Bistability of Membrane Conductance in Cell Adhesion Observed in a Neuron Transistor

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We attached individual nerve cells from leech ganglia to oxidized silicon using polylysine. We studied the electrical current through the cell membrane in the region of adhesion taking advantage of field-effect transistors integrated in the substrate. We found that a minute mechanical deformation of the cell triggered a bistable reversible switching of the attached membrane between states of high and low conductance. Feasible mechanisms of the nonlinear effect are discussed. [S0031-9007(97)04739-X]

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A neuron transistor is an elementary junction between the electronics of computers and the ionics of brains [1]. An individual nerve cell is attached to the open gate of a field-effect transistor (Fig. 1). Ohmic and capacitive currents through the attached membrane arise from intracellular voltage transients. They modulate the voltage on the gate due to the voltage drop in the seal resistance between cell and chip (Fig. 1). In previous studies two types of coupling were observed which differed in the conductance of the attached membrane by an order of magnitude [2–4]. In the present paper we describe a reversible switching of the attached membrane between states of low and high conductance. Transitions could be triggered by a minute mechanical deformation of the cell which resulted from lowering and lifting a glass microelectrode which was impaled into the cell. The observation reveals the existence of an unknown nonlinear electromechanical effect in the region of cell adhesion.

Chip.—We made chips (size 10 mm × 10 mm) from *n*-type silicon with a 100 surface (Wacker-Chemie, Burghausen, Germany). An array of 16 insulated-gate field-effect transistors was fabricated by standard techniques [4,5]. Source and drain were made by boron doping. Length and width of the *p*-type channel were 6 and 30 μm, respectively. An area of 17 μm × 30 μm was etched into the field oxide (1 μm thick) and covered with a gate oxide (12 nm thick). The chip was wire bonded and a circular chamber of perspex was attached (bottom diameter 3 mm). The surface of the chip was wiped several times with a hot (80 °C) aqueous solution of 6 vol. % ammonia and 5 vol. % hydrogen peroxide. After rinsing with water, a drop of an aqueous solution of poly-L-lysine (0.75 mg/ml, MW 15 000-30 000, Sigma) was applied to each gate and dried. Finally the chamber was filled with 600 μl Leibovitz-15 medium (Gibco, Eggenstein).

Neurons.—Retzius cells were dissociated from the ganglia of leeches (*Hirudo medicinalis*) [4,6]. They were kept in a Petri dish (Costar No. 3035) at 20 °C for several days with Leibovitz-15 medium (Sigma, Heidelberg, Germany) supplemented with 30 μg/ml gentamycinsulfate, 5 mg/ml glucose, and 2% fetal calf serum (Gibco). Then a single cell was placed onto a gate using a pipette with an opening of 100 μm. The cell adhered immediately to the surface.

Setup.—We impaled neurons on a transistor with a microelectrode filled with 4 M potassium acetate (resistance about 15 MΩ) as illustrated in Fig. 2. The electrode was held at an inclination of about 45° by a three axis micromanipulator (3D-Nanorobot, SPI, Oppenheim,

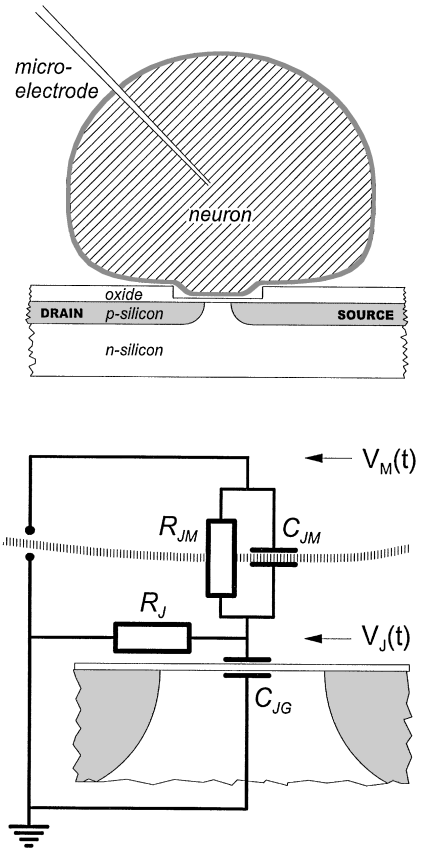

FIG. 1. *Neuron transistor.*—Top: Schematic cross section. Bottom: Circuit for ac signals in the linear regime with a capacitance C_{JM} and a resistance R_{JM} of the membrane in the junction, a capacitance C_{JG} of the gate region and a seal resistance R_J between membrane and chip. $V_M(t)$ is the intracellular voltage; $V_J(t)$ is the extracellular voltage in the junction.

