Neuron Transistor: Electrical Transfer Function Measured by the Patch-Clamp Technique

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Leech neurons are attached to metal-free field-effect transistors in electrolyte. The voltage in a neuron is controlled through a pipette fused to its membrane. Gaussian transients and ac voltages are applied. The response of the source-drain current is measured. Two types of neuron-transistor couplings are observed. (A) Efficient coupling above 100 Hz with a phase shift of 90°. (B) Efficient coupling from 0.1–1000 Hz without a phase shift. The responses are described by a circuit made of capacitance and resistance of the membrane, by the gate capacitance and by the resistance of the junction.

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Electrical signals are the basis of signal processing in brains and computers. It is suggested to connect the elements of these systems—neurons and transistors—directly on the level of the electrical voltage. Indeed we were able to record spontaneous electrical activity of a single neuron by the source-drain current in a field-effect transistor (FET) [1]. This construction of a microscopic metal-free neuron-silicon device is to be seen in relation to electrical recording in biological tissue by a large FET [2] and to silicon-supported metallic microelectrodes [3,4].

A physical characterization of the neuron-silicon junction requires a control of the voltage across the neuron membrane. This may be achieved by the patch-clamp technique with a micropipette fused to the cell membrane [5]. Our setup is shown in Fig. 1. The neuron is attached to the gate oxide of a metal-free transistor submerged in electrolyte. Current is injected through a patch pipette. The intracellular voltage is measured by an impaled microelectrode and the response of the source-drain current is observed.

We fabricated a 4×4 array of transistors (distance 300 μm) on a 10 mm×30 mm chip of *n*-type silicon (cf. Ref. [1]). The distances of source and drain were 6 μm. The openings in the field oxide (thickness 1 μm) had a size of 20 μm×34 μm. They were covered by a gate oxide of 12 nm thickness. We arranged the contacts to the 16 drains and the common source on a circle of 6 mm diameter. A Plexiglas chamber (bottom diameter 3 mm) was attached after wire bonding. We cleaned the chip by hot basic hydrogen peroxide and applied poly-L-lysine (MW 15 to 30 kD) to the gate as an adhesive for the neuron by drying a drop of an aqueous solution (1 mg/ml). The chamber was filled with a culture medium (Leibowitz-15, *p*H 7.4). Large secretory neurons of the leech *Hirudo medicinalis* (Retzius cells, diameter 50–70 μm) were placed on the gate [1]. We attached a patch pipette (tip diameter 3–4 μm, resistance 0.5 MΩ, filled with 140 mM KCl, 1.5 mM $MgCl_2$, 10 mM Hepes, 10 mM EGTA at *p*H 7.4)—with a relatively low seal resistance of about 10 MΩ due to the rough surface—and broke the membrane by suction [5]. We held the neuron at a voltage $V_M = -60$ to -75 mV such that stimulations remained below the threshold for a nonlinear response. We applied a bias voltage of $V_{ES} = -3.0$ to -3.5 V between electrolyte and source (and bulk silicon). The electrolyte was kept on ground potential (Ag/AgCl electrode in 1 M KCl/agar). At a drain-source voltage of $V_{DS} = -2.0$ V the source-drain current was $I_D = 100$ to 150 μA. A change $\Delta V_{ES} = +10$ mV altered the current by about $\Delta I_D = -1.2$ μA as tested without neuron. The resistance across the gate oxide in electrolyte was above 1 TΩ comparable with a dry oxide of good quality. We stimulated the neuron by Gaussian voltage pulses and by ac voltages. The voltage in the neuron did not follow the voltage program applied to the patch pipette—in particular at high frequencies—due to the impedances of patch pipette and neuron and due to the leaky seal. For that reason we impaled a microelec-

