A Neuron-Silicon Junction: A Retzius Cell of the Leech on an Insulated-Gate Field-Effect Transistor

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An identified neuron of the leech, a Retzius cell, has been attached to the open gate of a p-channel field-effect transistor. Action potentials, spontaneous or stimulated, modulate directly the source-drain current in silicon. The electronic signals match the shape of the action potential. The average voltage on the gate was up to 25 percent of the intracellular voltage change. Occasionally weak signals that resemble the first derivative of the action potential were observed. The junctions can be described by a model that includes capacitive coupling of the plasma membrane and the gate oxide and that accounts for variable resistance of the seal.

MULTISITE RECORDING OF THE electrical activity of neurons is a prerequisite to the study of signal processing in arborized neurons and in neural nets. Such a method must be noninvasive and should detect changes in intracellular voltage at a high spatial and temporal resolution. Optical recording by voltage-sensitive dyes (1, 2) and electrical recording by extracellular metallic electrodes on glass (3–6) or Si (7, 8) have been used. We report on a direct coupling of neural excitation and electrical current in Si in a transistor-type device. The assembly is a first step toward multiple recording in neurons and neural nets and toward the development of neural biosensors and neuroelectronic circuits.

A neuron is mounted on a thin insulating layer of a gate oxide on n-type Si in an electrolyte (Fig. 1). A positive-bias voltage is applied to the bulk Si to provide an accumulation of mobile, positive defect electrons near the surface (strong inversion). The positive voltage change in the neuron during an action potential lifts the surface potential of Si and reduces the density of mobile defect electrons. The neuron-Si interaction causes a modulation of the current along the inversion layer (p-channel) driven by a voltage between source and drain. The junction thus behaves like a field-effect transistor (FET).

The neuron-FET junction has certain features: (i) direct coupling of neuron and Si owing to an interaction of cell membrane and gate oxide without interfering metal; and (ii) well-defined structure and function made possible by the controlled attachment of an identified neuron to a microscopic transistor of matched design. These features can be compared to the characteristics of earlier approaches: A large (1 mm by 50 μm) FET with an open gate was used by Bergveld et al. to record extracellular voltage in muscle tissue (9). An FET with a metalized gate was used by Jobling et al. (10). An assembly of neuron and metalized Si was described by Regehr et al. (11).

Retzius cells (diameter about 60 μm) were isolated from segmental ganglia of the leech Hirudo medicinalis. The soma of the neuron together with a short neurite was dissociated after enzymatic treatment of opened ganglia (12, 13). In freshly dissociated cells the amplitude of the action potentials was 40 to 60 mV. Planar technology was used to fabricate p-channel insulated-gate FETs (14, 15). Source and drain were made on the (100) surface of n-Si by boron diffusion. The length of the channel between source and drain was 6 μm. Its width was 30 μm (Fig. 2). The channel region was insulated by a gate oxide 20 nm thick. A Perspex chamber was fixed on the Si plate (16). After the gate had been coated with poly-L-lysine, we filled the chamber with electrolyte (leech Ringer, pH 7.4). A neuron was attached on the tip of a glass pipette and mounted on the gate under visual control (Fig. 2) (17).

We performed four types of measurement (18) with the junctions (Fig. 3). (i) A single action potential was stimulated by current

Fig. 1. Neuron-Si junction (unscaled). A neuron (N) is attached to oxidized Si. A thin layer of gate oxide (Ox) marked by the heavy line) covers the source (S) and drain (D) of a p-type Si insulated by a thick field oxide (Og). The electrolyte (E) is maintained at ground potential (Ag/AgCl electrode). Bulk silicon (B), source, and drain are held at positive bias voltages (p-channel FET). The source-drain current is measured by a current-voltage converter. The neuron is impaled by a microelectrode (ME) (Ag/AgCl). Current (Isi) is injected to stimulate the cell. The membrane potential is measured by a voltage follower.
injection through an impaled microelectrode. The change of intracellular voltage $V_M$ (membrane potential) was up to 40 mV. The source-drain current $I_D$ was lowered and was well correlated with the increase of $V_M$ (Fig. 3A). The maximal change of current corresponded to the effect of a calibration voltage on the gate of $V_G = 9$ mV (compare the current-voltage relation of the FET in Fig. 3E). (ii) Using current pulses, we stimulated repetitive firing with a perfect match of $I_D$ and $V_M$ (Fig. 3B). (iii) We observed modulations of $I_D$ without impaled microelectrode (Fig. 3C). The shape and amplitude of the response resembled the signals obtained by stimulation. (iv) Frequently we found modulations that resembled the first derivative of an action potential (Fig. 3D).

