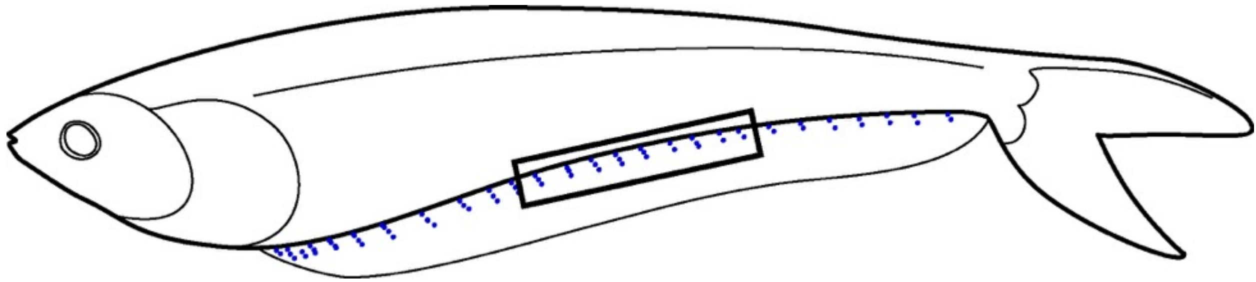
Histochemistry

Anal fins were fixed in formalin, processed for paraffin embedding using standard techniques, and 5-micron sections cut perpendicularly to the fin were mounted on slides. Odd-numbered slides were stained with hematoxylin and eosin (H&E). When electroreceptor organs were identified, the corresponding even-numbered slides were used for immunohistochemistry.

Electric Field Calculations

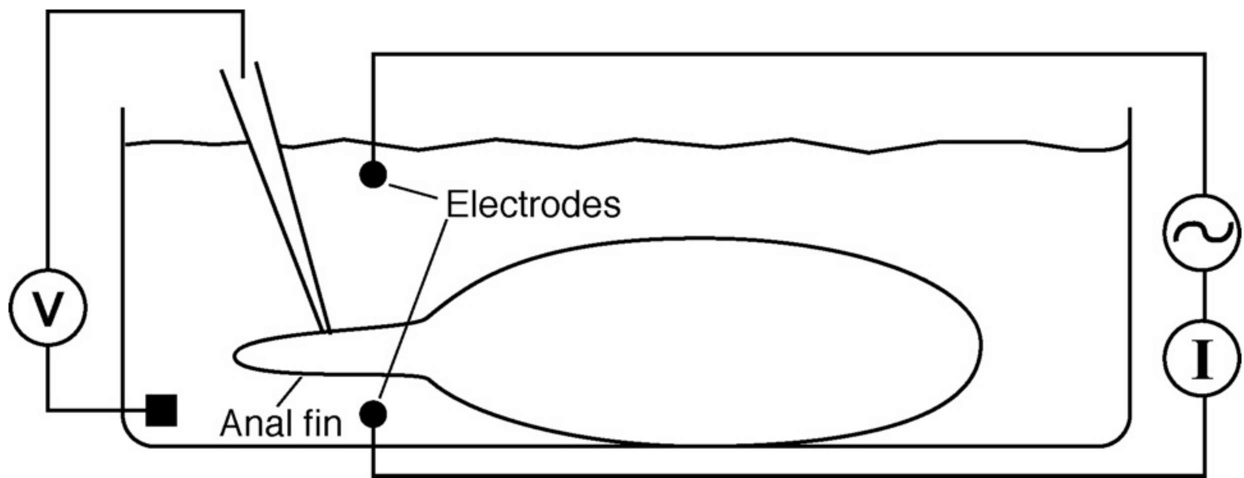
A representative fish was cut into a contiguous series of 1-mm thick transverse sections, and the shape and dimension of each section were used to build a mathematical model for *Kryptopterus*. Assumed conductivities of the skin and internal tissue were 0.8 mS/m and 100 mS/m, respectively; the conductivity of the water was the measured value (24 mS/m). The electric field was calculated in two stages, using Femlab (Cosmol, Los Angeles, CA). First, the field was determined at 10 µm above the surface of the anal fin in the vicinity of an electroreceptor organ, and those values were then used as boundary conditions to find the field within the organ. The apical face of each electroreceptor cell is exposed to the lumen of the organ, and the cell is sealed around its margin by tight junctions. The group of electroreceptor cells of the organ was therefore modeled as an oblate spheroid (semi-major and semi-minor axes of 26 and 7 µm, respectively) located at the bottom of a glycoprotein-filled spherical cavity 90 µm in diameter. The conductivity of the interior of the spheroid was taken as 100 mS/m; it was bounded by a 5-nm membrane having a conductivity of 0.07 µS/m. The lumen (24 mS/m) was separated from the internal tissue of the fish (100 mS/m) by a 5-µm thick membrane (0.8 mS/m) that lined the cavity and was continuous with the skin; the cavity was connected to the water by a circular opening of diameter 20 µm.