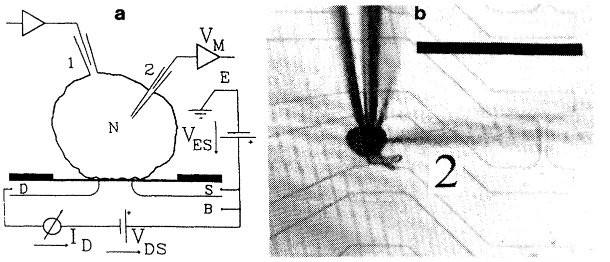

FIG. 1. Neuron-silicon junction. (a) Schematic cross section. The plasma membrane of a neuron is attached to the metal-free gate oxide (black) of a field-effect transistor in electrolyte. A voltage V_{ES} is applied between electrolyte *E* (on ground potential) and source *S* (*p* type). The bulk silicon *B* (*n* type) is kept on source potential. A voltage V_{DS} is applied between drain *D* and source. Voltage transients are applied to the neuron *N* by current injection through the patch pipette (1). The membrane voltage V_M is recorded by the impaled microelectrode (2). The response of the source-drain current I_D is observed. (b) Micrograph (scale bar 300 μm). The bent dark lines mark the boundaries of *p*-type sources and drains and of *n*-type bulk silicon. A Retzius neuron of the leech is attached to one of the two visible gate openings (out of 16 on the chip), connected to the patch-pipette and impaled by the microelectrode.

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trode (tip diameter < 1 μm, filled with 4 M K-acetate) to measure the voltage V_M. The effect of direct capacitive coupling of patch pipette and microelectrode was $< 1\%$. [Note: At high frequencies the measured voltage—in particular its phase—deviated from the actual voltage due to uncompensated effects of capacitance (15 pF) and resistance (20 MΩ) of the electrode.] We expressed the modulation of I_D caused by a change ΔV_M by the equivalent change ΔV_{ES}^{M} which led to the same modulation in the neuron-free system. The accuracy of the transfer ratio $\Delta V_{ES}^{M}/\Delta V_M$ was 10% for the amplitude and 5° for the phase.

Gaussian transients.—At first we simulated the spontaneous activity of neurons as studied in previous experiments [1]. We applied a Gaussian depolarization $\Delta V_M(t) = \Delta V_0 \exp(-t^2/2\sigma^2)$ with $\Delta V_0 = 30$ to 40 mV and $\sigma = 1.25$ ms. We found two types of responses in the source-drain current: (i) A type: The response was biphasic as shown in Fig. 2(a). The peaks of the equivalent voltage ΔV_{ES}^{M} were around 1 mV. The response resembles a damped negative first derivative of the stimulus. (ii) B type: The response resembles the inverted stimulus as shown in Fig. 2(c). The ratio of the equivalent voltage of the response ΔV_{ES}^{M} and of the stimulus ΔV_M was 20% to 40% in various junctions. We observed no intermediates of A-type and B-type junctions. In some cases a transition from A type to B type could be induced by pressing the neuron onto the transistor. We never found a transition from B type to A type. These observations confirm the results of the previous study [1].

