Serotonin–Transistor Biosensor

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Recombinant Serotonin Receptor on a Transistor as a Prototype for Cell-Based Biosensors**

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It is a goal of biosensor technology to combine the extraordinary specificity of biochemical receptors with general-purpose microelectronics to develop selective probes for diagnostics, drug screening, and toxin detection.[1] Numerous receptors are coupled to ion channels in the cell membrane directly or through G proteins. Usually, the ion current upon activation is recorded with classical or planar patch-clamp techniques that cause damage of the cells.[2–6] Herein, a proof-of-principle experiment demonstrates the feasibility of non-invasive receptor-cell–transistor (RCT) sensors as an alternative.[7] The ion current of a recombinant receptor is directly coupled to a microelectronic device in a cell–transistor junction (Figure 1 a). When ion channels are opened by an agonist, ion current flows into the cell along a narrow cleft between the cell and chip. An extracellular voltage V_J is created on the transistor that modulates the electronic source-drain current.[8,9]

In our experiments, we use the ionotropic serotonin receptor 5-HT3A, which is overexpressed in HEK293 cells. Serotonin receptors play an important role in the peripheral and central nervous system.[10,11] Clinically, specific blockers are used to inhibit chemotherapy-induced emesis and to treat irritable-bowel syndrome.[12] Different 5-HT3 subunits can be coexpressed to form heteropentamers.[13] The A subunit alone is able to assemble into the functional homopentameric 5-HT3A receptor with a cation-selective channel and with binding sites for serotonin in its extracellular domain.[12–14]

To achieve a physicochemical characterization of the receptor–transistor coupling, we impose two constraints in our present study (Figure 1 a): We control the agonist concentration c_A with a Θ-tube pipette and the intracellular voltage V_M with a whole-cell patch pipette. To probe the ligand-gated channel, a rather low membrane current must be detected without averaging repetitive signals. We solved this problem with a low-noise electrolyte-oxide-semiconductor (EOS) transistor in a buried-channel configuration.[15]

A silicon chip with HEK293 cells on an array of EOS transistors is depicted in Figure 1 b. The cells are cultured for 24 h on fibronectin. After application of an extracellular recording medium, a cell that covers the gate of a transistor is contacted with a patch pipette. The transistor is calibrated by voltage pulses to the Ag/AgCl electrode in the bath. We hold the cell at an intracellular voltage of −120 mV and apply serotonin at a concentration of 100 μM dissolved in the recording medium with a solution-switching system. Figure 2 shows, for two cells, transients of 10 nA for the whole-cell current that indicate an opening and desensitization of about 80 000 5-HT3A receptors with a single-channel conductance of 1 pS.[16,17] Simultaneously, we observe the transients of the transistor voltages with amplitudes around 1 mV. The waveforms are similar for the transistor voltage and the whole-cell current. When we lower the serotonin concentration from 100 μM to 5 μM (Figure 2 a) or lower the hyperpolarization from −120 mV to −100 mV and −80 mV (Figure 2 b), the pipette current and the transistor voltage become smaller. No

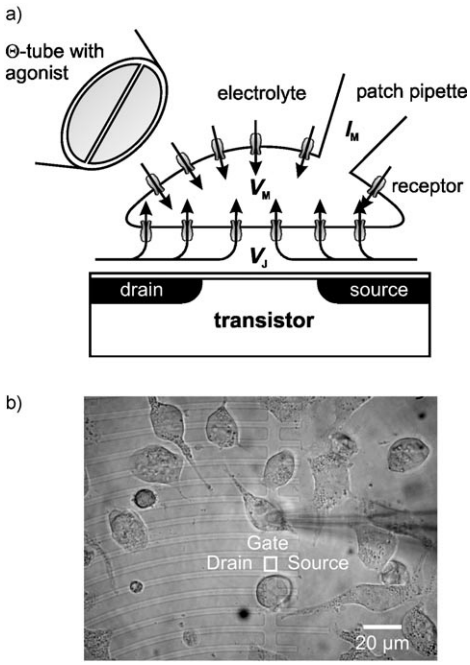

Figure 1. RCT biosensor with ligand-gated ion channel. a) Schematic cross section (not to scale) of the test experiment. A cell (diameter around 20 μm) is separated from the open gate of a field-effect transistor by a narrow cleft (width around 50 nm) with extracellular electrolyte. An agonist is applied with a Θ tube. The ion current flowing through open channels in the attached membrane gives rise to an extracellular voltage V_J in the cell–transistor junction that modulates the source-drain current. A patch pipette controls the intracellular voltage V_M and records the ion current I_M through the total membrane. b) HEK293 cells with the serotonin receptor 5-HT3A on a linear array of low-noise transistors. The leads of source and drain are indicated and the gate area is marked by a white square for one of the transistors. A selected cell is contacted with a patch pipette.

