Silicon-Neuron Junction: Capacitive Stimulation of an Individual Neuron on a Silicon Chip

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An identified nerve cell of the leech is attached to a planar silicon microstructure of *p*-doped silicon covered by a thin layer of insulating silicon oxide. A voltage step, applied between silicon and electrolyte, induces a capacitive transient in the cell which elicits an action potential. The capacitive extracellular stimulation is described by an equivalent electrical four-pole.

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The integration of electronic circuitry and neuronal networks requires a bidirectional electrical communication between silicon elements and nerve cells. In recent years, the successful assembly of a neuron-to-silicon junction was reported with direct signal transfer from an individual neuron to a microscopic metal-free field-effect transistor [1,2]. In the present paper, we describe a complementary system, the direct silicon-to-neuron junction. An individual Retzius cell of the leech (diameter about 70 μm) is stimulated extracellularly from a microspot (diameter about 50 μm) of *p*-type silicon which is perfectly insulated by silicon oxide (dc resistance $>$ 1 TΩ) [Fig. 1(a)]. The stimulation was achieved by capacitive coupling which is effective in our system due to the tight association of oxidized silicon and neuron. There is no Faradaic current flowing across the electrode/electrolyte interface in contrast to all systems with a metallic interface [3–8]. Thus any toxic electrochemical effects can be avoided.

Silicon electrode.—Chips (30×10 mm^2) were cut from *n*-type Si wafers with a (100) surface and a resistance of 5–10 Ω cm (Aurel GmbH, Landsberg, FRG). We opened 16 radial lanes in a mask oxide grown in wet O_2 at 1000 °C by etching with ammonium fluoride solution (AF 87.5.12,5, Merck, Darmstadt). The lanes were 2.5 mm long (from 0.5 to 3 mm from the center of chip). Their width was 60 μm for the first millimeter and widened up to 700 μm at the periphery. These lanes were *p*-doped with boron [1,2]. Their dc resistance was about 400 Ω. A field oxide (1 μm) was grown at 1000 °C. Stimulation spots (diameter 20–50 μm) were etched at the inner end of the lanes and covered by a thin layer of oxide (about 10 nm) grown in dry oxygen at 1100 °C in a rapid thermal processing oven (AST, Dornstadt, FRG). After opening contact sites at the peripheral ends of the lanes, they were wire bonded. Finally, a Plexiglass chamber (radius 1.5 mm) was attached using silicone glue (Raumedic, Rehau, FRG). It separates the region of the stimulating spots from the ends of the conductive lanes which were connected to external electronics.

Neuron-chip assembly.—Retzius cells were dissociated from *Hirudo medicinalis* [9] and kept in a plastic petri dish in a culture medium (L-15, Gibco, Life Technologies GmbH, Eggenstein, FRG) with 2% fetal calf serum (Gibco), 5 mg/ml glucose, and 50 μg/ml gentamycin sulfate (Sigma) for up to three days. Cells which were covered by material from the extracellular matrix were treated with dispase/collagenase (Boehringer, Mannheim; 2 mg/ml) for 30 min to remove it. We cleaned the