We prepared 54 neuron-Si contacts, and in most cases we studied each one for about 20 min. In eight samples we found a strong coupling of action potentials with an effective gate voltage of about 10 mV. In two of these contacts $V_M$ was checked with a microelectrode. In six samples we observed a weak coupling of spikes with the shape of derivatives (amplitudes around 2 mV). In two of these contacts the shape changed occasionally such that it resembled the spike itself. In 14 samples we observed a very weak coupling of action potentials (<1 mV). In 15 samples we found a strong coupling of depolarizations as induced by current injection without triggering a spike. In 11 samples we could not detect any coupling. In two of these contacts action potentials were recorded by a microelectrode.

Apparently there are two problems: variable strength of coupling and variable activity of the neurons themselves. The variable coupling may be caused by a variable contact area of membrane and gate and by a variable cleft width between membrane and gate. The neurons may be damaged by the isolation, by manipulations in the procedure of attachment, by a toxic effect of poly-1-lysine, and by the impalement of the microelectrode. The FETs themselves were sufficiently stable. Within the first few hours in Ringer solution, their threshold (Fig. 3E) drifted by about 0.5 V. No change was observed, however, within 60 hours of further use. We assign the satisfactory stability to the positive polarization of n-Si, which repels sodium ions from the Si-SiO$_2$ interface. It may be optimized by depositing silicon nitride on the gate. The noise of $I_D$ (Fig. 3) may be reduced by improved bond-

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**Fig. 2.** Structure of the neuron-Si junction. (A) Top view of the FET with a Retzius cell. The horizontal band (width, 50 μm) reflects source and drain. (B) Top view of the FET. The visible distance between source and drain is 16 μm. The contours mark the range of primary deposition of boron (groove of depth 150 nm), not the location of the $p_n$ junctions. In the center the insulating oxide (1 μm) is opened over an area 22 μm by 30 μm. This region is covered by the gate oxide (thickness 20 nm). (C) The $p_n$ junctions. The lower half of the FET is ground at an angle of 2° up to the edge marked by the two arrows. The geometry is illustrated at the right (not scaled). The stripes are interference fringes due to the field oxide. The two $p_n$ junctions of source and drain—as stained by copper—are marked by arrows. Their horizontal distance at the gate is 6 μm.

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**Fig. 3.** Electrical junction of the Retzius cell and the FET: (A) Single action potential. The source-drain current $I_D$ of the FET and the membrane potential $V_M$, as measured by an impaled microelectrode (ME), are shown. The effective gate voltage $V_G$ is determined by calibration before attachment of the neuron (see the current-voltage relation in (E)). The noise of the current is due to imperfections of FET fabrication. (B) $I_D$ and $V_M$ of a sequence of action potentials triggered by current pulses as marked at the bottom. The discontinuities in the trace of the $V_M$ are due to the voltage drop of injection current in the microelectrode (unbalanced bridge). (C) $I_D$ without impaled electrode. The signals resemble action potentials. (D) $I_D$ without impaled electrode. The signals resemble first derivatives of action potentials. (E) Current-voltage characteristic of the FET in Ringer at pH 7.4. $I_D$ versus $V_G$ for various source-drain voltages $V_{DS}$. 
ing and suppression of surface states.

