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Extracellular recording with transistors and the distribution of ionic conductances in a cell membrane

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Abstract Intracellular voltage transients of cultured cells are recorded by transistors and other planar electrodes as local extracellular voltages. The theoretical relationship between extra- and intracellular voltage is investigated with a two-compartment circuit using the approximation of a fast, weak and small cell-silicon junction. It is shown that extracellular recording relies on the difference of specific ionic conductances in the attached and free regions of the cell membrane. The result rationalizes various observations with neuron transistors. It guides the optimization of extracellular recording and the development of cell-based chemical sensors.

Key words Transistors · Cell membranes · Ionic conductance · Extracellular recording

Introduction

Open field-effect transistors are able to record electrical signals of attached cells in culture (Fromherz 1996; Jenkner and Fromherz 1997; Schätzthauer and Fromherz 1998; Vassanelli and Fromherz 1998). In this Letter, general relationships are derived between the extracellular response and the intracellular voltage transients in cells with voltage-gated and ligand-gated ionic conductances. Using the approximation of a fast, weak and small cell-silicon junction, it is shown how the extracellular record is determined by the spatial distribution of ionic conductances in the membrane. The results are important for the molecular design of cell-silicon junctions with the perspective to develop hybrid neuronal networks and efficient cell-based chemical sensors. The analysis is also relevant for planar metallic microelectrodes as they are used frequently for extracellular recording (Regehr et al. 1988; Breckenridge et al. 1995).

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Results and Discussion

Point circuit model

When a cell (diameter of 10–100 μm) is attached to oxidized silicon, the membrane and the chip are separated by 10–100 nm of electrolyte, depending on the cell type and the coating (Braun and Fromherz 1997, 1998). The extended cleft of the electrolyte is insulated from the cytoplasm by the cell membrane and from the silicon by a layer of silicon dioxide (Fig. 1). The bath, cytoplasm and silicon are each equipotential. The junction forms a two-dimensional core-coat conductor and obeys a two-dimensional analog of the cable equation (Weis and Fromherz 1997). Its crucial features can be described by a circuit with two compartments, cell and junction, as shown in Fig. 1 (Weis and Fromherz 1997; Schätzthauer and Fromherz 1998). The areas A_{FM} and A_{JM} of the free membrane and of the membrane in the junction have various (*i*-type) specific ionic conductances (per unit area) g^i_{FM} and g^i_{JM} which may depend on the voltage across the membrane and on the concentration of chemical agonists. The specific membrane capacitance c_M is the same all over the cell. The seal between the cell and the chip is described by a specific conductance g_J, defined as a global seal conductance divided by the area of the junction ($g_J \equiv G_J/A_{JM}$). The specific capacitance of the microelectrode is c_{EL}. The intracellular voltage V_M and the local extracellular voltage V_J are determined by the current balance in the cell and the junction, respectively. V_J is recorded by an integrated transistor.

Fast, weak, small junction

The extracellular voltage V_J is determined by the current through the attached membrane, through the silicon dioxide and along the seal (Fig. 1). The intracellular voltage V_M depends on the current through the attached and free membranes and on the injection current I_{INJ} through a micropipette (Fig. 1). The current balance in the junction and the cell defines two coupled differential equations for $V_J(t)$

