# Two-way silicon-neuron interface by electrical induction

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A nerve cell is placed onto a combined silicon microstructure of an insulated spot of doped silicon and an insulated-gate field-effect transistor. Voltage pulses are applied to the insulated spot. They elicit neural activity which in turn modulates the transistor. The bidirectional interface between the ionics of neurons and the electronics of silicon is based on electrical induction mediated by an electrochemically safe interface. [S1063-651X(97)12401-1]

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## INTRODUCTION

Large scale integration of electronic and neural circuitry will provide an improved analysis of neural nets and may enable their control and support. It requires individual junctions to stimulate single neurons and to record their electrical activity. In this paper, to our knowledge, we present, the first bidirectional connection of a nerve cell and a silicon microstructure on the basis of electrical induction. The study is the final result of previous studies which showed that capacitive recording and capacitive stimulation of neural activity are possible with an insulated-gate field-effect transistor and with an insulated spot of silicon, respectively [1–3] (Fig. 1). The two-way junction is obtained by reducing the size of the stimulation spot and field-effect transistor by an order of magnitude, and by placing them close to each other beneath a single neuron (Fig. 1). Retzius neurons from the leech were used, as they are large and easy to handle.

## ASSEMBLY

Stimulation spots were made of $p$-doped silicon on a $n$-type substrate. They were insulated by a thin thermal oxide. Their area was varied between 100 and 400 $\mu m^2$. Field-effect transistors were made with $p$-doped source and drain. The area of the insulated gate was $1.8\times2.2$ $\mu m^2$. Short circuits between the units were suppressed by recessed silicon dioxide and by a reversed bias of the $p$-$n$ junctions. A section of the chip with three stimulation spots and one transistor is shown in Fig. 2(b). The main steps of processing were as follows: (i) Etching of grooves in silicon and growth of recessed oxide. (ii) Doping of the lanes for stimulation and of source and drain by boron implantation (resistance 600 Ω). (iii) Passivation by a thick oxide layer (580 nm). (iv) Opening of the contract area with stimulation spots and gates. (v) Growth of a thin oxide layer (12.6 nm) and etching down to a thickness of 6.5 nm using fluoric acid (specific capacitance 0.52 $\mu F/cm^2$. Details of the processing are described elsewhere [4]. A profile of the final surface is shown in Fig. 2(c). The whole chip had 16 stimulation spots and ten transistors. Doped lanes of contact for the stimulation spots and for the drains of the transistors and for their common source were arranged radially such that their ends could be wire bonded to a circuit board. A conical chamber of plexiglas (bottom diameter 3 mm) was attached to contain an electrolyte and to shield the bond contacts.

The surface of the chip was cleaned with hot (80 °C) basic hydrogen peroxide (30% hydrogen peroxide, 25% ammonia, and water at a volume ratio 1:1:5) to make it hydrophilic. The chamber was filled with serum-free culture medium ( L-15 Gibco, Eggenstein, FRG) with 5-mg/ml glucose and 50-$\mu$g/ml gentamycin sulfate (Sigma). Retzius cells

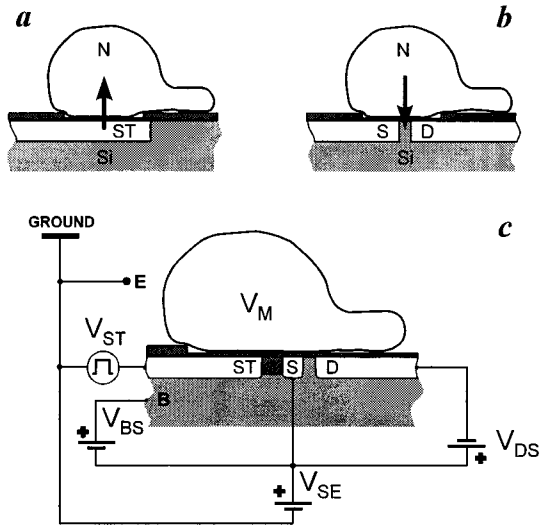


FIG. 1. Neuron-silicon interfaces. (a) Silicon-neuron junction. A neuron ( $N$) is attached to a stimulation spot (ST) of $p$-doped silicon covered with a thin layer of silicon dioxide. Voltage pulses applied to the silicon elicit an action potential in the neuron. (b) Neuron-silicon junction. A neuron is attached to the gate oxide of a field-effect transistor ($S$, source; $D$, drain). A change of the voltage in the neuron modulates the source-drain current. (c) Two-way interface. A single neuron covers a stimulation spot and a transistor, separated by recessed oxide. The electrolyte ($E$) is on ground potential, the source is held at a voltage $V_{SE}$, the drain is at a voltage $V_{DS}$ and the bulk silicon ($B$) at a voltage $V_{BS}$ to the source. The voltage $V_{ST}$ of the $p$-doped stimulation spots is such that the reversed $p$-$n$ junction prevents a spread of the stimuli in the chip.

