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An artificial membrane-cable: decay and delay of electrical potentials along a lipid bilayer with ion channels

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A model membrane is described which exhibits the properties of a neurite with respect to passive propagation of electrical potentials. A groove in a glass plate is covered by a black lipid membrane of glycerol monooleate. Gramicidin is incorporated. The stationary and transient response of the assembly is tested by two experiments: (i) One end of the groove is clamped at a constant voltage. The voltage at the other end and the total electrical current are measured. (ii) A charge pulse is injected at one end of the groove. The time-dependent voltage at the other end is measured. The results with respect to the lateral decay and delay of voltage are in quantitative agreement with the stationary and transient solutions of Kelvin's equation for a homogeneous cable. If gramicidin is incorporated unevenly along the membrane, the lateral decay of voltage is found to be asymmetric with respect to both directions. The cable is a partial one-way transmission line.

Introduction

The extensive studies on planar model membranes during the last 25 years were directed to reconstitute the local electrical properties of biological membranes as their capacitance and conductance.

Crucial features of biological membranes are related, however, to lateral spread of electrical signals. Typical examples are the propagation of an action potential in neural axons [1], linear and nonlinear signal processing in neural dendrites [2,3] and pattern formation by dissipative selforganization [4,5].

In the present paper we describe a model membrane which is suitable to study processes where the lateral spread of the electrical membrane potential plays a role. We use a variant of the BLM technique [6–11]. The system exhibits all features of a core-coat conductor as described by Kelvin's cable equation with respect to the propagation of stationary and transient polarizations.

At first we summarize the pertinent solutions of the cable equation and discuss the design of an artificial cable as based on the technique of planar lipid bilayers. Then the experimental method is explained. The observations on lateral decay of a tonic polarization and the lateral delay of a phasic polarization are presented. They are compared with the solutions of the cable equation.

Theory

Kelvin equation

Let us consider an electrolytic 'wire' of resistance R_I per unit length as separated by a membrane of capacitance C_M and leak conductance G_M per unit length from an electrolytic bath of negligible resistance (Fig. 1). The electrical voltage $V_M(x, t)$ across the membrane as a function of space x and time t is determined by the balance of current densities according to Eqn. 1 (Kelvin's cable equation) [12]. In a finite cable of length l_M constraints at both ends $x = 0$ and $x = l_M$ have to be defined with respect to the gradient $\partial V_M/\partial x$ or the voltage V_M itself.

$$C_M \cdot \frac{\partial V_M}{\partial t} = \frac{1}{R_I} \cdot \frac{\partial^2 V_M}{\partial x^2} - G_M \cdot V_M \qquad (1)$$

Voltage clamp

Let us apply a local voltage clamp at one end ($x = 0$) as $V_M(0) = V_0$. The stationary profile $V_M^{SE}(x)$ of voltage for a sealed distal end is given by Eqn. 2 as obtained from Eqn. 1 with the constraint $(dV_M/dx)_{l_M} = 0$ [12]. Similarly the profile $V_M^{OE}(x)$ for an open distal end is given by Eqn. 3 as obtained with the constraint $V_M(l_M)$

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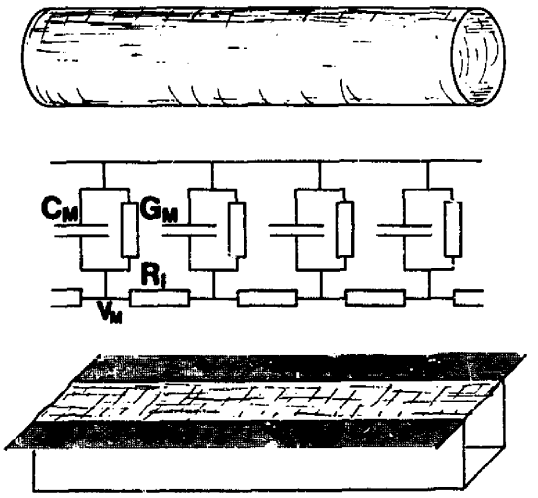

Fig. 1. Cables. Top: Conventional cable of a cylindrical membrane isolating a cylindrical electrolytic core from an electrolytic bath. Bottom: Prismatic cable of a planar membrane isolating the electrolyte in a groove in an isolating support from an electrolytic bath. Center: Equivalent circuit of a cable in discrete representation as defined by the resistance R_i per unit length of core, by the capacitance C_M per unit length of membrane and by the conductance G_M per unit length of membrane. The resistance of the bath is neglected. The state of the cable at each moment is defined by the profile of the voltage V_M along the core.

$= 0$. The length constant $\lambda = (G_M \cdot R_i)^{-1/2}$ characterizes the range of polarization.

$$V_M^{SE}(x) = V_0 \cdot \frac{\cosh[(l_M - x)/\lambda]}{\cosh(l_M/\lambda)} \tag{2}$$

$$V_M^{OE}(x) = V_0 \cdot \frac{\sinh[(l_M - x)/\lambda]}{\sinh(l_M/\lambda)} \tag{3}$$

Eqn. 2 defines the voltage V_{SE} at the sealed end as $V_{SE} = V_M^{SE}(l_M)$. The total (outward) currents I_{SE} and I_{OE} for sealed and open end are obtained from Eqns. 2 and 3 by integration. V_{SE}, I_{SE} and I_{OE} depend on the total resistance $R = R_i \cdot l_M$ of the core and the total conductance $G = G_M \cdot l_M$ of the membrane according to Eqns. 4, 5 and 6.

$$V_{SE} = V_0 \cdot \frac{1}{\cosh\sqrt{RG}} \tag{4}$$

$$I_{SE} = V_0 \cdot G \cdot \frac{\tgh\sqrt{RG}}{\sqrt{RG}} \tag{5}$$

$$I_{OE} = (V_0/R) \cdot \frac{\sqrt{RG}}{\tgh\sqrt{RG}} \tag{6}$$

In the limit $RG \ll 1$ (low core resistance, low membrane conductance) a sealed cable is isopotential with $V_{SE} = V_0$ and the currents through sealed and open cable depend on the membrane conductance and core resistance alone as $I_{SE} = V_0 \cdot G$ and $I_{OE} = V_0/R$, respectively.