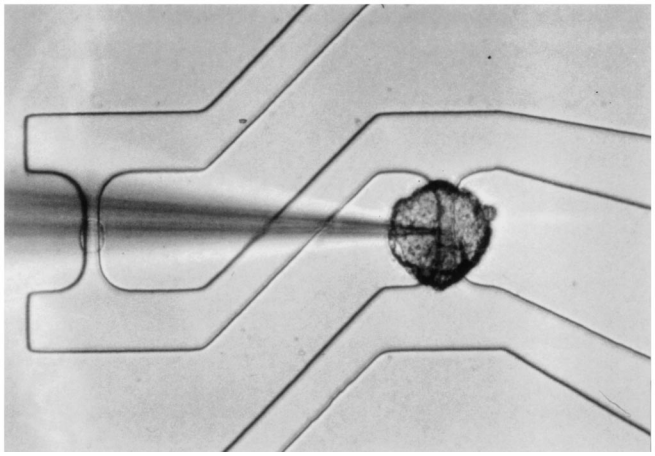

FIG. 2. Micrograph of a neuron transistor. The diameter of the nerve cell is about 80 μm. Gate, source, and drain are visible through the cell. A microelectrode is impaled at an angle of about 45°. It is moved up and down by a micromanipulator.

Germany) which defined the spatial position with sub-μm accuracy. We elicited action potentials by current injection for 10 ms. The intracellular voltage was recorded with a bridge amplifier (BA-1S, npi-electronic, Tamm, Germany). Bulk silicon and source were kept at -3 V with respect to the bath at ground potential. The drain-to-source voltage was -2 V. The source-drain currents (100–150 μA) were fed into current-voltage converters and amplified. We calibrated each transistor at its working point by applying defined voltage steps to the bath before attachment of a cell. The time courses of the intracellular voltage and of the source-drain current were displayed during the experiments. After a stimulation, we moved the microelectrode at a speed of 1 μm/s up or down normal to the surface.

Results.—An action potential was elicited as shown in the left of Fig. 3 (stimulus No. 1). The response of the transistor was weak and resembled a first derivative of the action potential. Such a response was dubbed A-type coupling [3,4]. Then we lowered the pipette in increments of about 1 μm and checked the coupling by eliciting further action potentials. After a displacement of 6 μm the transistor recorded reproducibly strong monophasic signals (stimulus No. 2 in Fig. 3). The shape of the response was indistinguishable from the action potential itself. Such a response was dubbed B-type coupling [3,4]. Then we lifted the pipette again. The response switched to an A-type signal after a displacement of 3 μm (stimulus No. 3 in Fig. 3). The transition from A-type (derivative response) to B-type (direct response) and vice versa was induced 10 times by similar displacements of the micropipette as shown in Fig. 3 from left to right.

The changing level of the intracellular voltage in the ten experiments (Fig. 3) was due to an adjustment of the stationary injection current. The intracellular response of the neuron differed somewhat from stimulation to stimulation.