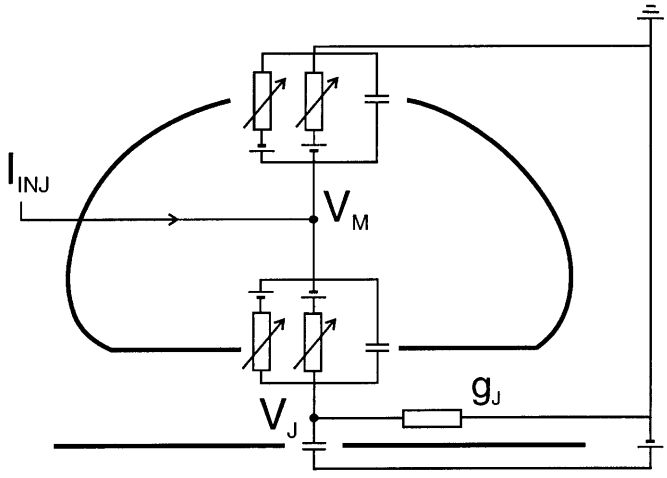

Fig. 1 Cell-silicon junction with two-compartment circuit (point-contact model). The cell membrane and silicon dioxide are marked as *heavy lines*. The actual distance between the membrane and the oxide is 10–100 nm, whereas the diameter of the junction is 10–100 μm. The attached and free membranes of the cell are described by a capacitance, by various (here two) specific ionic conductances which are driven by reversal voltages and which may be voltage-gated or ligand-gated. The oxide is described by a capacitance, the junction by a seal conductance (g_J). I_{INJ} is the injection current through a pipette. The extracellular voltage V_J is recorded by a transistor in the silicon kept at a bias potential. The intracellular voltage V_M may be monitored by a micropipette

and $V_M(t)$ (t time) (Schätzthauer and Fromherz 1998; Vassanelli and Fromherz 1998).

Here we consider three approximations:

1. The capacitive current of the response is small with $(c_{EL}+c_M)\,dV_J/dt \ll g_J V_J$. This approximation implies that the relaxation of the junction is fast compared to the dynamics of the response. It is valid for records in the millisecond range with $c_{EL}+c_M \approx 1\ \mu F/cm^2$ and with $g_J \approx 1000\ mS/cm^2$ (Vassanelli and Fromherz 1998).
2. The conductance of the attached membrane is small with $\sum g^i_{JM} \ll g_J$. This approximation is valid for usual membrane conductances with $\sum g^i_{JM} < 100\ mS/cm^2$ at $g_J \approx 1000\ mS/cm^2$. It implies that the voltage across the attached membrane is similar to the intracellular voltage with $V_M - V_J \approx V_M$, or that the junction is weak with $V_J \ll V_M$. For a weak junction it is adequate to assume that the ion concentrations in the cleft are not changed with respect to the bulk electrolyte, implying unchanged reversal voltages V^i_0.
3. The area of the attached membrane is small with $A_{JM}/A_{FM} = \beta \ll 1$. This approximation is usually compatible with the geometry of cell transistors made with invertebrate and mammalian neurons (Weis and Fromherz 1997; Schätzthauer and Fromherz 1998; Vassanelli and Fromherz 1998).

Current balance

We apply Kirchhoff's law to the nodes of the junction and the cell (Fig. 1). For a fast, weak and small junction we obtain Eqs. (1) and (2):

$$g_J V_J = \sum_i g^i_{JM}(V_M - V^i_0) + c_M \frac{dV_M}{dt} \quad (1)$$

$$\frac{I_{INJ}}{A_{FM}} = \sum_i (g^i_{FM} + \beta g^i_{JM})(V_M - V^i_0) + c_M \frac{dV_M}{dt} \quad (2)$$

In Eq. (1) the current along the seal results from the ionic currents and from the charging current of the attached membrane, which depend on the intracellular voltage $V_M(t)$. In general, Eq. (1) expresses a nonlinear relationship between extracellular and intracellular voltage, because the conductances g^i_{JM} may depend on the membrane voltage. The dynamics of $V_M(t)$ [Eq. (2)] is dominated by the ionic currents of the free membrane and by the injection current. The attached membrane contributes only if its small area with $\beta \ll 1$ is compensated by excessive local conductances $g^i_{JM} \gg g^i_{FM}$.

Voltage clamp

The intracellular voltage may be kept constant by appropriate control of the injection current with $dV_M/dt = 0$ in Eqs. (1) and (2). Then the extracellular voltage V_J reflects the voltage-gated ionic currents through the attached membrane [Eq. (1)]. On the other hand, the injection current I_{INJ} indicates mainly the ionic currents through the free membrane [Eq. (2)]. A comparison of $g_J V_J$ with I_{INJ}/A_{FM} reveals modifications of the attached membrane with respect to the ionic conductances. (The scaling factors g_J and A_{FM} can be obtained from the response of V_J and I_{INJ} to a transient dV_M/dt under conditions of closed ion channels, given the value of c_M.) A superposition of a small AC voltage $\underline{V}_M(\omega)$ (angular frequency ω) to the holding DC potential would give rise to an extracellular modulation $\underline{V}_J(\omega)$. The complex transfer function $\underline{V}_J(\omega)/\underline{V}_M(\omega)$ then describes the linear response of the attached membrane, which varies with the holding potential if voltage-gated conductances are present in the attached membrane.