By combining Eqns. 4 and 5 we may eliminate the conductance G and obtain an assignment of the voltage V_{SE} to the current I_{SE} for a sealed cable. The relation $V_{SE}(I_{SE})$ depends on the core resistance R alone. We may eliminate both parameters, conductance G and resistance R, by combining Eqns. 4 and 5 with Eqn. 6 and obtain a unique relation between the voltage V_{SE} and the currents I_{SE} and I_{OE} at a given V_0 according to Eqn. 7.

$$(V_{SE}/V_0)^2 = 1 - (I_{SE}/I_{OE}) \tag{7}$$

Charge pulse

Let us inject a narrow pulse of charge Q_0 at one end ($x = 0$) of a sealed cable. The constraints are $(\partial V_M/\partial x)_0 = -Q_0 \cdot R_i \delta(t)$ at the site of injection ($\delta(t)$ Dirac's delta-function) and $(\partial V_M/\partial x)_{l_M} = 0$ at the distal end. The voltage as a function of space and time is obtained from Eqn. 1 by Laplace-transformation [12] according to Eqn. 8.

$$V_M(x,t) = (Q_0/C_M l_M) \cdot e^{-t/\tau_M} \cdot \left\{1 + 2\sum_1^\infty (-1)^n \cdot e^{-n^2 t/\tau_S l_M^2} \cdot \cos[n\pi(1 - x/l_M)]\right\} \tag{8}$$

The time constant $\tau_M = C_M/G_M$ describes the relaxation of charge across the membrane. The diffusion constant $D_S = 1/R_i C_M$ describes the spread of charge along the membrane. From Eqn. 8 we obtain the time course of the voltage at the sealed distal end $V_{SE}(t)$ according to Eqn. 9.

$$V_{SE}(t) = (Q_0/C) \cdot e^{-t/\tau_M} \cdot \left\{1 + 2\sum_1^\infty (-1)^n \cdot e^{-n^2 t/\tau_S}\right\} \tag{9}$$

$C = C_M \cdot l_M$ is the total capacitance of the membrane. The voltage increases with some delay due to diffusive spread of charge as described by the time constant $\tau_S = R \cdot C/\pi^2$. It decays subsequently due to relaxation of charge as described by the time constant $\tau_M = C/G$. If the relaxation is slow as compared to spread for $\tau_M \gg \tau_S$ the voltage reaches transiently the amplitude Q_0/C for homogeneous distribution of injected charge.

Design of a cable

Geometrically a cable is distinguished by the anisometric shape of its membrane. Electrically a cable is distinguished by a short length constant $\lambda^2 = l_M^2/RG$ and a low diffusion constant $D_S = l_M^2/RC$ with respect to tonic and phasic perturbations.

Let us consider planar lipid bilayers spanning an opening in a septum between two aqueous phases [6–11]. An anisometric membrane, with a width w_M distinctly shorter than a length l_M, is obtained by spanning a slot in a septum as described in a previous paper [13].

A length resistance R appears if the membrane spans a subphase of finite depth d_S (Fig. 1). The resistance R depends on l_M, w_M and d_S and on the specific resistance ρ of the subphase as $R = \rho \cdot l_M / w_M \cdot d_S$. The subphase is an electrolyte of concentration [E] and molar conductivity Λ_E. Thus we obtain $\rho = (\Lambda_E \cdot [E])^{-1}$ and $R = l_M / w_M \cdot d_S \cdot \Lambda_E \cdot [E]$.

The conductance G depends on l_M and w_M and the specific conductivity g_M of the membrane as $G = g_M \cdot l_M \cdot w_M$. The membrane contains ion channels of density n_{CH} and conductance Λ_{CH}. Thus, we obtain $g_M = n_{CH} \cdot \Lambda_{CH}$. If the channel conductance is proportional to the concentration of the electrolyte as $\Lambda_{CH} = \Lambda'_{CH} \cdot [E]$ we obtain finally $G = n_{CH} \cdot \Lambda'_{CH} \cdot [E] \cdot l_M \cdot w_M$. The capacitance C depends on l_M and w_M and on the specific capacitance c_M of the membrane as $C = c_M \cdot l_M \cdot w_M$.

From the expressions for R, G and C we calculate the length constant λ and the diffusion constant D_S according to Eqns. 10 and 11.

$$\lambda^2 = d_S \cdot \Lambda_E / \Lambda'_{CH} \cdot n_{CH} \tag{10}$$

$$D_S = d_S \cdot [E] \cdot \Lambda_E / c_M \tag{11}$$

Apparently a shallow subphase (small d_S) is the prerequisite to attain a short length constant and a low diffusion constant, i.e., to attain cable properties of an anisometric planar lipid membrane. The length constant may be shortened by application of a high density n_{CH} of channels, whereas the diffusion constant may be reduced by application of a low concentration [E] of salt.