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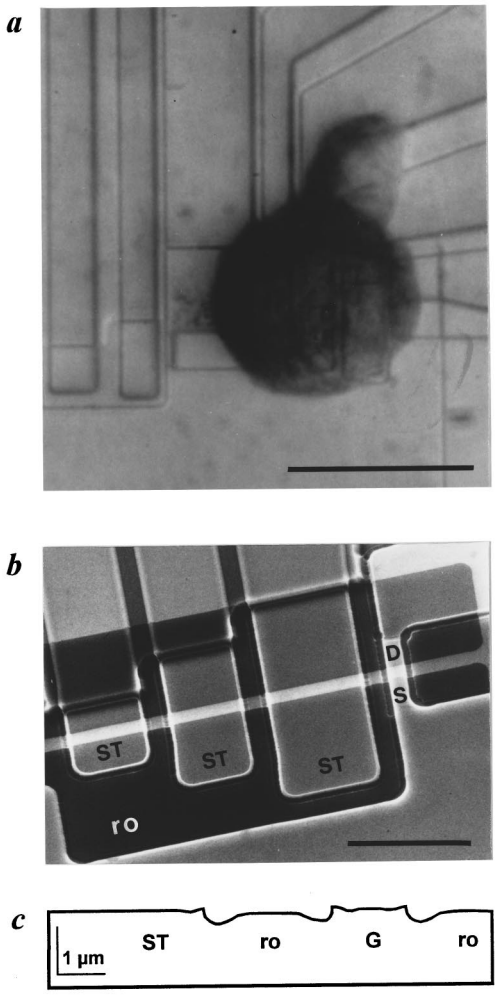


FIG. 2. Two-way interface. (a) Retzius cell attached to stimulation spots and a transistor which shine through the cell body. Scale bar 60 $\mu$m. (b) Scanning electron micrograph of the chip with three stimulation spots (ST) and one transistor (*S*, source; *D*, drain). Scale bar 15 $\mu$m. The dark area (ro) is recessed oxide which separates the active components. (The bright streak is due to microscopic contrast of secondary *p* doping.) (c) Surface profile across a stimulation spot, recessed oxide and gate (*G*).

were dissected from the leech *hirudo medicinalis* [5]. After a few hours they were transferred into the chamber using a glass pipette, and blown onto the contact sites such that one transistor and one or several stimulation spots were covered. No adhesive such as polylysine was applied. An example of an attached neuron is shown in Fig. 2(a). The chips were kept at 20 °C up to 65 h. As the cells dissociated partly from the surface during incubation, they are readjusted using an impaled microelectrode.

## MEASUREMENT

The electrolyte was kept at ground potential (Ag–AgCl–agar–1*M* KCl electrode). The neurons were impaled by a microelectrode filled with 4*M* K acetate (resistance 15 M$\Omega$). To suppress spontaneous firing, we held the intracellular voltage at $-50$ to $-70$ mV. Activity of the neurons was checked by current injection. The signal of the microelectrode was recorded by an amplifier (10 kHz bandwidth) and sampled by a computer controlled analog-to-digital converter (25 kHz, 12 bit). We kept the bulk silicon at a constant voltage $V_{BE}=+6$ V. Voltage changes of $0<\Delta V_{ST}\leqslant+5$ were applied to the stimulation spots. Stimulating wave forms were generated by a pulse generator with programmable random-access memory digital-to-analog converter (RAM-DAC) (4000 points, 12 bit, 200 kHz, slope 2.2 V/$\mu$s). The working point of the transistor was defined by a source voltage $V_{SE}=2.2$ V and a drain-source $V_{DS}=-2.2$ V using 12-bit digital-to-analog converters. The source-drain current $I_{SD}$ was detected with a current-voltage converter, amplified and sampled with a 12-bit analog-to-digital converter. In a calibration experiment a change $\Delta V_{SE}=10$ mV with $V_{SD}=0$ and $\Delta V_{BS}=-10$ mV caused by modulation of $\Delta I_{SD}=1.2$ $\mu$A. The bandwidth of the electronics was 27 kHz. Electrical couplings were reproduced in each neuron-silicon assembly for several minutes. The chips themselves were stable for weeks under the conditions of the measurement.