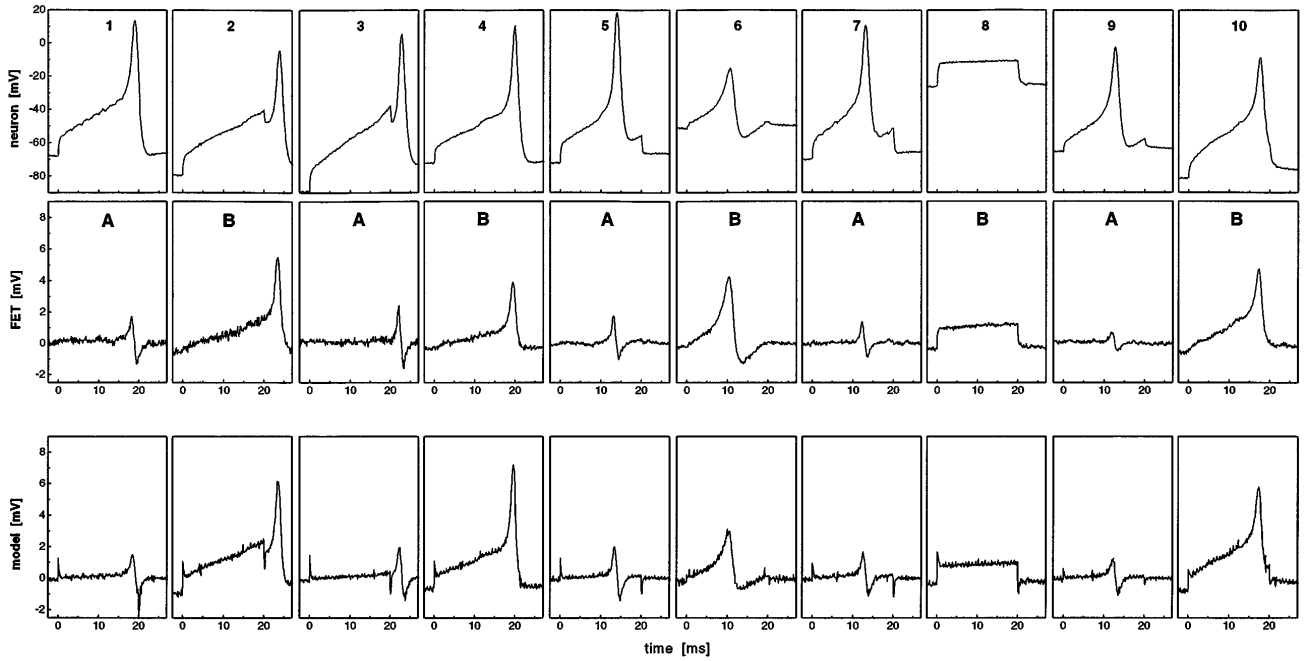

FIG. 3. Reversible switching of the cell membrane revealed by different relations of the intracellular voltage $V_M(t)$ (traces in the first row, records of impaled microelectrode) and the extracellular voltage $V_J(t)$ (traces in the second row, records of field-effect transistor). The microelectrode is lowered by a few micrometers after stimulations No. 1, 3, 5, 7, and 9 and lifted after stimulations No. 2, 4, 6, and 8. (The intracellular voltage is superposed by a voltage drop in the electrode during current injection for 10 ms.) Simulations of the extracellular voltage $V_J(t)$ are shown in the third row using the record of the microelectrode as a stimulus. The resistances of membrane and seal are $R_{JM} = 300$ MΩ, $R_J = 2$ MΩ for No. 1, 3, 5, 7, 9, and $R_{JM} = 40$ MΩ, $R_J = 3.5$ MΩ for No. 2, 4, 6, 8, and 10.

An action potential appeared in nine cases. In experiment No. 8 the cell was not excitable. Note that the voltage drop of the stimulation current in the pipette caused a rectangular perturbation of the intracellular record. The neurons remained attached to the chip during the whole experiment. Global deformations of the cell were not observed. The manipulations of the nerve cells impaired their electrical activity—due to direct mechanical damage by the pipette or due to the enhanced membrane permeability in the B-state. For that reason a multiple reversibility of the transition was rare. A sequence as that shown in Fig. 3 was observed twice.

Circuit.—Neuron-silicon junctions are described by a circuit (Fig. 1) defined by the capacitance C_{JM} and the resistance R_{JM} of the membrane in the junction, by the capacitance C_{JG} of the gate oxide in the junction, and by the seal resistance R_{J} between membrane and gate. This circuit is valid for ac signals in the linear regime [4]. The four parameters depend on the geometry and the materials of the junction. We consider the model of a homogeneous circular junction with radius a_{J} and distance d_{J} between membrane and obtain Eqs. (1) with the specific capacitance c_{JM} and conductance g_{JM} of the membrane, with an average specific capacitance c_{JG} of the oxide in the junction and with the specific resistance ρ_{J} of the electrolyte. [The seal resistance R_{J} is proportional to an effective distance $(1/3)a_{\mathrm{J}}$ of the adhesion area from the bath and inversely proportional to a corresponding effective contact length $(5/6)2\pi a_{\mathrm{J}}$ to the bath [4].]