Coupled dynamics

We consider an attached cell without or with constant injection current (current clamp). In that case the dynamics of extracellular and intracellular voltage are coupled. The free membrane with the injection current creates a signal $V_M(t)$ according to Eq. (2), and the junction reacts with a record $V_J(t)$. We can substitute the charging current of the attached membrane in Eq. (1) by the ionic currents of the free membrane according to Eq. (2) and with $\beta \ll 1$ obtain Eq. (3):

$$g_J V_J = \sum_i (g^i_{JM} - g^i_{FM})(V_M - V^i_0) + \frac{I_{INJ}}{A_{FM}} \quad (3)$$

The record $V_J(t)$ is not an independent superposition of ionic and capacitive current as suggested by Eq. (1). It appears as a superposition of ionic currents only, beside the

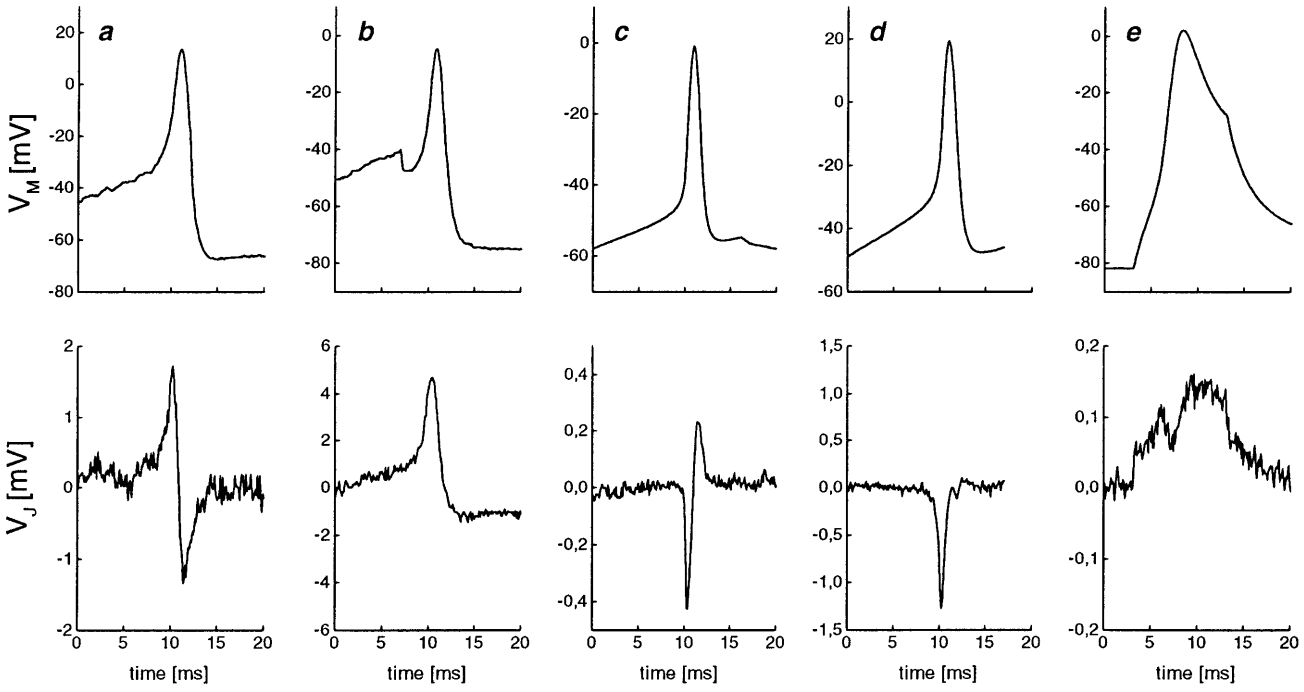

Fig. 2a–e Observations. *Upper row*: intracellular voltage $V_M(t)$ measured with a micropipette. *Lower row*: extracellular records $V_J(t)$ measured with a transistor. **a** Capacitive response (soma of leech neuron) (Jenkner and Fromherz 1997). Interpretation: low ionic conductance in the junction. **b** Ohmic response (soma of leech neuron) (Jenkner and Fromherz 1997). Interpretation: voltage-independent ionic conductance in the junction. **c** Anti-capacitive response (axon stump of leech neuron) (Schätzthauer and Fromherz 1998). Interpretation: accumulation of sodium and of potassium conductance in the junction. **d** Early anti-capacitive response (axon stump of leech neuron) (Schätzthauer and Fromherz 1998). Interpretation: accumulation of sodium conductance in the junction. **e** Early capacitive response with secondary positive response (rat neuron) (Vassanelli and Fromherz 1998). Interpretation: depletion of sodium conductance and accumulation of potassium conductance in the junction. (The two jumps at an interval of 10 ms mark the duration of the injection current)

injection current. The response depends on the differences of the specific ion conductances g^i_{JM}–g^i_{FM} in the attached and free membranes, weighted by the driving voltages V_M–V^i_0. So an inhomogeneity of the cell membrane with respect to specific ionic conductances is the prerequisite for extracellular recording! The strength of the response is given by the differences of the ionic currents, scaled by the seal conductance g_J. The inhomogeneities may be due to changed molecular properties of the ion channels, to a different state of the activation of the channels or to their accumulation and depletion in the attached membrane. We illustrate Eq. (3) by several examples.