## INTERFACES

We observed three types of interfaces (Fig. 3): (I) A voltage step of $+5$ V was applied to the stimulation spot. It elicited a delayed action potential which induced a modulation of the source-drain current with the shape of its mirror image [Fig. 3(b)]. This interface is a combination of a ''strong coupling'' observed in previous stimulation experiments [3], and of a ''strong coupling'' observed in neuron transistors [1,2]. The formation of this ''strong interface'' was related with a reduced vitality of the neuron as indicated by a small amplitude of the action potential. (II) The most common interface is shown in Fig. 3(b). An action potential appeared after applying a burst of 100 short 5-V pulses of 20-$\mu$s width within 4 ms to the stimulation spot. A single voltage step was ineffective. The action potential also affected the source-drain current here. This modulation, however, was biphasic. Its midpoint matched the maximum of the action potential. This interface is a combination of a ''weak coupling'' observed in previous experiments of stimulation [3] and of a ''weak coupling'' observed in neuron transistors [1,2]. The ''weak interface'' was not connected with a loss of vitality of the neuron. (III) In a third type of interface we could also stimulate the neuron by a burst of pulses [Fig. 3(c)]. There we observed a positive modulation of the source-drain current. With respect to the action potential the signal was delayed by about 0.5 ms.

We made 56 successful attachments of a neuron on a stimulation-detection device. After incubation up to 65 h, we found that 25 of the neurons were still adjusted adequately to form a two-way interface *and* exhibited normal excitability by intracellular current injection. Among the 11 assemblies on a chip with a 12.6-nm-thick oxide, we found no capacitance stimulation but six recordings after intracellular stimulation. Among the 14 assemblies on a chip with 6.5-nm oxide, we observed seven stimulations and ten recordings with the formation of seven two-way interfaces. Five of the interfaces were weak, one was strong, and one was of type III. We assign the improved yield of the stimulation on the chip with the thin oxide to the twofold higher capacitance of the stimulation spot. The number of data is not sufficient to decide whether the formation of both junctions in a single assembly is statistically correlated.