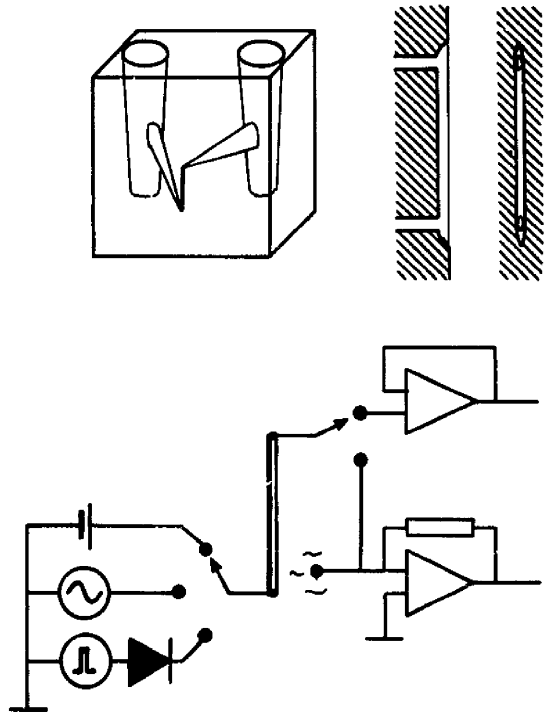

Fig. 2. Upper left: Glass block as a support for a cable-like structure. The vertical line in the center marks the groove. Ag/AgCl electrodes are inserted through the vertical NS5 joints and the inclined borings down to both ends of the groove. Upper right: Blow-up of the groove. Its depth is 0.3 mm, its width is 0.25 mm and its length between the connections is 4.5 mm. The inner surfaces of the groove are hydrophilic. The surface of the block is hydrophobic. The groove is covered by a black lipid membrane. Lower: Schematic diagram of the electrical circuit (i) to apply various waveforms of voltage to one end (DC-voltage, AC-voltage, charge pulse), (ii) to keep the distal end sealed or open at both potential and to measure the voltage at the sealed end by a voltage-follower and (iii) to measure the total current by a current voltage-converter keeping the bath at virtual ground.

Materials and Methods

Cell

The prototype of a cell (Fig. 2) is made by cutting a groove in a block of lead-glass (Lohmaier, Gussenstadt, F.R.G.) using a diamond saw (Brasseler, Lemgo, F.R.G.). The overall length of the groove with tapered ends is 6 mm. The edges of the groove are rough. The groove is contacted by two NS5 joints and two holes as drilled from the back (Fig. 2) using carbide tools (Brasseler, Lemgo, F.R.G.). Depth and width of the groove are constant between the two contacts as $d_S = 0.3$ mm and $w_M = 0.25$ mm. The length of the homogeneous part between the contacts is $l_M = 4.5$ mm.

In a second stage of experimentation the grooves are assembled from quartz plates of a thickness 0.3 mm as fused on top of a quartz block at appropriate distance. The edges are smooth. All results presented in this paper are obtained with the prototype. First experiments with the improved cells show that the lifetime of the membranes is distinctly extended.

The cells are cleaned with chromic acid (Merck, Darmstadt) for 24 h and rinsed extensively with pure water (Millipore). The surface is coated by octadecyltrichlorosilane (OTS, Fluka, Neu-Ulm, F.R.G.) [14] as applied for 10–15 min as a 2 vol% solution in chloroform/CCl_4 (4:1, v/v). The cells are rinsed with chloroform, methanol and water. The wetting angles are $105 \pm 5°$ (advancing) and $95 \pm 5°$ (receding) for water and $40 \pm 3°$ (advancing and receding) for decane. The silane in the groove is removed by local application of chromic acid for one hour and extensive rinsing with water such that the groove is hydrophilic again. Coated blocks may be used repeatedly without recoating after cleaning with water, methanol, chloroform, methanol and water.

Membrane

The two NS5-joints in the glass-block which contact the groove (Fig. 2) are connected to two syringes through two PTFE-stop-cocks. Using T-joints two Ag/AgCl electrodes are introduced such that they are in direct contact to the groove. The assembly, with the groove in vertical orientation, is mounted to a Langmuir-Blodgett lift [15] on a damped table in a box made of metal-grid covered by transparent plastic. The cell may be dipped into a beaker (volume 50 ml, surface 15 cm^2) which is filled with a 5 mM or 50 mM solution of NaCl (Suprapure, Merck-Darmstadt, kept for 5 h at 600 °C in an oven). The bath is contacted by an Ag/AgCl electrode.

The cell is submersed. Using the syringes, the groove and the joints are filled with electrolyte avoiding any bubbles. The stop-cocks are closed. 100 μl of a 10 mM solution of glycerol monooleate (Sigma, Heidelberg, F.R.G.) in decane (Merck) are applied to the water surface. After about 10 min the cell is lifted at a speed of 0.3 mm/s across the water/air interface. The water volume in the groove is adjusted using a screw pressing onto a piece of flexible tube between the cell and stop-cocks. The block is submersed again until the upper end of the groove is a few millimeters below the surface. The procedure is monitored by a video camera (Sony, Tokio) through a stereomicroscope (Zeiss, Oberkochen). The bilayer forms within a few minutes by thinning of an oil lamella (Mueller-Rudin mechanism [6,7]), not by direct junction of lipid monolayers (Montal-Mueller mechanism [13,16]). The lifetime of the membranes varies from 3 to 200 min. Utmost care is required to avoid any impurities.

The conductance of the membrane is enhanced by gramicidin [17–19]. We use gramicidin D (Sigma, Heidelberg, F.R.G.) as dissolved in methanol at a concentration of 500 ng/ml. Two procedures are applied: (i) Samples of 1–5 μl (Hamilton syringe) are added to the bath close to the bottom of the groove after formation of the membrane. (ii) Samples of 1–5 μl are added to the surface of the bath before formation of the membrane. The system is homogenized by fast dipping of the cell about twenty times in and out. After 10 min the membrane is drawn.

Circuit

We apply three types of electrical polarization to the upper or lower end of the groove (Fig. 2): (i) A DC-voltage of $V_0 = 15$–50 mV. The level of the voltage is adjusted to attain on one hand large signals and to avoid on the other hand excessive currents which damage the electrodes. (ii) An AC-voltage of a frequency of 1 kHz and an amplitude 10 mV. This voltage may be applied also to both ends simultaneously. (iii) A charge pulse as obtained by a voltage pulse (width 1 μs, height 5 V, Phillips PM 5712) and a diode (1 N 4448) [8].