Location of electroreceptor organs (shown as dots) on the anal fin of *Kryptopterus bicirrhus*.

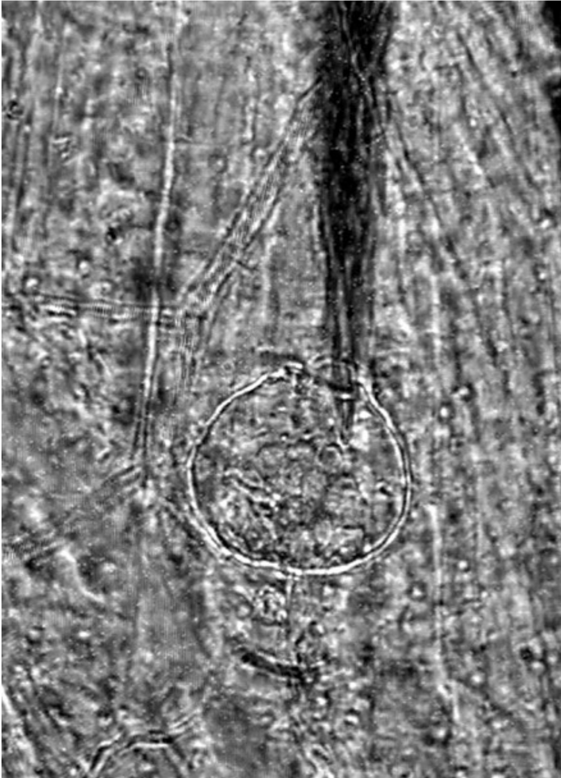


Rectangle depicts the relative position of the stimulus electrodes (1 cm long, 0,5 mm in diameter; center-to-center separation, 8 mm).

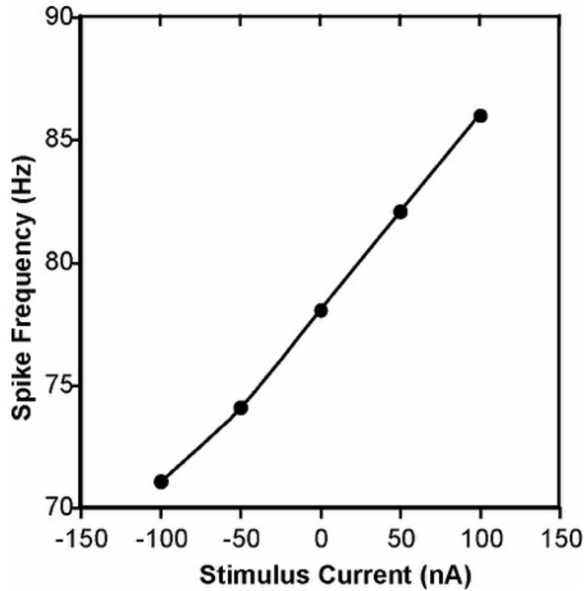
Cross-sectional view depicting voltage measurement inside an electro-receptor organ of an anesthetized fish exposed to an electric field.