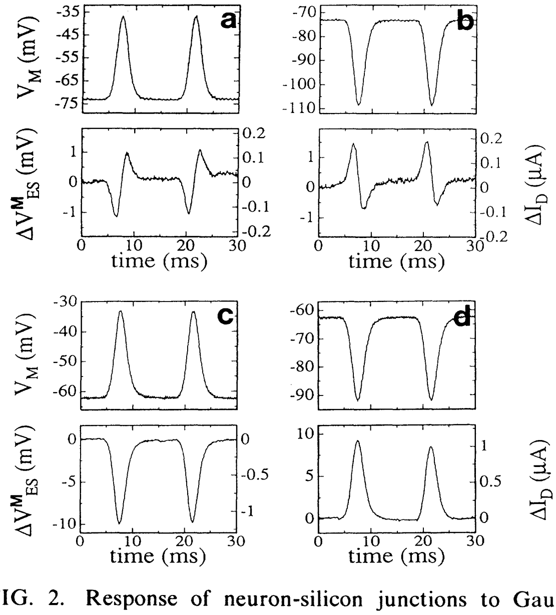

FIG. 2. Response of neuron-silicon junctions to Gaussian transients of the membrane voltage. (a) A-type junction with depolarizing Gaussian. (b) A-type junction with hyperpolarizing Gaussian. (c) B-type junction with depolarizing Gaussian. (d) B-type junction with hyperpolarizing Gaussian. The upper traces show the stimulation of the neuron—the membrane voltage $V_M(t)$ versus time. The lower traces show the response of the transistor—the modulation of source-drain current $I_D(t)$ and the equivalent voltage change $\Delta V_{ES}^{M}(t)$.

In a second experiment we tested the linearity by comparing the response to positive (depolarizing) and to negative (hyperpolarizing) Gaussians. We found that inversion of the stimulus leads to an inversion of the response in A-type and in B-type junctions as shown in Figs. 2(b) and 2(d). The result shows that the concept of linear electrical networks may be used to describe the coupling. In a third experiment we compared the response of the transistor beneath an attached neuron with the response of another transistor at a distance of 300 μm. We observed in no case any significant signal in the distant transistor. These results show that the response of the transistors is due to local coupling of neuron and silicon; i.e., it is not due to a shift of the bath potential.

Spectral transfer function.—We applied ac voltages $\Delta V_M(t) = \Delta V_0 \cos\omega t$ with an amplitude $\Delta V_0 = 10$–45 mV in the frequency range $\omega/2\pi = 0.1$–1000 Hz. We determined the complex yield $\Delta V_{ES}^{M}/\Delta V_M$ of transfer. The amplitude of the yield $|\Delta V_{ES}^{M}/\Delta V_M|$ is shown in Fig. 3(a); the phase difference between ΔV_{ES}^{M} and ΔV_M is shown in Fig. 3(b). We observed two types of spectral response in correlation to the results with Gaussian stimulation. Each is represented by one example in Fig. 3. A type: The amplitude is weak from 0.1 to 100 Hz. It increases up to about 0.3 at higher frequencies. The phase difference is zero at low frequencies and increases up to 90° between 10 and 100 Hz. B type: Here the amplitude is almost constant between 0.1 and 1000 Hz—around 0.4. (We found B-type junctions with a constant yield in

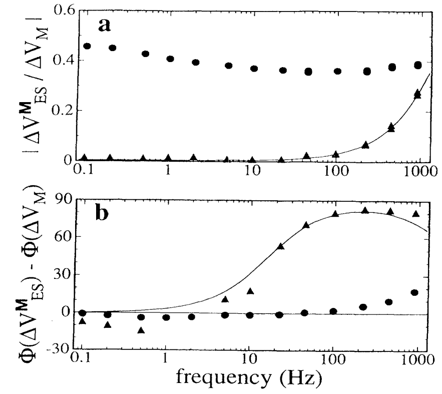

FIG. 3. Spectral transfer function of two neuron-silicon junctions. (a) Amplitude of the ratio $|\Delta V_{ES}^{M}/\Delta V_M|$ of the equivalent voltage and of the applied membrane voltage versus frequency. (b) Phase difference between the response ΔV_{ES}^{M} and the stimulus ΔV_M. The triangles refer to an A-type junction, the dots to a B-type junction. The data of the A-type junction are fitted by the point-junction model. For the parameters see text.

the range of 0.2 to 0.4.) The phase difference is zero within the accuracy of the experiment. We assign the deviation at high frequency to an interference of the recording system which may cause an increase of phase above 500 Hz.

The features of the spectral response are compatible with the features of the response to the Gaussians as expected for a linear system: The frequency band of the Gaussians extends up to about 500 Hz. In a B-type junction all these frequencies are transferred without phase shift. In an A-type junction only spectral components above 100 Hz are transferred with notable efficiency. As the phase shift is 90° there, the response is a damped first derivative.