The second (distal) end of the groove is connected to a voltage-follower (Fig. 2) [20] (amplifier TL 071, input resistance > 1 GΩ, condition of sealed end). Alternatively the second end is kept at bath potential (condition of open end). The bath electrode is connected to a current/voltage converter (Fig. 2) [20] (operational amplifier OPA 121) and kept on virtual ground.

Measurement

In a voltage-clamp experiment we observe the voltage V_0 applied to one end, the output of the voltage-follower at the other end and the output of the current/voltage-converter. These signals may be read into a microcomputer (Apple II) using a 12-bit AD-converter. The computer may switch periodically between the condition of sealed and open end. Furthermore it may switch periodically the voltage clamp from the upper to the lower end of the groove. Thus the computer reads, at intervals of 1 s, the voltage V_{SE} at the sealed end, the currents I_{SE} and I_{OE} for sealed and open end and the V_{SE}, I_{SE} and I_{OE} for reversed contacts. After three cycles the applied voltage V_0 is read and the zero-points of the amplifiers are checked.

AC-voltage is used to determine the complex admittance. It is applied separately to both ends of the groove to check for electrical symmetry. It is applied to both ends simultaneously to evaluate the capacitance of the membrane. The output of the current/voltage converter is sent to two lock-in amplifiers (PAR, Princeton, Mod. 186A) set at 90° phase difference. The complex admittance $Y_{SE}^{(2)}$ of a cable, with sealed ends and voltage applied to both ends, depends on the parameters R, G and C and on the angular frequency ω as derived in Appendix A. We evaluate the admittance with respect to the capacitance C at a given resistance R and a negligible conductance G. The calibration curves for $\mathrm{Im}(Y_{SE}^{(2)})$ and for $\phi(Y_{SE}^{(2)})$ are given in Appendix A.

Charge pulses are applied at a repetition frequency of 2–5 Hz. The output of the voltage-follower is observed on an oscilloscope. Photographs of the screen are evaluated.

Results and Discussion

Resistance R

We prepare a BLM of monooleate on the groove in 50 mM NaCl without gramicidin. If we apply a voltage of $V_0 = 25$ mV to one end with the other end kept open we measure a current of $I_{OE} = 230 \pm 10$ nA. At low conductance G, i.e., for $RG \ll 1$ the current depends only on the resistance R according to Eqn. 6. From the ratio V_0/I_{OE} we obtain a resistance $R = 0.11$ MΩ.

We may calculate the resistance $R = l_M/w_M \cdot d_S \cdot \Lambda_E \cdot [E]$ from the length $l_M = 4.5$ mm, width $w_M = 0.25$ mm and depth $d_S = 0.3$ mm of the groove, the concentration $[E] = 0.05$ mol/l of NaCl and the molar conductivity

$\Lambda_E = 111\ S \cdot cm^2 \cdot mol^{-1}$ [21]. We obtain $R = 0.11\ M\Omega$. The agreement of the experimental and calculated resistance indicates that the groove is filled completely with electrolyte and covered by an isolating coat.

Capacitance C

We prepare a BLM of monooleate on the groove in 50 mM NaCl without gramicidin. We apply an AC-voltage of 1 kHz to both sealed ends. We measure an imaginary part of the admittance of $\mathrm{Im}(Y_{SE}^{(2)}) = 32 \pm 3$ μS and a phase of $\phi(Y_{SE}^{(2)}) = 77 \pm 3°$. Using the expressions of $\mathrm{Im}(Y_{SE}^{(2)})$ and $\phi(Y_{SE}^{(2)})$ for a cable of resistance $R = 0.11\ M\Omega$ as a function of the capacitance (Appendix A) we obtain C = 7.7 nF. (Note: From a naive evaluation which neglects the lateral resistance, using $\mathrm{Im}(Y_{SE}^{(2)}) = \omega C$, we would obtain a capacitance of $C = 5$ nF.)

We may calculate the capacitance $C = c_M \cdot w_M \cdot l_M$ for the length $l_M = 4.5$ mm and width $w_M = 0.25$ mm of the groove and the specific capacitance $c_M = 8.5 \pm 0.4$ nF/mm^2 of a BLM of monooleate [22]. We obtain $C = 9.5$ nF. The similarity of the experimental and calculated capacitance indicates that the overwhelming part of the groove is covered by a lipid bilayer. The deviation of 20% may be due to (i) a torus of solvent along the edges of the groove, (ii) an enhanced solvent content of the bilayer and (iii) a geometry which deviates from the one-dimensional cable with terminal contacts.

For control we apply an AC-voltage to each end of the groove separately. The admittance is found to be identical for both experiments. Thus, whatever the origin of the smaller capacitance may be, the distribution of capacitance along the groove is symmetrical.

Conductance G

We prepare a BLM of monooleate on the groove in 50 mM NaCl without gramicidin. We apply a voltage of $V_0 = 50$ mV to one end with the other end sealed (with disconnected voltage-follower). We measure a current of $I_{SE} = 5$–50 pA. Using Eqn. 5 with $R = 0.11\ M\Omega$ we obtain a total conductance $G = 0.1$–1 nS. (As $RG \ll 1$ the conductance G is identical with the input conductance I_{SE}/V_0.)

We may calculate the specific conductivity $g_M = G/(l_M \cdot w_M)$ from the length $l_M = 4.5$ mm and width $w_M = 0.25$ mm of the groove. We obtain $g_M = 0.1$–1 nS/mm^2, a value which is well in a range for bilayers of monooleate [10,22].