Linear junction

We consider a situation when voltage-gated conductances g^i_{FM} are active in the free membrane, but suppressed in the attached membrane with $g^i_{JM} \approx 0$. We assume that an ohmic conductance g^i_{JM} (reversal voltage V_0) is induced in the junction (with $g_{FM} \approx 0$). Then the response $V_J(t)$ in Eq. (3) appears as the difference of the ohmic current in the attached and of the voltage-gated currents in the free membrane. Eqs. (1) and (2) lead to Eqs. (4) and (5) with $I_{INJ} = 0$ for modest ohmic conductance: the free membrane creates an action potential $V_M(t)$ [Eq. (5)] which drives the junction [Eq. (4)]:

$$g_J V_J = g_{JM}(V_M - V_0) + c_M \frac{dV_M}{dt} \tag{4}$$

$$c_M \frac{dV_M}{dt} = -\sum_i g^i_{FM}(V_M - V^i_0) \tag{5}$$

We may distinguish two limits with dominating voltage-gated current or ohmic current, respectively (Fromherz 1996; Weis and Fromherz 1997). In the first case, the response $V_J(t)$ reflects the first derivative of the signal according to Eqs. (4) and (5) (capacitive response). It corresponds to the experimental A-type coupling as shown in Fig. 2a (Jenkner and Fromherz 1997). In the second case, the capacitive current is small with $c_M\ dV_M/dt \ll g_{JM}$ $(V_M$–$V_0)$, with fast relaxation of the attached membrane. The response is proportional to the signal itself according to Eqs. (4) and (5) (ohmic response). It corresponds to the experimental B-type coupling as shown in Fig. 2b (Jenkner and Fromherz 1997). For the linear junctions, $V_J(t)$ may be computed from $V_M(t)$ using an experimental spectral transfer function (Weis and Fromherz 1997).

Single conductance

We consider a single ion channel that is activated or accumulated in the junction, with a background of other homogeneous conductances. Without an injection current,

Eq. (3) is reduced to Eq. (6):

$$V_J = \frac{g^i_{JM} - g^i_{FM}}{g_J}(V_M - V^i_0) \quad (6)$$

Only the current through the accumulated or activated channels contributes to the response. All homogeneous conductances have no effect. This simple case is important for the design of cell-based chemical sensors with ligand-gated channels. The response is optimal for a cell with complete activation or accumulation in the attached membrane, and also for complete inhibition or depletion. Prerequisite is a difference of membrane potential and reversal voltage. For channels with $V_M > V^i_0$ the response is positive for accumulation, for channels with $V_M < V^i_0$ the record is positive for depletion.