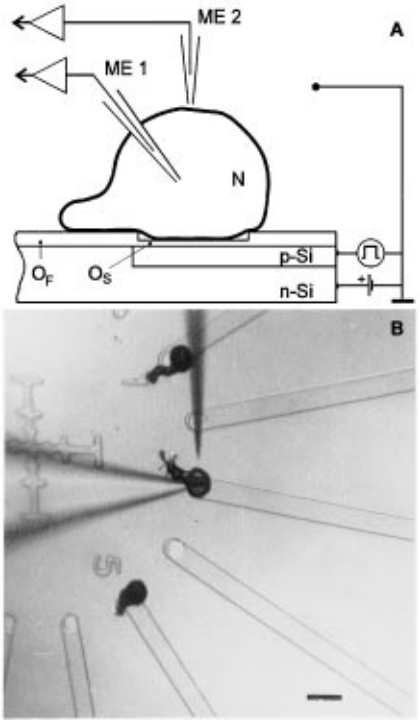

FIG. 1. Silicon-neuron junction. (a) Schematic cross section (unscaled). A neuron (N) is attached to a "stimulation spot" at the end of a *p*-doped lane, which is covered by a thin layer (10 nm) of "spot oxide" (O_S). The surroundings are covered by a thick (1 μm) field oxide (O_F). A bias voltage applied to the *n*-type bulk silicon gives rise to a blocked *p-n* junction. For extracellular stimulation, voltage wave forms are applied to the *p* silicon. Responses to the stimuli are detected intracellular by an impaled microelectrode (ME 1), and an extracellular microelectrode (ME 2). (b) Micrograph of a neuron chip. Scale bar 100 μm. Three Retzius cells are attached to circular stimulation spots at the ends of radial lanes. One of the neurons is impaled by a microelectrode (from the left). A second microelectrode is placed close the neuron.

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chip with hot (80 °C) basic hydrogen peroxide (30% hydrogen peroxide:25% ammonia/water = 1:1.5). As an adhesive we applied drops of polylysine [1 mg/ml in water, molecular weight (MW) 15 000–30 000, Sigma/Deisenhofen, FRG] to the stimulation spots and dried them for 2–3 h. Then the chamber was rinsed with water for up to 3 h and filled with a serum-free culture medium. A neuron was sucked into a glass pipette (tip diameter about 100 μm), transferred into the chamber, and blown onto a stimulation spot under visual control in a stereomicroscope.

Electrical setup.—The electrolyte was kept at ground potential (Ag/AgCl/agar in 1*M* KCl). We applied positive voltage steps or pulses with an amplitude up to 5 V (slope 2.2 V/μs) to the *p*-doped lanes using a computer controlled wave-form generator. The bulk *n*-type silicon was kept at +5 V such that a blocking *p*-*n* junction confined the stimulus to the lanes. Under these conditions we never observed any corrosion of the stimulation spot, even after many days of use. (Prerequisite for this stability is a careful preparation of the thermally grown oxide and a positive polarization of the silicon.) The neuron was impaled by a microelectrode (4*M* K acetate, resistance 15 MΩ). A second microelectrode, placed close to the cell, was used to record extracellular transients [Fig. 1(b)]. The signals of both microelectrodes were recorded by an amplifier (10 kHz bandwidth) with capacitance compensation and stored by a digital oscilloscope (100 MHz, Tektronix 2221A), a computer (25 kHz, 12 bit), or a tape recorder (5 kHz, *V* store, Racal Elektronik/Bergisch Gladbach, FRG). Before the experiments we checked the activity of attached neurons, eliciting action potentials by intracellular current injection. During the experiments spontaneous firing was suppressed by continuous hyperpolarization (−60 to −70 mV).

Silicon-to-neuron coupling.—By applying a voltage step to the stimulation spot, an attached neuron was excited electrically as shown in Fig. 2(a). The amplitudes were 4.8, 4.9, and 5.0 V. There is a sharp threshold for eliciting action potentials. While the smallest stimulus had no effect, the two other stimuli gave rise to an action potential, appearing with delays of 130 and 65 ms, respectively. When the voltage was applied as a pulse of 10 ms duration, the initiation of the action potential by the initial step was not interrupted by the negative transient as shown in Fig. 2(b) for another neuron. As a third type of stimulus we used a sequence of bursts of short pulses, which elicited a train of action potentials [Fig. 2(c)]. The extracellular stimuli lead reproducibly to action potentials, if the neurons were placed precisely on the stimulation spots. 75% of the junctions made of an active neuron on a stimulation spot with thin oxide (around 10 nm) responded as shown in Fig. 2. The stimulations of single neurons were effective up to 3 h. Then they lost their excitability because of the injury by the penetrating microelectrode.