We prepare a membrane of monooleate in 50 mM NaCl after the addition of 2.5 ng of gramicidin to the surface of the bath and homogenization. The symmetry of the membrane is checked by observing the admittance with an AC-voltage applied to either end. At a voltage of e.g. $V_0 = 19.7$ mV as applied to one end, with the other end sealed, we observe a current of $I_{SE} = 230$ nA. Using Eqn. 5, with $R = 0.11\ M\Omega$, we obtain a total conductance $G = 19$ μS. This value is the highest as attained reproducibly in these experiments. (Note that G exceeds the input conductance $I_{SE}/V_0 = 12$ μS.)

We may calculate the specific conductivity $g_M = G/(l_M \cdot w_M)$ from the length $l_M = 4.5$ mm and width $w_M = 0.25$ mm of the groove. We obtain $g_M = 19$ $\mu S/mm^2$. We assume the channel conductance to be $\Lambda_{CH} = 1.7$ pS at 50 mM NaCl. (We have evaluated $\Lambda_{CH} = 17$ pS in 500 mM NaCl from current fluctuations at a low density of gramicidin [17].) Thus the highest density of channels attained in these experiments is around $n_{CH} = 10^7\ mm^{-2}$.

Channels form by dimerization with an association constant $K_A = n_{CH}/(n_G \cdot n_G)$ where n_G is the density of monomers. From $n_{CH} = 10^7\ mm^{-2}$ and $K_A < 2 \cdot 10^{-10}\ mm^2$ [19] we estimate a total density $n_G = 2n_{CH} > 2.5 \cdot 10^8\ mm^{-2}$. The density of monooleate in a bilayer is around $n_{MO} = 5 \cdot 10^{12}\ mm^{-2}$ such that the molar ratio lipid/protein is in the order of 10^4. The lamellar phase in the system phosphatidylcholine/gramicidin becomes instable with respect to a hexagonal-II phase at a molar ratio lipid/protein of 10^2 [23]. Enhanced flip-rate sets in at a ratio of 10^3 [23]. The inability to obtain membranes of higher conductance may be related to this phase transition.

Cable parameters

We assign the total resistance $R = 0.11\ M\Omega$, the total capacitance $C = 7.7$ nF, and the total maximal conductance $G = 19$ μS to groove and membrane between the electrical contacts at a distance of $l_M = 4.5$ mm. As the ends of the groove are tapered we neglect their contribution to the electrical properties. Assuming the membrane between the contacts to be homogeneous we obtain the following cable parameters per unit length (Fig. 1): Resistance $R_1 = 25\ k\Omega/mm$, capacitance $C_M = 1.7$ nF/mm, maximal conductance $G_M = 4.2$ $\mu S/mm$.

We expect for the length constant $\lambda = (R_1 G_M)^{-1/2}$ a shortest value of $\lambda = 2.9$ mm. A voltage V_0 at one end of the grove should decay to a value of $V_{SE} = 0.4 \cdot V_0$ at the sealed end at a distance of $l_M = 4.5$ mm (Eqn. 4). Such an effect is expected to be detectable.

For the diffusion coefficient $D_S = (R_1 C_M)^{-1}$ we expect a value of $D_S = 2.4 \cdot 10^4\ mm^2/s$. A pulse applied to one end should arrive at the sealed and at distance $l_M = 4.5$ mm after a delay time of $\tau_S = l_M^2/(\pi^2 \cdot D_S) = 0.1$ ms. An extension of this time, using a lower salt concentration, is discussed below.

Voltage clamp of homogeneous cable

We prepare bilayers in 50 mM NaCl after addition of a variable amount of gramicidin to the surface of the bath and after homogenization. The admittance is checked with AC-voltage applied to both ends separately. The systems are found to be symmetric.

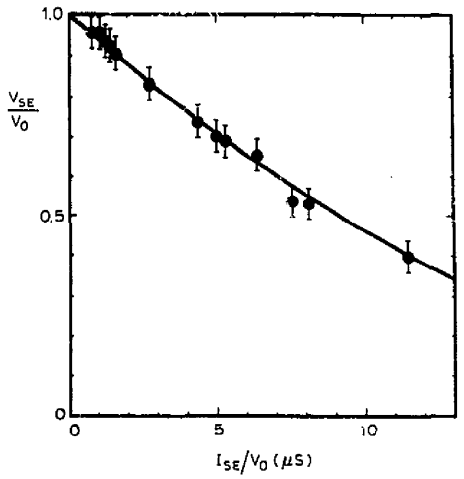

Fig. 3. Voltage V_{SE} at sealed distal end versus total current I_{SE}, both normalized by the voltage V_0 applied to the proximal end. Each data point refers to a membrane as prepared after addition of gramicidin and homogenization. The bars indicate maximal estimated errors. Line: Theory for a homogeneous cable of resistance $R = 0.11$ MΩ.

We apply a voltage of $V_0 = 15$–50 mV to one end with the other end kept sealed or open. The voltage V_{SE} at the sealed distal end and the total currents I_{SE} and I_{OE} for sealed and open distal end are measured. The signals remain almost constant during the lifetime of the membrane. The measurements are repeated with an exchanged connection of the electrical contacts to both ends. The signals are found to be unchanged.

For sealed distal end the voltage V_{SE} is plotted versus the current I_{SE} in Fig. 3. The data points are obtained for one set of membranes drawn after a stepwise addition of gramicidin and subsequent homogenization. The more gramicidin is added, the higher is the current I_{SE} across the membrane and the lower is the voltage V_{SE} arriving at the distal end of the groove.

In Fig. 3 the relation of V_{SE} and I_{SE} for an ideal cable of resistance $R = 0.11$ MΩ is plotted as calculated from Eqns. 4 and 5. The agreement of experiment and theory is satisfactory within the error of the measurements.