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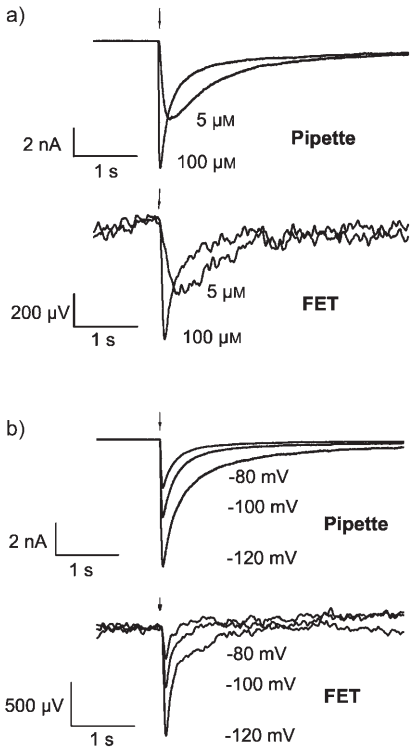

Figure 2. Records of whole-cell current (pipette) and voltage of field-effect transistor (FET) upon application of serotonin (arrows) at a constant intracellular voltage without signal averaging. a) Intracellular voltage $V_M = -120$ mV. Application of two different serotonin concentrations (100 μM, 5 μM). b) Application of a serotonin concentration $c_{serotonin} = 100$ μM at three different intracellular voltages (−80 mV, −100 mV, −120 mV).

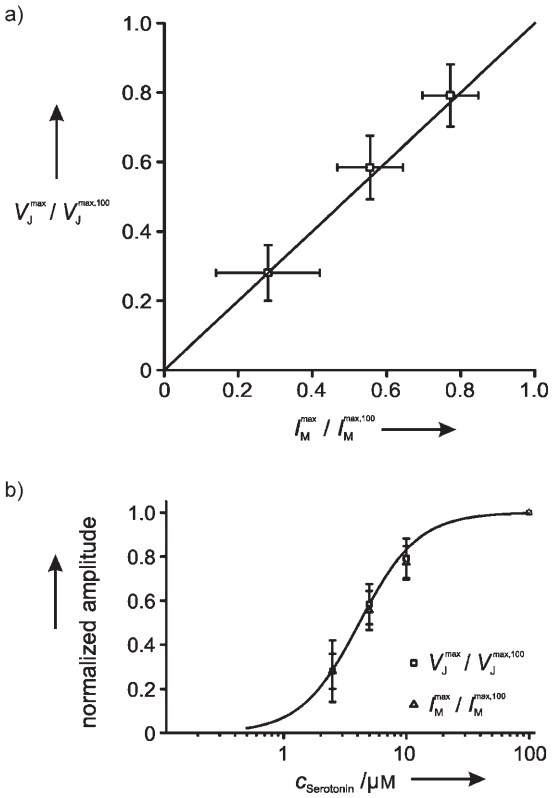

Figure 3. Dose–response relationship. a) Amplitude of the transistor signal V_J versus the amplitude of the membrane current I_M at three concentrations of serotonin normalized to the amplitude at a concentration of 100 μM (five measurements at each concentration). b) Normalized amplitude of transistor signal and normalized amplitude of whole-cell current versus concentration of serotonin. The drawn line is an isotherm computed with a concentration of 4.2 μM for half-maximum channel activation and with a Hill coefficient of 1.8 as obtained from separate patch-clamp experiments.

signals are observed when a solution switching is applied without serotonin.