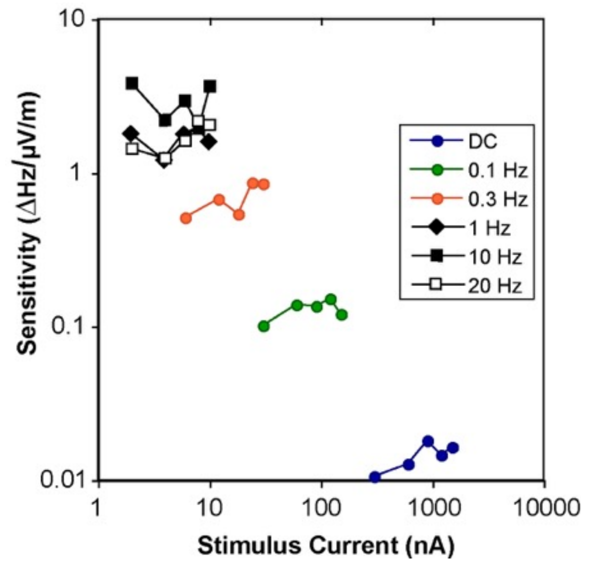
Microelectrode inserted into the opening of an electroreceptor organ.



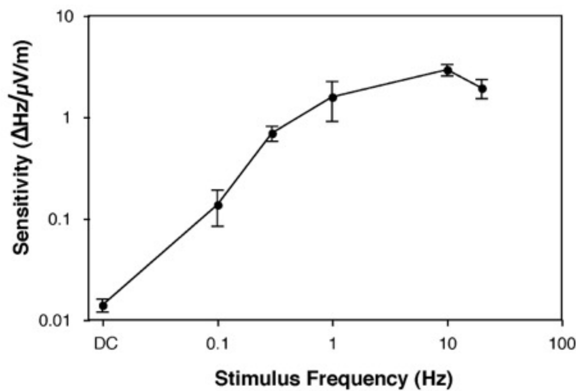
Spike frequency of the afferent nerve of an electroreceptor organ in a typical fish as a function of stimulus magnitude and polarity.



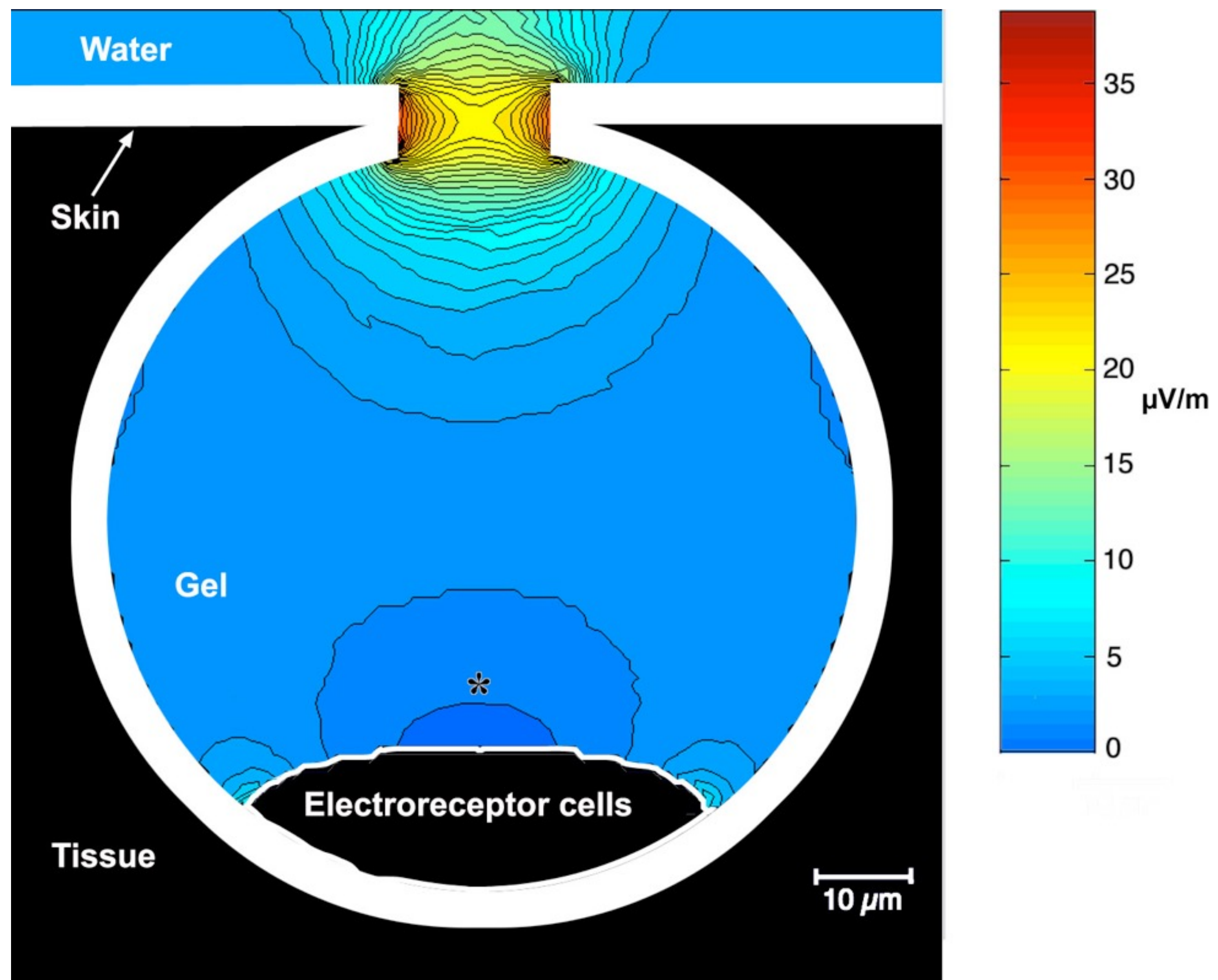
Sensitivity (change in spike frequency per unit field) of the same fish as a function of stimulus intensity and frequency (insert).