The square of the voltage V_{SE} at the sealed distal end is plotted versus the ratio I_{SE}/I_{OE} of the currents for sealed and open end in Fig. 4. The data points are obtained from the same set of membranes as used above. The voltage V_{SE} at the sealed end drops as more gramicidin is added. The current I_{OE} through an open cable increases more than the current I_{SE} through a sealed cable with $I_{SE}/I_{OE} < 1$. However, the more gramicidin is added, the closer are both currents to each other.

In Fig. 4 the parameter-free relation of V_{SE}/V_0 and I_{SE}/I_{OE} for an ideal cable is plotted according to Eqn. 7. The agreement of experiment and theory is satisfactory within the error of the measurements.

We conclude from the results that our groove as covered by monooleate with gramicidin behaves as a homogeneous, finite, one-dimensional core-coat conductor with respect to the propagation of stationary electrical signals.

Voltage clamp of inhomogeneous cable

We prepare a bilayer in 50 mM NaCl without gramicidin in the system. The symmetry and the capacitance of the membrane are checked by measurement of the admittance. We add a certain amount of gramicidin (1–5 μl) to the bath, close to the bottom of the groove, without homogenization. We apply a voltage $V_0 = 15$–50 mV to each end of the groove alternately. The voltage V_{SE} at the sealed distal end and the current I_{SE} are measured.

We observe a continuous increase of the current and a continuous decrease of the voltage during the first 15 minutes. The current I_{SE} and the voltage V_{SE} are significantly higher if the voltage V_0 is applied to the upper end of the groove than if the same V_0 is applied to the lower end. These results are found in all membranes prepared. The details of the dynamics and of the asymmetry vary somewhat from membrane to membrane.

For a typical experiment the voltage V_{SE} at the sealed distal end is plotted in Fig. 5 versus the current I_{SE}. Some data points as measured at the same time, with V_0 applied to the upper and lower end of the groove, are connected.

At a given time the efficiency of signal transduction along the cable depends on the direction. The assembly exhibits features of a 'valve' for electrotonic spread.

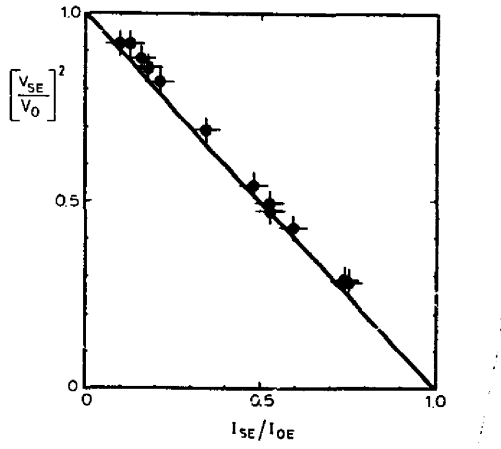

Fig. 4. Squared voltage V_{SE} at sealed distal end as normalized by the voltage V_0 applied to the proximal end versus the ratio of the total current I_{SE} with sealed end and total current I_{OE} with open end. The bars indicate maximal estimated errors. Line: Theory for homogeneous cable.

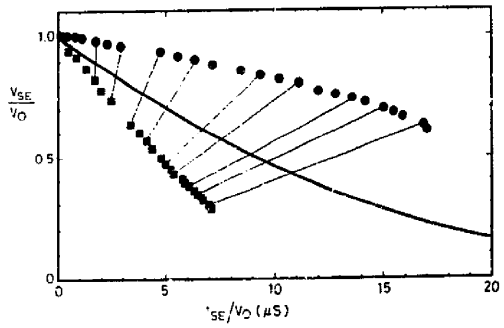

Fig. 5. Voltage V_{SE} at sealed distal end versus total current I_{SE}, both normalized by the voltage V_0 applied to the proximal end. Experimental data as obtained during one experiment after local application of gramicidin. Circles: voltage clamp at upper end of the groove. Squares: Voltage clamp at lower end of the groove. Some points are connected which are measured at the same time. Line: Theory for homogeneous cable of resistance $R = 0.11$ MΩ.

Both sets of experimental data differ significantly from the theory for a homogeneous cable as shown in Fig. 5. At later times a large current I_{SE} is related to a high potential V_{SE} if the upper end of the groove is clamped at V_0. A low current I_{SE} is related to a low potential V_{SE} if the lower end of the groove is clamped. We assign the effect to an inhomogeneous incorporation of gramicidin into the bilayer.

As a most simple model of an inhomogeneous cable we consider a linear gradient of conductance $G_M(x) = 2(x/l_M)(G/l_M)$ where G is the total conductance of the cable. This model has no free parameter as compared to a homogeneous cable of the same total conductance G.

The stationary cable equation is solved with the linear profile of conductance using a power series (Appendix B). The constraint of sealed end is considered with both polarities: (i) Voltage clamp at the end of maximal conductance ($x = l_M$) and (ii) voltage clamp at the end of minimal conductance ($x = 0$). The solutions $V_M(x)$ provide the voltages V_{SE} at the sealed distal end. The currents I_{SE} are obtained by integration.

The voltage V_{SE} is plotted versus the current I_{SE} in Fig. 6 as calculated for various conductances G. (i) Current and voltage are higher than in a homogeneous cable if the end of maximal conductance is clamped. (ii) Current I_{SE} and voltage V_{SE} are lower than in a homogeneous cable if the end of minimal conductance is clamped. Points belonging to the same total conductance G are connected. The efficiency of signal transduction is higher for case (i) as compared to case (ii). The result resembles closely the experimental data of Fig. 5. We avoid a fit of the data by an arbitrary profile of the conductivity.

We conclude from the results that local application of gramicidin gives rise to a gradient of conductance from the upper end of the membrane down to its lower end. The partial one-way transmission of a stationary signal is compatible with a finite one-dimensional core-coat conductor with graded conductance.