Equivalent circuit.—The change of the total source-drain current ΔI_D (and the equivalent voltage change $\Delta V_{\rm ES}^{M}$) is the integral response of the transistor channel to all local modulations caused by the neuron. The contact of neuron and gate oxide may be inhomogeneous: (i) The conductance of the membrane and the width of the cleft between membrane and oxide may vary. (ii) Even in a structurally homogeneous junction, the voltage change may vary due to current flow along the cleft. As we have no information about the local structural and electrical properties of the junction, we try to describe the system by the minimal model of a "point junction."

The circuit of the point junction is given in Fig. 4. It is defined by the capacitance $C_{\rm JM}$ and the resistance $R_{\rm JM}$ of the membrane in the junction, by the capacitance $C_{\rm JG}$ of the gate oxide in the junction and by the resistance R_J of the cleft between membrane and gate oxide. A change of voltage V_M across the membrane results in a change of voltage V_J in the junction. The complex yield $\Theta = V_J/V_M$ of the frequency dependent transfer is given by

$$\Theta = \left\{ 1 + \frac{R_{\rm JM}}{R_J} \, \frac{1 + i\omega R_J C_{\rm JG}}{1 + i\omega R_{\rm JM} C_{\rm JM}} \right\}^{-1} . \qquad (1)$$

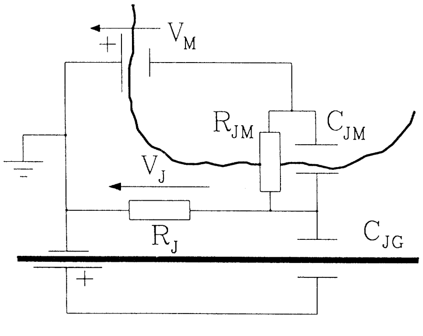

FIG. 4. Circuit of a point junction described by capacitance $C_{\rm JM}$ and resistance $R_{\rm JM}$ of the membrane in the junction, by the capacitance $C_{\rm JG}$ of the gate oxide in the junction and by the resistance R_J of the junction. The response of the system to a change of the membrane voltage V_M is given by the change of the voltage V_J in the junction.

At low frequencies the junction is controlled by the two resistances $R_{\rm JM}$ and R_J with the real yield $\Theta_0 = R_J/(R_J + R_{\rm JM})$. At high frequencies the junction is controlled by the two capacitances $C_{\rm JM}$ and $C_{\rm JG}$ with the real yield $\Theta_\infty = C_{\rm JM}/(C_{\rm JG} + C_{\rm JM})$. Thus, there is no phase shift for both limits. We try to describe the spectral data of A-type and B-type junctions—given by the ratio $\Delta V_{\rm ES}^{M}/\Delta V_M$ of equivalent voltage and membrane voltage—by the yield $\Theta = V_J/V_M$ of the point junction choosing appropriate values for $C_{\rm JM}$, $R_{\rm JM}$, $C_{\rm JG}$, and R_J.

A-type junction.—In A-type junctions we observe a weak response without phase shift at low frequency (Fig. 3). From the experimental data we estimate a yield $\Theta_0 < 0.01$, i.e., $R_J < 0.01 R_{\rm JM}$. Thus, instead of the general Eq. (1) we may apply the approximation of a "leaky junction" for $R_J \ll R_{\rm JM}$. Its amplitude of yield $|\Theta|$ and its shift of phase $\Delta\Phi$ are described by