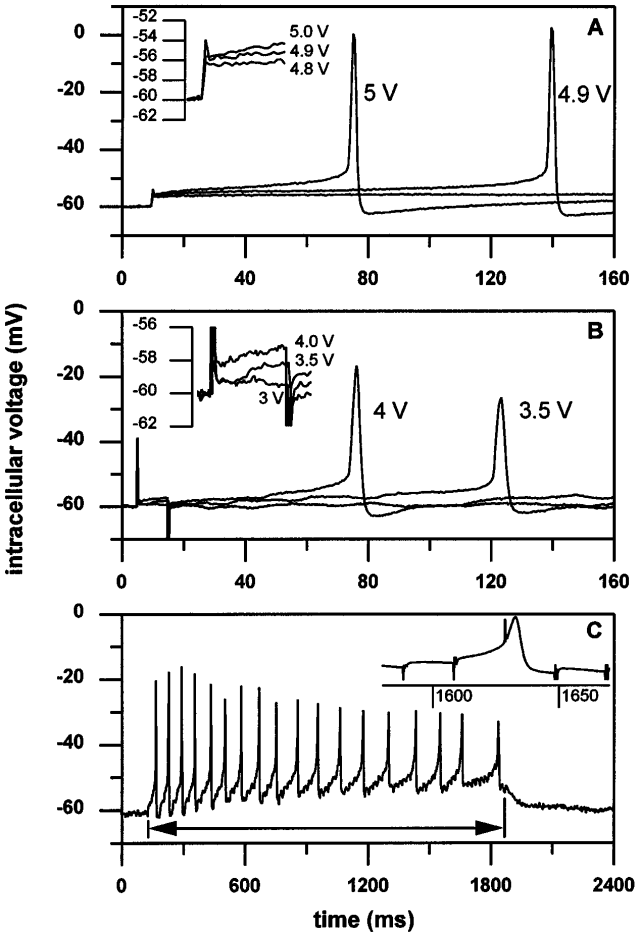

FIG. 2. Extracellular stimulation. Response of Retzius cells to various pulse programs applied to the stimulation spot. (a) Step stimulation (cell with 1.3–1.6 nF and 20 MΩ, spot diameter 50 μm, 9 nm thick oxide). The step of 4.8 V height is a subthreshold stimulus. Action potentials are elicited by steps of 4.9 and 5 V height. For the stronger stimulus the delay is shorter. In the inset the stimulation phase is shown at an expanded scale. (The true, fast capacitive transient is not resolved because of the low sampling time of the electronics.) (b) Single-pulse stimulation (cell with 600 pF and 30–40 MΩ, spot diameter 40 μm, 9 nm thick oxide). Pulses of 10 ms duration and various amplitude are applied. The pulse with an amplitude of 3 V is a subthreshold stimulus. Action potentials are elicited by pulses with amplitudes of 3.5 and 4 V. The inset shows the stimulation phase at an expanded scale with the capacitive transients. (c) Repetitive stimulation. Bursts (each with 70 pulses of 10 μs duration) are applied at a frequency of 50 Hz for a period of 1.8 s (double arrow). A train of action potentials is elicited during the stimulation (cell parameters not determined, spot diameter 50 μm, oxide of 15 nm thickness). The inset shows a single action potential with the superposed capacitive transients.

The long delay between the stimulus and the action potential was rather surprising. We checked whether this effect is an artifact caused by the silicon-neuron contact or whether it is due to the type of stimulation. For comparison we injected short and weak depolarizing current pulses into a Retzius cell in standard cell culture.

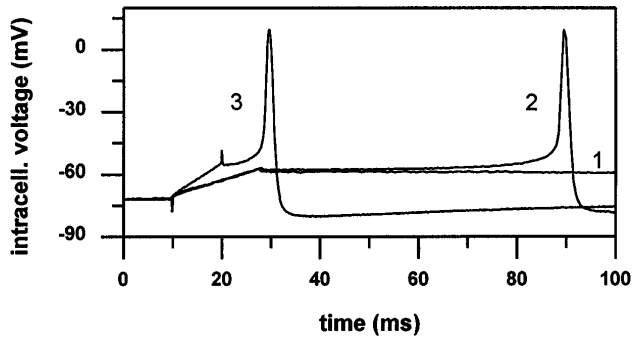

FIG. 3. Response of a hyperpolarized Retzius cell (kept at −70 mV) to intracellular current pulses. (1) Subthreshold stimulus with 1 nA amplitude and 18 ms duration (injected charge 18 pA s). (2) Weak suprathreshold pulse with 1 nA height and 18.5 ms duration (injected charge 18.5 pA s). (3) Strong pulse with 2 nA amplitude and 10 ms duration (injected charge 20 pA s). The width of the pulses is shorter than the time constant of the neuron (51 ms at a resistance of 34 MΩ and a capacitance of 1490 pF). For weak stimuli (trace 2) there is a long delay between stimulus and action potential which shortens for stronger stimuli (trace 3), similar to the extracellular stimulations in Fig. 2.