Effect of stimulus frequency on sensitivity (mean ± SD) of the electroreceptor organ of *Kryptopterus* (electroreceptor organs from 10 fish).

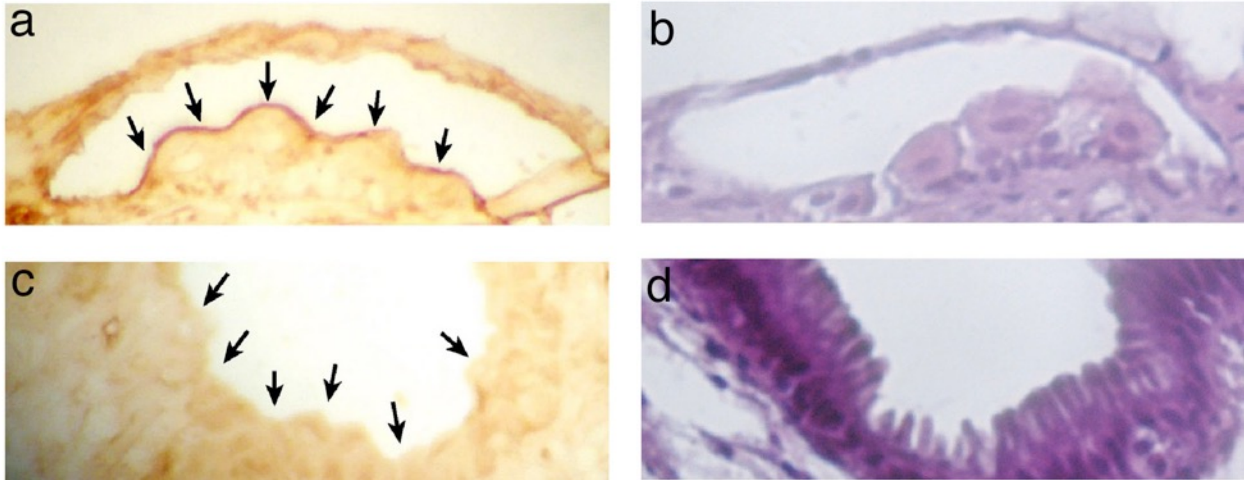


Calculated electric field inside an electroreceptor organ corresponding to the application of 2 nA.