$$|\Theta| = \Theta_\infty / \sqrt{1 + (\omega\tau_J)^{-2}} \, , \qquad (2a)$$

$$\Delta\Phi = 1/[(\omega\tau_{\rm JM})^{-1} + \omega\tau_J] \, , \qquad (2b)$$

$$\Theta_\infty = C_{\rm JM}/(C_{\rm JM} + C_{\rm JG}) \, , \qquad (2c)$$

$$\tau_J = R_J (C_{\rm JM} + C_{\rm JG}) \, , \qquad (2d)$$

$$\tau_{\rm JM} = R_{\rm JM} C_{\rm JM} \, , \qquad (2e)$$

The "leaky junction" is defined by three independent parameters: (i) The yield at infinite frequency Θ_∞, (ii) the time constant τ_J which describes the charging of the capacitances through the junction, and (iii) the time constant $\tau_{\rm JM}$ of the membrane in the junction. These three parameters define the fourth parameter—the amplitude $\Theta_0 \ll 1$ at low frequencies—by

$$\Theta_0/\Theta_\infty = \tau_J/\tau_{\rm JM} \, . \qquad (3)$$

The two time constants of the leaky junction differ by orders of magnitude with $\tau_J \ll \tau_{\rm JM}$ according to Eq. (3). Thus, the increase of phase at $\omega = 1/\tau_{\rm JM}$ and the decrease of phase at $\omega = 1/\tau_J$ [Eq. 2(b)] are separated by a plateau with $\Delta\Phi = 90°$. The decrease of the phase from 90° to 0° around $\omega = 1/\tau_J$ coincides with an increase of the amplitude from Θ_0 to Θ_∞ according to Eq. 2(a).

Certain aspects of the A-type junction (Fig. 3) match the spectral features of a leaky junction nicely: (i) We observe an increase of the phase from 0° to a plateau at 90° around a frequency of 16 Hz. (ii) We observe an increase of the amplitude up to 0.3 between 100 and 1000 Hz. Unfortunately we do not see a concomitant drop of phase. This discrepancy is due to the interference of recording microelectrode and amplifier as mentioned above. High-pass shunting may cause a positive phase shift above 500 Hz which compensates the expected drop of phase.

We may fit a time constant $\tau_{\rm JM} = 10$ ms to the increase of phase around 16 Hz using Eq. 2(b). Without indication for a saturation of the amplitude we are not able, however, to fit a maximal yield Θ_∞ and a time constant

τ_J using Eq. 2(a). [We may estimate an upper bound for τ_J from Eq. (3): With $\Theta_\infty > 0.3$ and $\Theta_0 < 0.01$ we obtain $\tau_J < 0.33$ ms. Thus, saturation is expected to occur clearly above 500 Hz—beyond the range of the experiment.] We introduce an assumption for Θ_∞: If the junction is structurally homogeneous the ratio of the capacitances $C_{\mathrm{JM}}/C_{\mathrm{JG}}$ [Eq. 2(c)] is given by the ratio of the specific capacitances c_M/c_G of cell membrane and gate oxide. We obtain $\Theta_\infty = 0.9$ with $c_G = 0.3$ μF/cm^2 for an oxide of thickness 12 nm and dielectric constant 3.9 and with $c_M = 3$ μF/cm^2 as determined from the capacitance and size of intact neurons. (The relatively large value of c_M is due to submicroscopic folding of the plasma membrane of Retzius cells.) With $\Theta_\infty = 0.9$ and $\tau_{\mathrm{JM}} = 10$ ms we obtain a satisfactory fit of the complete transfer spectrum (yield amplitude and phase shift) by Eq. (2) as shown in Fig. 3 using a fast time constant $\tau_J = 55$ μs. From these values for τ_{JM}, τ_J, and Θ_∞ we obtain $\Theta_0 = 0.005$ from Eq. (3) in good agreement with the estimate $\Theta_0 < 0.01$. To evaluate the four elements C_{JM}, C_{JG}, R_{JM}, and R_J from the three independent parameters $\tau_{\mathrm{JM}} = 10$ ms, $\tau_J = 55$ μs, and $\Theta_\infty = 0.9$ we make an assumption about the area of the junction. An upper limit is the opening in the field oxide $A_J = 680$ μm^2. Using $C_{\mathrm{JG}} = A_J c_G$ we obtain $C_{\mathrm{JG}} = 2$ pF, $C_{\mathrm{JM}} = 20$ pF, $R_{\mathrm{JM}} = 0.5$ GΩ, and $R_J = 2.5$ MΩ.