We observed long delays between stimulus and action potential for weak stimuli (Fig. 3). The delay effect is an intrinsic feature of hyperpolarized Retzius cells under conditions of weak transient depolarization.

Four-pole.—To rationalize the crucial features of the experimental results (capacitive transients, threshold to elicit action potentials), we consider the circuit shown in Fig. 4. It consists of the capacitance C_{JS} of the spot oxide, the capacitance C_{JM} and the resistance R_{JM} of the contacted neuron membrane, and the seal resistance R_J in the junction. The free part of the membrane is represented by the capacitance C_{FM} and the voltage-dependent conductances of the membrane as described, e.g., by the Hodgkin-Huxley model [10]. By changing the voltage V_S between stimulation spot and electrolyte the system is stimulated. The response is given by a change of the voltage V_M between cell and electrolyte. This voltage controls the Hodgkin-Huxley type dynamics of the membrane. An analogous circuit was used to describe the capacitive coupling of a neuron and a transistor [1,2]. It differs from models for capacitive stimulation with macroscopic electrodes, where the voltage-drop across the extracellular medium of a neuronal tissue is essential [11,12].

Capacitive transients.—A voltage step of an amplitude ΔV_S^0 at the stimulation spot induces exponentially decreasing voltage transients ΔV_J and ΔV_M in the junction and the neuron, respectively. From the equivalent circuit we obtain for their amplitudes

$$\Delta V_J^0 = \frac{C_{JS}}{C_{JS} + C_{JM}} \Delta V_S^0, \tag{1}$$

$$\Delta V_M^0 = \frac{C_{JM}}{C_{JM} + C_{FM}} \Delta V_J^0. \tag{2}$$

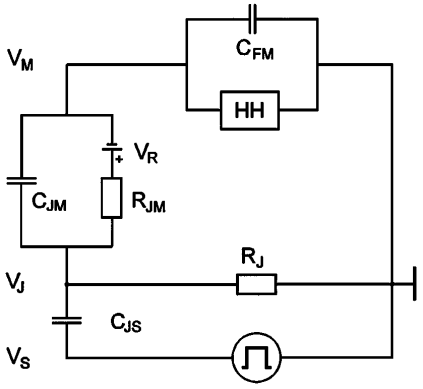

FIG. 4. Equivalent circuit of the silicon-neuron system. The junction is described by the capacitance C_{JS} of the stimulation spot, by the seal resistance R_J, and by the capacitance C_{JM} and resistance R_{JM} of the bound membrane around the resting voltage V_R. The free membrane is represented by the capacitance C_{FM} and a system of voltage-gated conductances according to the Hodgkin-Huxley model.

The relaxation time τ_J^* of the transients is given by the discharge of the capacitances in the junction C_{JM} and C_{JS} through the resistances R_{JM} and R_J

$$\frac{1}{\tau_J^*} = \frac{1}{C_{JM} + C_{JS}} \left(\frac{1}{R_{JM}} + \frac{1}{R_J} \right). \tag{3}$$

Typically, a stimulation spot had a capacitance of $C_{JS} = 4.7$ pF at an area of 1.3×10^{-5} cm^2. The total capacitance and resistance of a tested Retzius cell was around 500 pF and 40 MΩ, at a surface area of about 1.5×10^{-4} cm^2. From these data we estimate for the capacitance and resistance in the junction $C_{JM} = 40$ pF and $R_{JM} = 490$ MΩ, assuming that the stimulation spot is covered completely by the membrane. For a positive stimulus $\Delta V_S^0 = 5$ V we expect voltage peaks $\Delta V_J^0 = 516$ mV and $\Delta V_M^0 = 42$ mV in the junction and in the cell, respectively, according to Eqs. (1) and (2). The voltage drop across the membrane in the junction is about 470 mV, well below the threshold of electrical breakthrough, which is about 1 V [12]. (Note: The electrical field across the membrane in the junction corresponds to an intracellular hyperpolarization.) With a seal resistance $R_J = 2.5$ MΩ, as in a weak A-type coupling of Retzius cell and transistor [2], we estimate a time constant $\tau_J^* = 113$ μs.