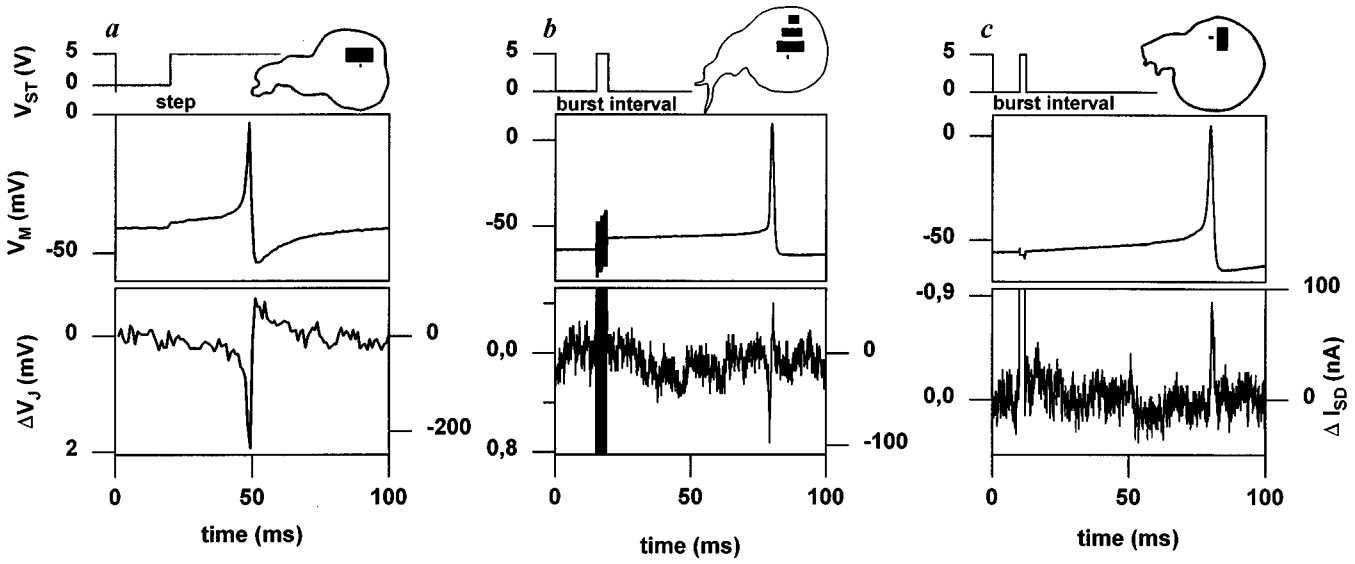


FIG. 3. Silicon-neuron loops. The upper lane of each figure shows the voltage applied to the stimulation spot. The intracellular voltage is shown in the central row. At the bottom the response of the transistor is depicted, the source-drain current (right scaling) and junction voltage (left scaling) as obtained from the slope of the transistor. The arrangements of neuron, stimulation spot and transistor are sketched in the upper right corners. (a) Strong loop. A voltage step of $\Delta V_{\mathrm{ST}} = +5$ V was applied. An action potential appeared with a delay of 25 ms. The response of the transistor (filtered by a 1-kHz bandpass) resembled an inverted action potential. (b) Weak loop. A burst of 100 voltage pulses (width 20 $\mu$s) was applied within 4 ms to three stimulation spots. An action potential appeared after 60 ms. The weak response of the transistor resembles the first derivative of an action potential. Stimulation artifacts are seen in the source drain current as well as in the intracellular voltage. (c) Type-III loop. A burst of 50 pulses (width 20 $\mu$s) was applied. An action potential appeared after 70 ms. The weak response of the transistor was positive monophasic and delayed by 0.5 ms.

## CIRCUIT

We discuss stimulation and recording on the basis of the same representative circuit (Fig. 4). It consists of the capacitance $C_{\mathrm{JOX}}$ of the oxide in the junction, the capacitance $C_{JM}$, and resistance $R_{JM}$ of the attached membrane and the seal resistance $R_J$ to the bath as formed by the electrolyte between membrane and oxide. The silicon is represented by the capacitance $C_{SI}$ of its boundary layer. The free part of the neuron is described by a capacitance $C_{FM}$ and a resistance $R_{FM}$.

## STIMULATION

The simplest type of stimulation is a positive voltage step of height $\Delta V^0_{\mathrm{ST}}$ applied to the doped silicon. This voltage directly polarizes the silicon dioxide because the interfacial capacitance $C_{\mathrm{SI}}$ of silicon is large due to high doping. It induces a negative charge at the intracellular side of the attached membrane and a positive charge on the free membrane (Fig. 4). The intracellular response $\Delta V_M$ is exponential [3]. Its amplitude $\Delta V^0_M$ and time constant $\tau_J$ are

$$\Delta V^0_M \approx \frac{C_{\mathrm{JOX}}}{C_{FM}}\,\Delta V^0_{\mathrm{SI}}, \tag{1a}$$

$$\tau_J \approx R_J C_{JM}\,. \tag{1b}$$

The approximations are valid if the specific capacitance of the oxide is distinctly smaller (about 0.5 $\mu$F/cm$^2$) than that of the membrane (about 5 $\mu$F/cm$^2$), if the area of the junction is small with respect to the area of the whole cell membrane and if the resistance of the junction is low with respect to the resistance of the attached membrane [2,3]. The exponential transient $\Delta V_M$ may trigger an action potential if the amplitude is high and lasts long enough. This kind of stimulation was observed frequently with large stimulation spots [3]. In the present study it was effective only in the strong interface [Fig. 3(a)]. Apparently in most junctions the seal resistance $R_J$ was too low, such that the transients were too short [Eq. (1b)] considering the small amplitudes expected with the low capacitance $C_{\mathrm{JOX}}$ of small stimulation spots [Eq. (1a)].

The fact that repetitive pulsing was effective in the weak junctions can be explained by the presence of voltage-gated ion channels. The amplitude of the voltage transient across the attached membrane is $\Delta V^0_{MJ} \approx (C_{\mathrm{JOX}}/C_{JM})\Delta V^0_{\mathrm{ST}} \approx -500$ mV. A sequence of such strong hyperpolarizing pulses may close potassium channels ($n$ gates) and open inhibiting gates of sodium channels ($h$ gates) as described by the Hodgkin-Huxley model [6]. As a result an action potential is elicited locally.