B-type junction.—The amplitude at low frequencies is not small in a B-type junction (Fig. 3): Thus the model of a leaky junction with $R_J \ll R_{\mathrm{JM}}$ is not valid. The yield around 0.4 is almost constant over the observed spectral range without significant phase shift; there is no visible transition between a low frequency limit, where the resistances R_J and R_{JM} control the amplitude and a high frequency limit, where the capacitances C_{JM} and C_{JG} dominate. We consider two tentative interpretations: (i) A "degenerated junction" with equal ratio of resistances and capacitances as $R_J/R_{\mathrm{JM}} = C_{\mathrm{JM}}/C_{\mathrm{JG}}$. (ii) A "leaky-membrane junction" with a small membrane resistance $\omega R_{\mathrm{JM}} C_{\mathrm{JM}} \ll 1$.

(i) *Degenerated junction.*—For $R_J/R_{\mathrm{JM}} = C_{\mathrm{JM}}/C_{\mathrm{JG}}$ the amplitudes at low and high frequency are identical, $\Theta_0 = \Theta_\infty$. In fact the amplitude is constant and the phase vanishes all over the spectral range with $|\Theta| = \Theta_0$ and $\Delta\Phi = 0$ according to Eq. (1). Using $R_{\mathrm{JM}} = 0.5$ GΩ and $C_{\mathrm{JM}}/C_{\mathrm{JG}} = 10$—as assigned to the A junction—we obtain $R_J = 5$ GΩ. Thus the degenerated junction would be a "tight seal" junction with close adhesion of membrane and gate. The yield is $\Theta = 0.9$. This value differs significantly from the experimental value $\Theta = 0.4$.

(ii) *Leaky-membrane junction.*—The properties of the cell membrane may be changed by the attachement to silicon oxide. For a membrane with reduced resistance the limit of low frequency—with $\Theta_0 = R_J/(R_J + R_{\mathrm{JM}})$ and $\Delta\Phi = 0$—could extend up to a frequency of 1000 Hz according to Eq. (1) for 2π 1000 Hz $R_{\mathrm{JM}} C_{\mathrm{JM}} \ll 1$. With $C_{\mathrm{JM}} = 20$ pF we estimate a resistance $R_{\mathrm{JM}} \ll 8$ MΩ which is far lower than $R_{\mathrm{JM}} = 0.5$ GΩ of the intact membrane in an A-type junction. For the experimental yield $\Theta = 0.4$ we have to assume a seal resistance $R_J \ll 5.5$ MΩ. Thus the leaky-membrane junction would also be a "leaky-seal" junction. It is questionable whether such a leaky junction would allow neurons to remain intact on the gate as indicated by their electrical activity [1].

The patch-clamp technique permits the determination of the spectral transfer function of neuron-silicon junctions. On this experimental basis it is possible to discuss the junctions in terms of the simple circuit of a point junction. A-type junctions (weak biphasic response) can be described quantitatively by a model with a leaky seal. For B-type junctions (strong monophasic response) the model with a leaky membrane seems to be attractive. An improved understanding in terms of more sophisticated models will be possible when the spectral range of the measurements is extended up to 5 kHz and when structural information—on the membrane and on the membrane-oxide distance—is available.

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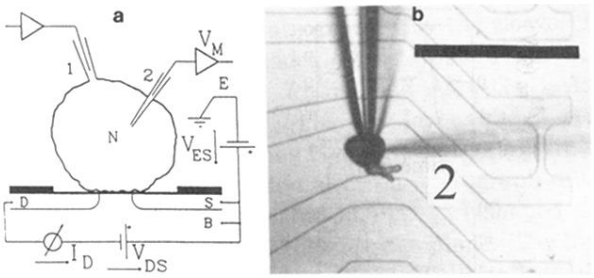

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