We tried to check the features of capacitive stimulation in recordings with enhanced time resolution (Fig. 2, insets). We were faced with two problems. (i) Electrodes and amplifiers shunted spectral components above 1000 Hz. (ii) A substantial part of the signal in the intracellular electrode was caused by a capacitive coupling of chip and micropipette "through-space." To estimate the intracellular transient we subtracted from the intracellular signal the transient measured with the extracellular microelectrode. In a typical case we obtained an amplitude of $\Delta V_M^0 = 10$ mV and a relaxation time of $\tau_J^* = 130$ μs. The simplest way to explain the relatively low amplitude

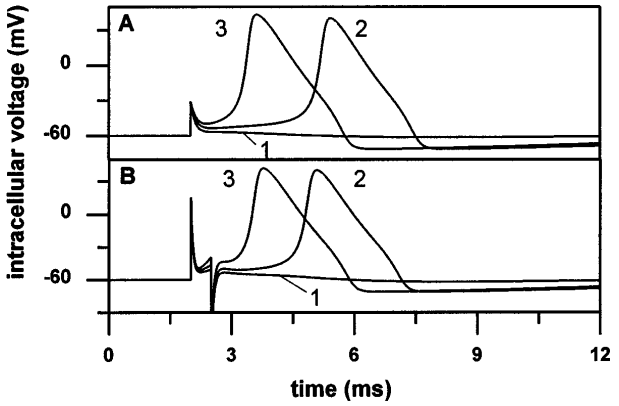

FIG. 5. Simulation of the silicon-neuron. A model neuron with a surface area of 10^{-4} cm^2, a specific capacitance of the membrane of 1 μF/cm^2 and a specific resistance in the resting state of 1.47 kΩ cm^2 is attached to a stimulation spot with an area of 10^{-5} cm^2 and a specific capacitance of 0.37 μF/cm^2. The unattached part of the membrane is described by the original Hodgkin-Huxley model. (a) Stimulation by a voltage step of 1 V at various seal resistances of the junction: (1) 10 MΩ, (2) 14 MΩ, and (3) 18 MΩ. (b) Stimulation by a voltage pulse of 0.5 ms duration with the amplitudes (1) 2.2 V, (2) 2.4 V, and (3) 2.6 V at a seal resistance of 5 MΩ.

is to assume an incomplete coverage—about 25%—of the stimulation spot by the membrane.

Action potentials.—The experiments indicate that the exponentially decreasing, depolarizing intracellular transient ΔV_M is able to trigger an action potential. To check that concept, we evaluated the complete equivalent circuit (Fig. 4). However, a parametrization of the current across the membrane of Retzius cells is not available [14]. In order to avoid an arbitrary variation of the parameters, we used the original Hodgkin-Huxley model of the squid axon [10]. Figure 5(a) shows a simulation of the intracellular voltage induced by a voltage step of 1 V applied to a stimulation spot completely covered by a neuron. The maximal depolarization of the capacitive transient is around 30 mV. There is a threshold for the junction resistance, which must be surpassed for the elicitation of an action potential. The response to rectangular voltage pulses is given in Fig. 5(b), showing that the negative capacitive transient at the end of the stimulus is not able to stop the autonomous dynamics of the Hodgkin-Huxley model if the depolarization of the membrane reaches a certain threshold. As the stimulations reproduce the crucial features of the experiments, we conclude that the stimulation by the silicon microelectrode can be attributed to capacitive coupling across the silicon-neuron junction. The simulations do not reproduce the long delay of the action potential, which is an intrinsic feature of the Retzius cell.

Outlook.—Extracellular stimulation of an individual neuron is possible from a microscopic silicon element with perfectly insulating silicon oxide. Thus it is now possible to interface individual neurons with silicon microstructures in both directions by capacitive coupling—from silicon to neuron by a stimulation spot and from neuron to silicon using a metal-free field-effect transistor [1,2]. The physical nature of capacitive coupling will permit an application to smaller sizes of neurons and silicon microstructures and thus to large scale integration. A next stage will be the assembly of a bidirectional device with a stimulation spot and a metal-free field-effect transistor under a single neuron. How far such defined systems, with individual neurons and silicon elements, will be helpful for selective stimulations and recordings in neuronal tissue with silicon chips remains to be seen.

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