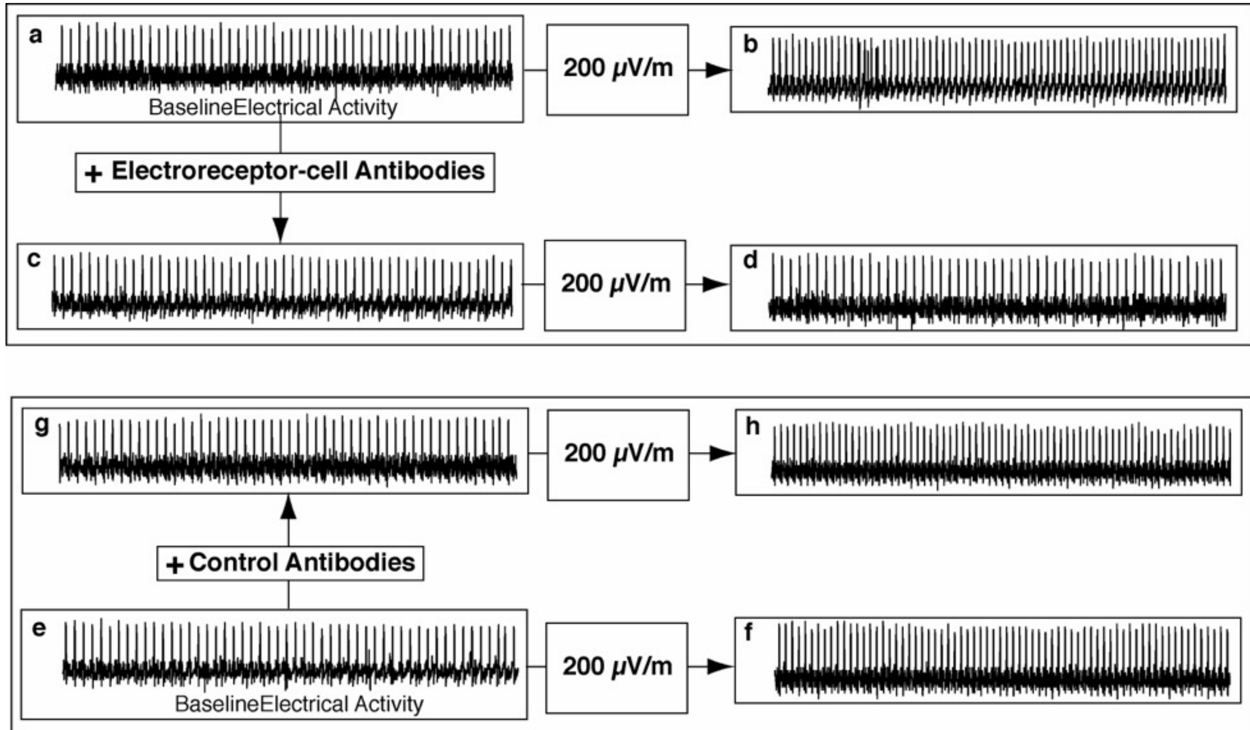
The fields associated with higher currents were proportionally greater. Resolution of contour lines, 2 μV/m (first contour (*), 1.5 μV/m). Assumed conductivities: water and gel, 24 mS/m; tissue, 100 mS/m; cell interior, 100 mS/m; cell membrane, 0.07 μS/m; skin, 0.8 mS/m. Under our model, the electric field inside an electroreceptor organ is the effector agent of transduction. Therefore, for clarity, the electric field in the tissue and in the interior of the electroreceptor cells was set to zero in the illustration.

Histochemistry of tissues from *Kryptopterus* (60x).



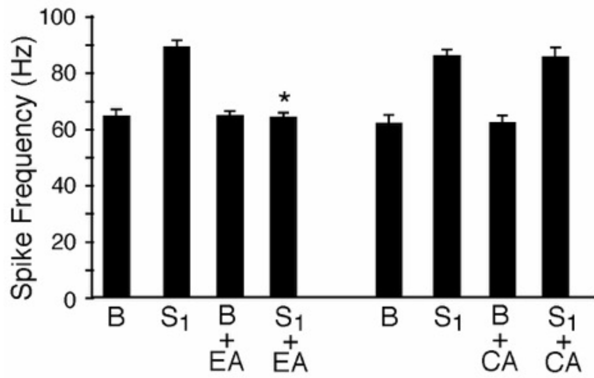
a, Section through electroreceptor organ showing staining of the apical membranes of the electroreceptor cells with electroreceptor-cell antibodies (arrows). **b,** A section 15 μm distant, stained with H&E to show individual cells. **c,** A section through the gut to show that the same antibodies did not stain the gut epithelial layer (arrows). **d,** An adjacent section of the gut stained with H&E to show that the epithelial layer was intact.