We obtain dose–response relationships for the pipette current and the transistor voltage by measurements at different serotonin concentrations. Owing to the finite lifetime of whole-cell patches, we have to combine the data from different cells. For each cell, we measure the maximum response at a certain concentration and normalize it by the maximum response at 100 μM at which all channels are open. The normalized amplitudes of the transistor voltage and of the pipette current are plotted versus each other in Figure 3a and versus the concentration in Figure 3b. The signals exhibit a perfect proportionality, and the dose response relations are in agreement with an isotherm obtained from separate patch-clamp recordings with a half-maximum channel activation at 4.2 μM and a Hill coefficient of 1.8. The parameters are close to published values of 3.4 μM and 1.8, respectively.[17]

The experiments demonstrate that an EOS transistor is able to record the activation of 5-HT3A receptors and that the transistor probes the ion current of activated channels similar to a patch pipette. Two peculiar aspects of the method, however, must be taken into account: 1) The transistor signal is created by the ion current through the attached membrane, whereas a pipette signal reflects the ion current through the whole cell membrane. 2) The voltage signal of the transistor implies a scaling factor for the current that is determined by the electrical resistance of the cell–transistor junction. In the following sections we consider the timing as well as the correlation of the transistor and pipette signals.

In Figure 4a, a transistor voltage $V_J(t)$ and a pipette current $I_M(t)$ are aligned in a single plot. The transistor signal in that experiment is delayed by about 70 ms. The effect is illustrated in Figure 4b in which $V_J(t)$ is plotted versus $I_M(t)$. The average delay time over the 16 experiments is 40 ms. We distinguish three phases: I) The pipette signal increases without the transistor signal. II) The pipette signal decays with a rise in the transistor signal. III) The pipette signal and the transistor signal both decay. This result indicates that the receptors in the attached membrane are activated with a delay when compared with the receptors in the free membrane.

In the area of cell adhesion, the serotonin concentration increases only after diffusion into the cleft between the cell and the chip. For an estimate, we consider a circular cell–chip junction of radius a_J and with a narrow extracellular space in the order of approximately 50 nm.[18] After an initial jump in the concentration of the bath, the concentration profile in the junction is given by an infinite series of exponentials.[19] The time constant of the slowest component—that dominates the

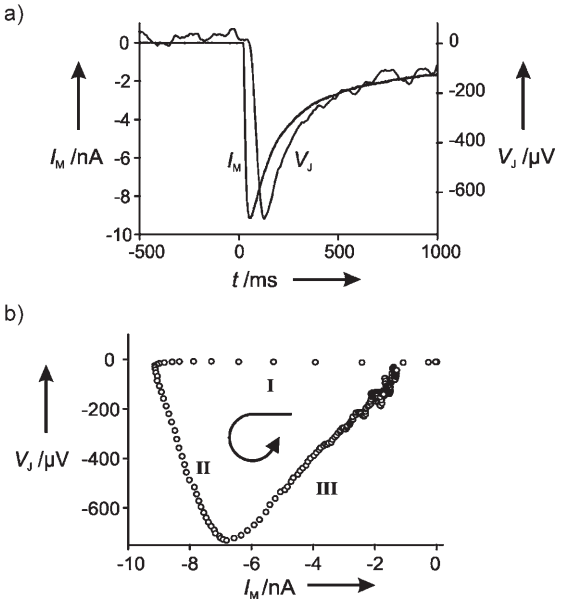

Figure 4. Delay between transistor signal and whole-cell current. a) Transistor signal $V_J(t)$ and pipette signal $I_M(t)$ versus time at an intracellular voltage of −120 mV and a serotonin concentration of 100 μM. b) Transistor signal $V_J(t)$ versus the pipette signal $I_M(t)$ with three phases: I) increasing whole-cell current and no transistor signal, II) increasing transistor signal and decreasing whole-cell current, and III) decay of whole-cell current and transistor signal. The direction of the recording time is indicated by the arrow (total measuring time = 5 s, intervals = 2.5 ms).

central region of adhesion—is $a_J^2/5.783\,D_A$ with the diffusion coefficient D_A and the first zero $\sqrt{5.783}$ of the Bessel function J_0. For a radius of 10 to 15 μm and a diffusion coefficient of 10^{-5} cm^2 s^{-1}, we obtain a time constant of 20 to 40 ms, which lies in the order of the experimental delays. A remaining difference may be due to a lowered diffusion coefficient in the narrow extracellular space.[20]

The delayed activation of receptors in the cell–transistor has two important aspects for RCT biosensors: 1) The delay does not impair the dose-response relationship as shown in Figure 3. 2) The delay provides a difference in the ion conductance between the attached and free membrane, which is a prerequisite for transistor recording without a patch pipette, to avoid a compensation of ionic and capacitive currents.[7,8] Thus, a structural polarity with an accumulation or depletion of ion channels is not required.