Charge pulse

At the present moment we are not able to resolve the delay time of voltage in a charge pulse experiment with membranes prepared in 50 mM NaCl. We apply a lower concentration of salt to reduce the diffusion coefficient D_S of charge spread (Eqn. 11). We use 5 mM NaCl. We expect a diffusion coefficient which is by a factor of 10 longer than in 50 mM NaCl, i.e., a delay time of around $\tau_S = 1$ ms. Unfortunately the formation of the BLM as well as its stability are not satisfactory in this medium.

If we apply a voltage $V_0 = 25$ mV to one end with the other end open we measure a current $I_{OE} = 23$ nS. Thus the resistance of the core $R = 1.1$ MΩ is higher by a factor of ten as compared to 50 mM NaCl as expected.

From the admittance we evaluate a capacitance of $C = 4.3 \pm 0.7$ nF. This value is distinctly lower than for membranes in 50 mM NaCl. It is only about 50% of the capacitance as expected for a bilayer of monooleate spanned over the whole groove. We assign the discrepancy to incomplete thinning of the membrane. As the lifetime of the membranes is rather short (up to 15 min) the measurements have to be performed at a very early stage.

From the resistance $R = 1.1$ MΩ and the capacitance $C = 4.3$ nF we estimate the delay time of charge spread $\tau_S = RC/\pi^2$ as $\tau_S = 0.5$ ms.

We apply a charge pulse of a width of 1 μs. The time course of the voltage $V_{SE}(t)$ at the sealed distal end is shown in Fig. 7. The voltage increases after a delay of a few milliseconds and decays within a few hundred milliseconds.

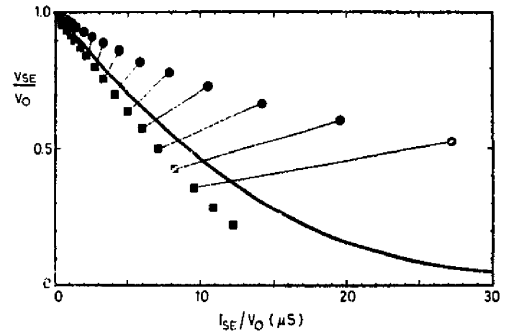

Fig. 6. Voltage V_{SE} at sealed distal end versus total current I_{SE}, both normalized by the voltage V_0 applied to the proximal end. Theoretical relation $V_{SE}(I_{SE})$ as obtained from Kelvin's equation for a linear gradient of conductance of the membrane. The hidden parameter is the total conductance G of the membrane. The core resistance is $R = 0.11$ MΩ. Circles: Voltage clamp at end of maximal conductance. Squares: Voltage clamp at end of minimal conductance. Some points are connected which are calculated for the same total conductance. Line: Theory for homogeneous cable.

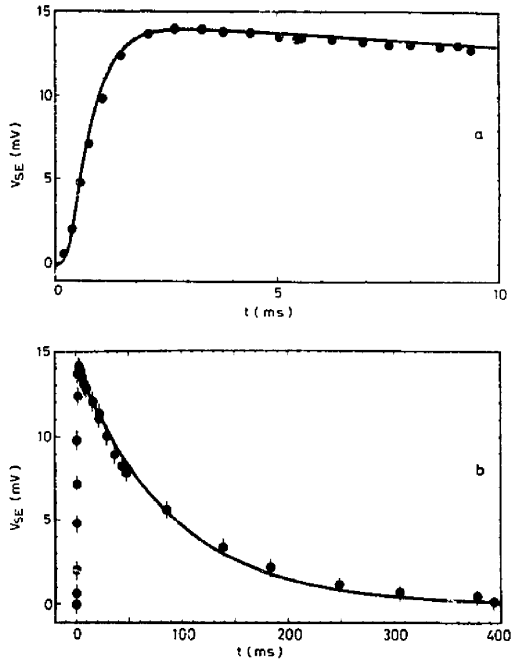

Fig. 7. Voltage V_{SE} at sealed distal end of artificial cable as a function of time t after injection of a charge pulse. Upper: Short time range. Lower: Long time range. Lines: Theory for homogeneous cable with three parameters, the time constant of charge spread $\tau_S = 0.5$ ms (calculated from cable parameters), the time constant of charge relaxation $\tau_M = 87$ ms (fitted to the exponential decay) and the injected charge $Q_0 = 65$ pA·s (fitted to the amplitude of the signal).

The time course of the voltage $V_{SE}(t)$ as calculated from the cable theory (Eqn. 9) is shown in Fig. 7 as well. A time constant of spread $\tau_S = 0.5$ ms is used as calculated from the values of resistance R and capacitance C. The time constant $\tau_M = 87$ ms of relaxation is fitted to the exponential decay of the signal. The injected charge of $Q_0 = 65$ pA · s is fitted to the amplitude of the signal.

From the relaxation time $\tau_M = C/G$ we calculate a conductance of $G = 50$ nS. It corresponds to a relatively high specific conductivity $g_M = G/w_M l_M = 44$ nS/mm^2. The enhanced leakage may be related to the instability of the membranes at the low salt concentration.

The result of the charge pulse experiment is fitted perfectly by the theory. Note in particular that the crucial delay of the voltage, which expresses the cable property of the system, is described on the basis of the time constant τ_S of charge spread which is obtained from independent measurements of R and C. The stages of charge spread and of charge relaxation are well separated. The transient voltage reaches almost the amplitude $Q_0/C = 14.5$ mV for homogeneous distribution of charge along the membrane.

We conclude from the results that our groove as covered by monooleate behaves as a homogeneous, finite, one-dimensional core-coat conductor with respect to the propagation of transient electrical signals.