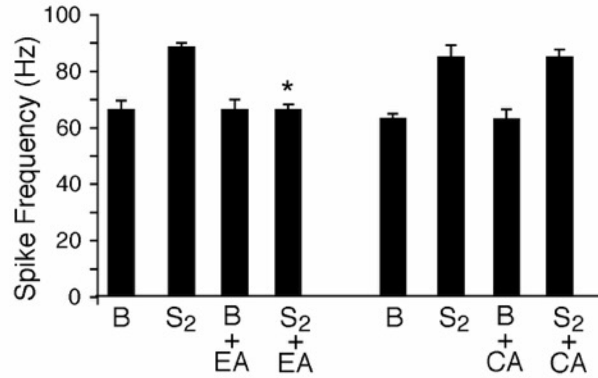
Effect of antibodies on the response of the electroreceptor nerve to the application of 200 μV/m, DC (200 nA).

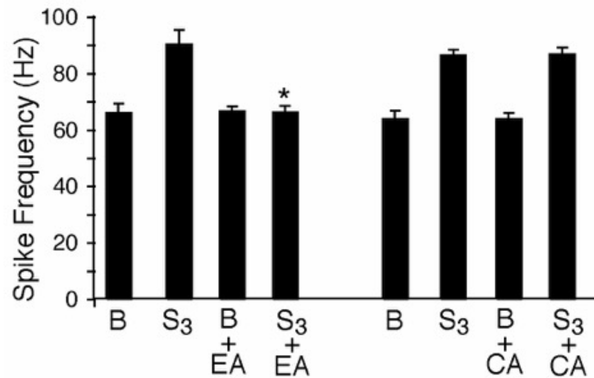


a, Baseline (pre-stimulus) electrical activity. **b,** Application of electric field. **c,** Baseline activity after addition of mouse antibodies raised against *Kryptopterus* electroreceptor-cell membrane fragments. **d,** Electrical activity in the presence of both the antibodies and the electric field. **e, f, g, h,** Same conditions as in **a, b, c, d** respectively (using another fish), except using control antibodies. The duration of each record was 0.8 seconds.

Effect of antibodies on the response of the electroreceptor nerve to low-frequency electric fields.







Each group of 4 measurements was made using a different fish. B, baseline electrical activity. S_1, 10 μV/m (10 nA), 5 Hz; S_2, 10 μV/m (10 nA), 10 Hz; S_3, 10 μV/m (10 nA), 20 Hz. EA, electroreceptor-cell antibodies. CA, control antibodies. *$P < 0.05$, compared with electric field alone.

Discussion

According to our model, the minimal mass of glycoproteins needed to detect a field of 2 μV is $M \approx 1.4 \times 10^{-6}/2 \times 10^{-6} \approx 0.7 \times 10^{-12}$ kg, which corresponds to a sphere of about 11 μm in diameter. Electroreceptor cells have diameters of 10-20 μm. If we assume that the mass of glycoproteins on the cell surface is ellipsoidal, it is easy to see that it could control the opening of 10-20 ion channels per cell, which could be sufficient to initiate transduction by the same mechanism as that occurring in stretch receptors.

The target in the membrane need not be an ion channel. It could be, for example, an integral membrane protein whose extracellular portion binds to glycoproteins and undergoes a structural modification in the presence of a field resulting in activation of an enzyme at the intracellular terminus of the protein.

Antibodies against membrane structures blocked transduction of the field. One possibility was that the antibodies became bound to charged structures that directly interacted with the field or were involved in the early part of the signaling pathway, thereby blocking transmission of a signal that coded for the presence of the field.

Although the results showed that an important component of the system responsible for detecting the field was located on the apical membrane, they did not prove that the component necessarily played an active role. The possibility remained that the antibodies merely plugged hypothetical unregulated pores in the apical membrane, thereby preventing the electric current from passing through the apical membrane into the cell. If unregulated pores were actually present in the apical membrane, it can be shown that the electric field there would propagate to the inner surface of the basolateral membrane. The proposed model could then explain transduction on the basis of ion channels in the basolateral membrane.