The ion current I_{JM} through the attached membrane is translated into an extracellular voltage V_J by the resistance of the cell–transistor junction with $V_J = (r_J/\eta_J)I_{JM}$, where r_J is the sheet resistance of the extracellular space between cell and chip and η_J accounts for the position of the transistor.[9] When we assume that the currents through the attached and total membrane are proportional to the membrane areas, expressed by a relation $I_{JM}/I_M = A_{JM}/A_M$, we obtain Equation (1) as a relationship between the extracellular voltage V_J

$$V_J = R_J^* I_M,\ R_J^* = \frac{R_J}{\eta_J}\frac{A_{JM}}{A_M} \qquad (1)$$

(probed with the transistor) and the total membrane current I_M (measured with the pipette) with an effective resistance R_J^*.

The geometry of the cell–transistor junction is not controlled in our approach. Thus, there is a wide variability in the effective resistances that gives rise to variability in the transistor records. To check this implication, we determine R_J^* by transistor recordings with alternate-current (ac) stimulation of the cell and by a measurement of the membrane capacitance.[9] We scale the transistor signal of receptor activation by the total membrane current and plot the ratio V_J^{max}/I_M^{max} versus the effective resistance R_J^* in Figure 5. We

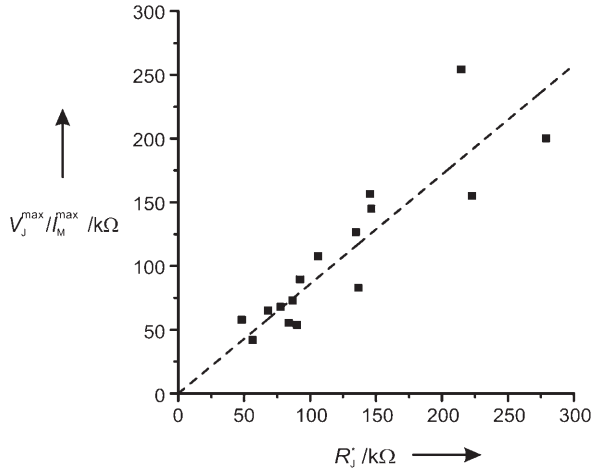

Figure 5. The ratio V_J^{max}/I_M^{max} for the amplitudes of transistor voltage and pipette current at a serotonin concentration of 100 μM versus the effective resistance R_J^* of the cell–transistor junction. The dashed line obtained by linear regression has a slope 0.86 ($r^2 = 0.78$). The correlation indicates that the variability of transistor recording is dominated by a variability in the cell–transistor coupling owing to different positions of the gate in the area of adhesion.

find a wide variability of both parameters in a range from 50 to 250 kΩ. Linear regression leads to a slope of 0.86 ($r^2 = 0.78$), which is in good agreement with Equation (1). Thus, the variability of the transistor records is dominated by the effective resistance, R_J^*, and in particular by the parameter η_J, which accounts for the position of the transistor and the cell.[9] There is a minor contribution by the variability of channel expression as reflected by the membrane current.

In conclusion, we have solved the fundamental problem of RCT biosensors by interfacing a ligand-gated ion channel to field-effect transistors on the level of an individual cell. For constant intracellular voltage ("voltage clamp") the extracellular voltage on the transistor is proportional to the whole-cell ion current of activated receptors. For RCT biosensors, two important problems remain to be solved: 1) We must avoid the application of a patch-pipette. However, without voltage clamp, the receptor current rapidly abolishes the driving force such that no transistor record can be observed. An overexpressed delayed-rectifier K^+ ion channel may provide efficient repolarization. 2) The variability of transistor recording must be overcome for a random cell culture. Most promising is a statistical evaluation with a large number

of cells on a large array of closely packed transistors as fabricated by CMOS (complementary metal oxide semiconductor) technology.[21]

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