Summary

A groove spanned by a black lipid membrane with ion channels exhibits the properties of an electrical core-coat conductor with respect to the lateral decay of a tonic polarization and with respect to the lateral delay of a phasic polarization. In a membrane with graded distribution of channels the signal transduction depends on the direction of propagation.

Further development must be directed towards cables with defined lateral modulation, towards branched cables, towards miniaturization and towards a detection of the complete potential profile.

The system is suitable to model biological processes of lateral organization in membranes such as signal processing in neural dendrites, propagation of action potentials in neural axons and pattern formation in cellular development.

Appendix A

Admittance of a cable

We insert the ansatz of an AC-voltage profile $V_M(x, t) = U(x, \omega) \cdot \exp(i\omega t)$ (ω angular frequency) into Eqn. 1. We obtain for the complex amplitude $U(x, \omega)$ Eqn. A-1, where R, C and G are resistance, capacitance and conductance of a cable of length l_M.

$$l_M^2 \cdot \frac{d^2U}{dx^2} - (RG + i\omega RC) \cdot U = 0 \qquad \text{(A-1)}$$

We apply an AC-voltage $V_0 = U_0 \cdot \exp(i\omega t)$ to both ends of a sealed cable of length l_M. Such a system is equivalent to two sealed cables of length $l_M/2$ with the voltage applied to one end. The complex voltage $U_{SE}^{(2)}(x, \omega)$ is obtained from Eqn. A-1 (The index (2) refers to the application of voltage to both ends of the cable.). The complex current $I_{SE}^{(2)}(x, \omega)$ is obtained by integration. We define the admittance as $Y_{SE}^{(2)} = I_{SE}^{(2)}/U_0$ as given by Eqn. A-2 (formally related to Eqn. 5).

$$Y_{SE}^{(2)} = (2/R) \cdot \sqrt{RG + i\omega RC} \cdot \operatorname{tgh}[\sqrt{RG + i\omega RC}/2] \qquad \text{(A-2)}$$

We describe the admittance by its imaginary part $\mathrm{Im}(Y_{SE}^{(2)})$ and its phase angle $\phi(Y_{SE}^{(2)}) = \operatorname{arctg}[\mathrm{Im}(Y_{SE}^{(2)})/\mathrm{Re}(Y_{SE}^{(2)})]$. Evaluation of Eqn. A-2 for a low conductance G with $RG \ll 1$ leads to Eqn. A-3 and A-4 with the parameter $a = \sqrt{\omega RC/2}$.

$$\mathrm{Im}(Y_{SE}^{(2)}) = \omega C \cdot \frac{\sinh a + \sin a}{a \cdot (\cosh a + \cos a)} \qquad \text{(A-3)}$$

$$\phi(Y_{SE}^{(2)}) = \operatorname{arctg}\left\{\frac{\sinh a - \sin a}{\sinh a + \sin a}\right\} \qquad \text{(A-4)}$$

The Eqns. A-3 and A-4 are calibration curves for the capacitance C at given resistance R and frequency ω. In the limit $a \ll 1$ (i.e., for $\omega RC \ll 1$ or $\omega \cdot \tau_S \ll 1$ (fast charge spread)) $\mathrm{Im}(Y_{SE}^{(2)}) = \omega C$ grows linearly with the capacitance at a phase $\phi(Y_{SE}^{(2)}) = 90°$. In the limit $a \gg 1$ (i.e., for $\omega RC \gg 1$ or $\omega \cdot \tau_S \gg 1$ (slow charge spread)) $\mathrm{Im}(Y_{SE}^{(2)}) = \sqrt{2\omega C/R}$ grows with the square root of the capacitance at a phase $\phi(Y_{SE}^{(2)}) = 45°$.

Appendix B

Inhomogeneous cable

We consider a cable with a linear gradient of the conductance $G_M(x) = 2 \cdot (G/l_M)(x/l_M)$ where G is the total conductance and l_M is the length of the cable. The stationary potential profile $V_M(x)$ is defined by Eqn. B-1. R is the total resistance of the core.

$$l_M^2 \cdot \frac{d^2 V_M}{dx^2} - 2 \cdot RG \cdot (x/l_M) \cdot V_M = 0 \qquad \text{(B-1)}$$

We consider two kinds of local voltage clamp: (i) Clamp at the end of maximal conductance $x = l_M$ with the constraints $V_M(l_M) = V_0$ and $(dV_M/dx)_0 = 0$ and (ii) clamp at the end $x = 0$ of minimal conductance with the constraints $V_M(0) = V_0$ and $(dV_M/dx)_{l_M} = 0$.

Eqn. B-1 is solved by a power series according to Eqn. B-2. The first two coefficients a_0 and a_1 are defined by the constraints. The third coefficient vanishes as $a_2 = 0$. All other coefficients a_n are defined by Eqn. B-3.

$$V_M(x) = \sum_0^\infty a_n \cdot (x/l_M)^n \qquad \text{(B-2)}$$

$$a_{n+3} = a_n \cdot \frac{2RG}{(n+2)(n+3)} \qquad \text{(B-3)}$$

For the constraints of case (i) we obtain $a_1 = 0$. The value of a_0 is obtained from the relation $\Sigma a_n = V_0$ using Eqn. B-3. For the constraints of case (ii) we obtain $a_0 = V_0$. The value of a_1 is obtained from the relation $\Sigma n \cdot a_n = 0$ using Eqn. B-3. The potential profile is evaluated by numerical summation.

From the profiles $V_M(x)$ we obtain immediately the potentials V_{SE} at the sealed distal ends. The currents I_{SE} are calculated by integration of $V_M(x)$ term by term. From the functions $I_{SE}(R, G)$ and $V_{SE}(R, G)$ the conductance G may be eliminated such that a relation of voltage V_{SE} and the current I_{SE} is obtained which depends only on the resistance R.

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