## RECORDING

The voltage transient $\Delta V_M$ of an action potential creates positive charge on the internal side of the membrane. The charge is transferred to the gate of the transistor by induction through the capacitance, and by current through the conductance of the membrane (Fig. 4). The voltage $\Delta V_J$ in the junction polarizes the boundary layer of silicon by induction through the oxide and affects the source-drain current. The elation between $\Delta V_J$ and $\Delta V_M$ is linear if voltage-gated channels play no role. In the case of a high membrane resistance the capacitive current dominates the voltage in the junction. It is given by a first derivative [Eq. (2a)] for mod-

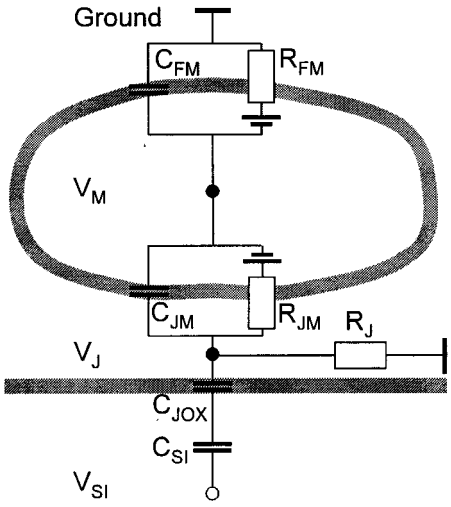


FIG. 4. Electrical circuit. $C_{\mathrm{JOX}}$ is the capacitance of silicon dioxide in the junction. $C_{JM}$ and $R_{JM}$ are the capacitance and resistance of the attached membrane. $R_J$ is the seal resistance. The free membrane is described by a capacitance $C_{FM}$ and a resistance $R_{FM}$, the boundary layer of silicon by a capacitance $C_{\mathrm{SI}}$. The resting voltage of the membrane is indicated by a battery. The voltages in the neuron, in the junction and in the silicon are $V_M$, $V_J$, and $V_{\mathrm{SI}}$. For stimulation $V_{\mathrm{SI}}$ refers to the $p$-doped stimulation spot, for recording to the $n$-doped bulk silicon. The electrical features of voltage-gated ion channels and of the transistor are not represented.

est values of the seal resistance [2]. If the membrane resistance is low we obtain the proportionality of an Ohmic system [Eq. (2b)] [2],

$$\Delta V_J \approx R_J C_{JM} \frac{d}{dt} \Delta V_M , \tag{2a}$$

$$\Delta V_J \approx \frac{R_J}{R_J + R_{JM}} V_M . \tag{2b}$$

The biphasic response in the weak interface II is compatible with Eq. (2a). Similar signals were observed with large transistors if an intact membrane was attached with a low seal resistance ( $A$-type junction) [1,2]. The amplitude of the biphasic response, however, is smaller in our two-way junctions. This may be due to the smaller contact area (smaller $C_{JM}$) or to a more leaky junction (smaller $R_J$). The monophasic recording in the strong interface I is compatible with Eq. (2b). Such signals were observed with large transistors when the membrane becomes leaky by adhesion ($B$-type junction) [1,2]. Again, the signals are much smaller in the two-way junction, which may also be due to a lower seal resistance $R_J$. In interface III we observed a negative voltage change $\Delta V_J$ during the late phase of the action potential. This response cannot be explained by the linear circuit. We have to invoke an effect of voltage-gated ion channels in the junction. A thorough simulation on the basis of the Hodgkin-Huxley equations is required, which is not the issue of the present paper.

## CONCLUSIONS

Our investigations show that electrical induction can mediate a two-way communication between a silicon chip and an individual neuron. We observed some interesting features of the stimulating and of the detecting junctions which are not yet completely understood. The differences from previous studies are due to the smaller size of the junctions or to the lack of an adhesive. The interfaces described in this study were made by assembly. A goal of future developments is the self-assembly of intimate neuron-silicon contacts, and a large-scale integration with many interfaced sites to study networks of neurons from invertebrates and mammals.

The two-way junction relies on the peculiar properties of silicon, the oxide interface with pure capacitive properties in a wide range of voltage, and the control of the device impedance by the technology of microintegration. The silicon system has advantages as compared to metallic electrodes [7]: (i) Due to the insulating properties of the interface there is no danger that the electrochemical reactions lead to a corrosion of the device and to a damage of the cells by toxic products. (ii) The spread of local extracellular voltage is not supported by the electrode itself. (iii) The pathways of stimulation and detections are separated. There is no long lasting perturbation of recording by a previous stimulation at the same site.

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