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Supporting Information

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Recombinant serotonin receptor on transistor, a prototype for cell-based biosensorics

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Experimental Section

HEK293 cells (DSMZ, Braunschweig, Germany) are transfected (Effectene, Qiagen) with the cDNA of the A subunit of the human serotonin receptor 5-HT3 (HORF clone collection, Invitrogen) in the pcDNA3.1(+) vector (Invitrogen, Karlsruhe, Germany). Stably transfected clones are obtained after 6 weeks of selection by cultivating at 37°C in 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% (vol/vol) heat inactivated bovine serum (Invitrogen) and 500 µg/ml geneticin (G418 disulfate salt, Sigma). The stably transfected cells are cultured in the same medium. They are split at 70–80% confluency and diluted with DMEM without geneticin and without bovine serum at a ratio 1:50 and replated on the silicon chips.

We use silicon chips with two linear arrays of p-type electrolyte-oxide-semiconductor field-effect transistors in a buried channel configuration.[14] The size of the metal-free gates is 6 µm x 7 µm. The chips are bonded to a ceramic package. A perspex chamber designed for laminar flow is attached. Before each use, the chips are

cleaned with a detergent (Ultrax 102 S, KLN, Heppenheim), rinsed with milli-Q water (Millipore, Bedford, MA) and dried with nitrogen. The surface is made hydrophobic by exposure to hexamethyldisilazane vapor (30 min), sterilized with UV light (30 min), coated with fibronectin (Sigma, Deisenhofen, Germany, 15 µg/ml in PBS, 2 hours, 4°C) and rinsed with PBS (phosphate buffered saline). Before replating the cells, the chips are equilibrated with serumfree DMEM for 2 hours at 37°C. 1 ml of the cell suspension is seeded and cultured for 24 hours at 37°C in 5% CO_2.

Electrophysiological measurements are performed with an Axopatch 200B amplifier (Axoclamp, Union City, CA) with a Ag/AgCl reference electrode (WPI, Sarasota, FL) at ground potential. The extracellular electrolyte contains (in mM) 145 NaCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 HEPES and 10 glucose (pH 7.4 adjusted with NaOH, 290 mOsmol/lit, 62.5 Ωcm) (all chemicals from Sigma). Micropipettes with 2-3 MΩ resistance are pulled from borosilicate glass (Science Products, Hofheim) and coated with Sylgard (Dow Corning, Midland, USA). They are filled with an intracellular electrolyte containing (in mM) 145 CsCl, 1 EGTA, 10 HEPES and 10 glucose (pH 7.4 adjusted with CsOH, 290 mOsmol/lit).

Serotonin (#H-9523, Sigma) is dissolved in extracellular electrolyte. Fast solution switching is achieved by the Θ-tube method.[15] We use glass tubes pulled to a tip diameter of 160-180 µm (Hilgenberg, Malsfeld, Germany; 2 mm outer diameter, 0.3 mm wall thickness, 0.117 mm septum) and the LSS-3100 solution switching system from Burleigh, (Fishers, NY). One channel of the Θ-tube is filled with serotonin solution, the other with extracellular electrolyte.

The transistors are controlled by a bias voltage V_{gs} = -0.5 V between gate (bath electrolyte) and bulk silicon and by a voltage V_{sd} = 0.5 V between source and drain with a source drain current around I_D = 10 µA. The transistors are calibrated before each measurement by modulating the bath potential with a transconductance around $g_m = \partial I_D / \partial V_{gs}$ = 20 µS. The current change induced by receptor activation, is expressed as an effective change of the gate voltage with the relation $\Delta V_{FET}(t) = \Delta I_D(t) / g_m$. That voltage signal is attributed to an extracellular voltage $V_J(t) = \Delta V_{FET}(t)$ between cell and chip, because the surface potential is not affected by a membrane current that is dominated by sodium ions.[9]

The experiments are performed at room temperature. During the experiments the culture chamber is flushed with extracellular electrolyte. Only solitary cells without connections to other cells are selected with a microsope (Axioskop 2 FS; Zeiss, Oberkochen), to ensure that the recorded signals are derived from individual cells and that capacitance compensation is not impaired. A cell-attached configuration is established with the patch-pipette and the stray capacitance is compensated. After the breakthrough, the membrane capacitance $C_M = c_M A_M$ is determined that yields the total membrane area A_M with c_M = 1 µF/cm^2. Then the capacitance and the access resistance are compensated. An alternating voltage $\underline{V}_M$ with an amplitude of 10 mV is superposed at eight frequencies between 400 and 1600 Hz to an intracellular holding voltage of -70 mV. The extracellular voltage $\underline{V}_J$ is obtained with the transistor. From a plot of $|\underline{V}_J / \underline{V}_M| = w(c_M + c_S)(r_J A_{JM} / h_J)$ versus the angular frequency w, we obtain the parameter $r_J A_{JM} / h_J$ with the chip capacitance c_S = 0.3 µF/cm^2.[9] Then the receptors are activated by application of serotonin for 5 s at a holding potential of -70..-120 mV. The

pipette current and the source-drain current are simultaneously recorded. The signals are filtered at 10 kHz and sampled at 30 kS/s. Between different experiments with the same cell, a period of at least 2 min allows for complete washing out of serotonin and for recovery of the receptors.