$$C_{\mathrm{JM}} = c_{\mathrm{JM}} a_{\mathrm{J}}^2 \pi\,, \tag{1a}$$

$$C_{\mathrm{JG}} = c_{\mathrm{JG}} a_{\mathrm{J}}^2 \pi\,, \tag{1b}$$

$$R_{\mathrm{JM}} = \frac{1}{g_{\mathrm{JM}} a_{\mathrm{J}}^2 \pi}\,, \tag{1c}$$

$$R_{\mathrm{J}} = \frac{\rho_{\mathrm{J}}}{5\pi d_{\mathrm{J}}}\,. \tag{1d}$$

The response of the voltage $V_{\mathrm{J}}(t)$ in the junction as a function of time t is controlled by the intracellular voltage $V_{\mathrm{M}}(t)$. From Kirchhoff's law we obtain

$$(C_{\mathrm{JM}} + C_{\mathrm{JG}})\frac{dV_{\mathrm{J}}}{dt} + \left(\frac{1}{R_{\mathrm{JM}}} + \frac{1}{R_{\mathrm{J}}}\right)V_J = C_{\mathrm{JM}}\frac{dV_{\mathrm{M}}}{dt} + \frac{1}{R_{\mathrm{JM}}}V_{\mathrm{M}}\,. \tag{2}$$

If the membrane resistance R_{JM} is high, the junction is driven by the first derivative of the intracellular voltage (A-type junction). If the membrane resistance is low, the Ohmic current through R_{JM} dominates: We expect a direct response to the intracellular voltage (B-type junction). In both cases the amplitude of the response depends on the seal resistance R_{J}.

Simulation.—We simulated the experiments on the basis of the representative circuit. As an intracellular stimulus $V_{\mathrm{M}}(t)$ in Eq. (2) we took the record of the microelectrode which includes the voltage drop in the electrode. We chose a capacitance $C_{\mathrm{JM}} = 50$ pF as estimated from the membrane capacitance $c_{\mathrm{JM}} = 5\ \mu\mathrm{F/cm}^2$ of Retzius cells using an average radius $a_{\mathrm{J}} = 18\ \mu$m of the junction [4]. The capacitance of the gate region was assumed to be $C_{\mathrm{JG}} = 2$ pF [4].

The computed response of A-type coupling in the stimulations No. 1, 3, 5, 7, and 9 is shown in the third row of Fig. 3. By trial and error we found that a membrane resistance $R_{\mathrm{JM}} = 300\ \mathrm{M\Omega}$ and a seal resistance $R_{\mathrm{J}} = 2\ \mathrm{M\Omega}$ lead to a fair description of the data. (The two narrow peaks in the computed response are artifacts of the voltage drop in the microelectrode.) A perfect agreement was obtained by an individual fit of the seal resistance (not shown in Fig. 3). These optimal values of R_{J} are plotted in Fig. 4. They are rather close to $R_{\mathrm{J}} = 2\ \mathrm{M\Omega}$.

We were able to simulate the monophasic response of the B-junctions by lowering the membrane resistance significantly. By trial and error we found that $R_{\mathrm{JM}} = 40\ \mathrm{M\Omega}$ was adequate. Computations of the stimulations No. 2, 4, 6, 8, and 10 are shown in the third row of Fig. 3 with a seal resistance $R_{\mathrm{J}} = 3.5\ \mathrm{M\Omega}$. Also response No. 8 was reproduced where no action potential appears. (Note that the voltage drop in the electrode gave rise to an artifact with two narrow peaks and a displacement of the response within 10 ms.) There were some discrepancies in the amplitudes of experiment and model, in particular, in the traces No. 4 and No. 6. An individual choice of the seal resistance R_{J} was essential to attain perfect fits (not shown in Fig. 3). These optimal values of R_{J} are plotted in Fig. 4. They scatter around the representative value