Scaled nonlinear junction

We consider a cell with several voltage-gated ion channels which are accumulated or depleted in the junction. We assume that this accumulation or depletion is identical for all channels, described by a common scaling constant as $g^i_{JM} = \mu_J g^i_{FM}$. In that case, all ionic currents can be eliminated from Eqs. (1) and (2). Without an injection current, Eq. (3) is replaced by Eq. (7):

$$V_J = \frac{1-\mu_J}{1+\beta\mu_J}\frac{c_M}{g_J}\frac{dV_M}{dt} \quad (7)$$

There exists a linear relation between extracellular and intracellular voltages despite the voltage-dependent conductances involved! However, Eq. (7) does not describe a linear property of the junction itself as did Eq. (4), because it relies on the coupled dynamics of extracellular and intracellular voltages. It is valid if the cytoplasm [Eq. (2)] and the junction [Eq. (1)] are driven by the same ionic currents up to a scaling constant. Complete depletion with $\mu_J = 0$ reproduces capacitive coupling. For partial depletion with $\mu_J < 1$, the response resembles a capacitive record with reduced amplitude. For accumulation with $\mu_J > 1$, the capacitive response is inverted. Junctions with an anti-capacitive response to action potentials were actually observed with the axon stump of leech neurons, as shown in Fig. 2c (Schätzthauer and Fromherz 1998). They are a prototype of nonlinear junctions, dubbed C-type. A transition from capacitive to anti-capacitive response was simulated also with the Hodgkin-Huxley model (Schätzthauer and Fromherz 1998).

Selective nonlinear junction

A complex response $V_J(t)$ to an action potential may appear if the ion channels are selectively accumulated or depleted in a junction, described by individual scaling constants as $g^i_{JM} = \mu^i_J g^i_{FM}$. The response can be written as a superposition of the ionic currents through the free membrane (weighted with positive or negative factors $\mu^i_J - 1$) according to Eq. (8), as obtained from Eq. (3) with $I_{INJ} = 0$:

$$V_J = \frac{1}{g_J}\sum_i (\mu^i_J - 1) g^i_{FM}(V_M - V^i_0) \quad (8)$$

We consider an excitable cell with sodium and potassium channels. The inward Na^+ current dominates in the early phase of an action potential with $g^K_{FM} \approx 0$. With $V_M < V^{Na}_0$ the response $V_J(t)$ is positive for depletion with $\mu^{Na}_J < 1$ and negative for accumulation with $\mu^{Na}_J > 1$. We expect a positive pulse or a negative pulse in the early phase of an action potential (early capacitive and early anti-capacitive response). Both types of records were actually observed with neurons from leech and rat as shown in Fig. 2d and Fig. 2e (Schätzthauer and Fromherz 1998; Vassanelli and Fromherz 1998). In the later phases of an action potential, the slower K^+ channels contribute to the response, too, if they are accumulated or depleted. There the response $V_J(t)$ may have almost any shape, depending on the relative strength of accumulation and depletion of the K^+ and Na^+ channels and on the nature of voltage-dependent activation (and inactivation) of the K^+ channels. In fact, various waveforms in the later phase of an action potential were observed with neurons from leech and rat as shown in Fig. 2c, 2d and 2e (Schätzthauer and Fromherz 1998; Vassanelli and Fromherz 1998). The effects were simulated with the Hodgkin-Huxley model (Schätzthauer and Fromherz 1998; Vassanelli and Fromherz 1998).

Metal electrodes

The results are valid for all planar electrodes which measure extracellular voltage by capacitive coupling. For metal electrodes, however, the Helmholtz capacitance may become relevant for fast voltage transients. It may be necessary to replace Eq. (3) by Eq. (9). The record $V_J(t)$ is given then by the differences of the ionic currents and by the injection current, convoluted with the exponential response to a current pulse:

$$c_{EL}\frac{dV_J}{dt} + g_J V_J = \sum_i (g^i_{JM} - g^i_{FM})(V_M - V^i_0) + \frac{I_{INJ}}{A_{FM}} \quad (9)$$

Conclusion

The model of a fast, weak and small junction reveals how extracellular recording depends on the inhomogeneity of ionic conductances in a cell membrane. Even if the three approximations may not be valid for every real situation, the approach provides a basis to rationalize extracellular recording in cell culture. The considerations may be relevant also for recording in a tissue with its close cell-cell contacts.

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