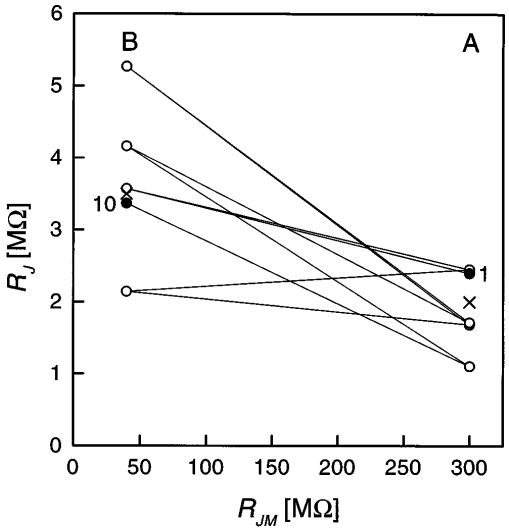

FIG. 4. Seal resistance R_{J} and membrane resistance R_{JM} for optimal simulations of the extracellular voltage $V_{\mathrm{J}}(t)$. The parameters of the first and tenth stimulations are marked by black dots. An identical membrane resistance R_{JM} was chosen for all A-type (300 MΩ) and B-type junctions (40 MΩ), respectively. The seal resistance R_{J} was varied individually. The crosses mark the parameters used in the simulations of Fig. 3.

$R_J = 3.5\ M\Omega$. The sequence of the ten pairs R_{JM}, R_J in Fig. 4 indicates a correlation of a decrease in the membrane resistance R_{JM} with an increase in the seal resistance R_J.

Structure of the junction.—A resistance $R_{JM} = 300\ M\Omega$ of A-type junctions corresponds to a membrane conductance of $g_{JM} = 0.36\ mS/cm^2$ according to Eq. (1). This value is in the range of free Retzius cells [4]. The transition to a B-type junction with $R_{JM} = 40\ M\Omega$ implies an increase of the conductance to $g_{JM} = 2.7\ mS/cm^2$. This enhancement is well within the range for opening of ion channels [7]. The seal resistance of $R_J = 2\ M\Omega$ corresponds to a distance $d_J = 32$ nm of membrane and gate [Eq. (1)], if a specific resistance $\rho_J = 100\ \Omega$ cm is assumed as in the bulk electrolyte. An increase up to $R_J = 3.5\ M\Omega$ in a B-type junction would correspond to $d_J = 21$ nm.

Mechanism of switching.—The evaluation of the experiments shows that a vertical displacement of the pipette switches the membrane conductance in the adhesion region. The primary trigger must be a deformation of the cell with its membrane and cell skeleton. The questions are as follow: How does this deformation affect the mechanical state of the membrane in the junction? How is the mechanics coupled to the electrical conductance? Why does the mechanoelectrical coupling exhibit bistability?

Mechanical control of ion channels is well known [8]. Coupling to the cell skeleton or to the lipid bilayer affects the chemical equilibrium between a closed and an open state of individual channels. Our present result differs from conventional mechanical gating in two aspects: (i) The modulation of conductance is localized in the region of adhesion as shown by comparison of transistor coupling and impedance in A- and B-type junctions [4]. (ii) There is no smooth modulation of the macroscopic conductance following mechanical stimulation, but a discrete switching between two macroscopic states of different conductance.

Nonetheless it is one possibility that a deformation of the cell skeleton or a tension in the membrane activate ion channels in the adhesion site. Such a mechanism would explain directly the correlation of mechanics and of membrane conductance, of course. However, special assumptions about nonlinear elastic properties of the membrane or of the cell skeleton are required in order to account for the feature of local bistability. Nothing is known about mechanosensitive ion channels in Retzius cells.

Another possibility is self-gating [9]: The pressure onto the cell would affect the width of the cleft between membrane and chip. The seal resistance would be enhanced and the current through weakly open channels would lead to an enhanced voltage drop in the junction. This voltage drop would then induce an opening of voltage-gated channels with concomitant increase of the membrane conductance. Because of positive feedback, small changes of the seal resistance would lead to a large effect on the membrane conductance with bistability [9]. Self-gating requires voltage-sensitive ion channels with a reversal voltage above the characteristic voltage of gating [9,10]. We do not know whether such channels exist in Retzius cells attached with polylysine. It is noteworthy, however, that our data indicate a correlation of seal resistance and membrane conductance (Fig. 4).

The bistability of neuron transistors reveals a novel and unexpected nonlinear mechanoelectric process in cell adhesion. To elucidate the physics on a molecular level we have to study the structure of the adhesion region and to control the ion channels in the attached membrane. In principle the first problem may be solved by fluorescence interference contrast microscopy [11,12] and the second problem by applying selective toxins or by transfection with appropriate channels. However, leech neurons are not suitable for such studies. Their membrane is so rough that the interference technique provides blurred pictures. Pharmacological tools are rather limited and genetic engineering is not developed for these cells.

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