To rationalize the junction, let us consider a minimal circuit of capacitive coupling as depicted in Fig. 4A. It consists of the capacitances $C_{JM}$ and $C_{IG}$ of membrane and gate and of the resistance $R_j$ of the cleft. It is driven by the voltage $V_M$ across the membrane capacitance $C_M$. The time course of the voltage $V_j(t)$ across $C_{IG}$ is determined by Eq. 1. In the case of high seal resistance $R_j$, we expect proportionality of output and input as $V_j = V_M C_{IM}/(C_{IM} + C_{IG})$. In the case of low resistance the output resembles the damped derivative of the input as $V_j = R_j C_{IM} dV_M/dt$.

$$\frac{dV_j}{dt} = \frac{C_{IM}}{C_{IM} + C_{IG}} \frac{dV_M}{dt} - \frac{1}{R_j(C_{IM} + C_{IG})} V_j$$

(1)

We assign the large signals of perfect shape (Fig. 3, A through C) to tight capacitive coupling and the low signals of biphasic shape (Fig. 3D) to a low seal resistance. In a tight junction we expect $V_j/V_M = C_{IM}/(C_{IM} + C_{IG}) = 0.86$ (specific capacitances 1 μF/sq centimeter of membrane and 0.16 μF/sq centimeter of gate oxide of thickness 20 nm and dielectric constant 3.9). We observe a ratio $V_G/V_M = 0.25$ with respect to the effective gate voltage $V_G$ (Fig. 3A). We assign the discrepancy of $V_G$ and $V_j$ to an incomplete gate-membrane contact. We may assume that the junction voltage $V_j$ is effective only along a width $w_j$ of the gate, whereas the calibration voltage $V_G$ modulates $I_D$ along the whole gate of width $w_G = 30$ μm. The effects of $V_j$ and $V_G$ are then identical per definition as expressed by $w_f V_j/w_f V_G$. Within this model the ratio $V_j/V_G = 0.3$ reflects the ratio $w_f(w_j/w_G)$, that is, the junction covers 10 μm of the gate. We evaluate $V_j(t)$ for such a junction of area 6 μm by 10 μm according to Eq. 1 with $C_{IM}/(C_{IM} + C_{IG}) = 0.86$, $C_{IM} + C_{IG} = 0.7$ pF, and $R_j = 0.25, 1, 4$, and 16 gigaohms. A match of the pulse shapes of input and output is attained for high resistances $R_j > 10$ gigaohms (Fig. 4B). For low resistance the output resembles the derivative of the input at a reduced amplitude.

Apparently a tight seal of membrane and gate is the prerequisite for efficient coupling. As a first step toward a structural analysis of the junction, we looked at samples fixed with glutaraldehyde using a scanning electron microscope. The neuron spreads over the Si (Fig. 5). An intimate local contact between membrane and gate is likely.

The neuron-Si junction outdoes metallic microwires where the amplitude of the extracellular voltage is in the range of 0.1 mV (3–6, 8) up to 1 mV (10, 11). We assign the good performance to the nature of capacitive coupling at high seal resistance as attained by tight adhesion of neuron and gate oxide without metallic conductor.

Owing to the simple geometry, no major problems should arise in the construction of patterns of FETs with gates of 10 μm on a single chip. Thus, multiple recording in cell cultures appears to be a feasible technique that may be applied to neurons in a network or to sites in a neuronal arborization. FET probes may be superior to impaled electrodes and voltage-sensitive dyes, which interfere with cell activity. Long-term recordings at high resolution and high signal-to-noise ratios with these neuron-Si junctions may be possible.

REFERENCES AND NOTES

13. Segmented ganglia of Hirudo medicinalis (Biopharm, Swansea) were dissected and pinned on a dish coated with Silgard ( Dow-Corning) in 1:15 medium (Gibco, Eggenstein) supplemented with 2% fetal cell serum, glucose (6 mg/ml), and penicillin (50 μg/ml).
15. Samples were cut from n-type Czochralski-Si wafers ([100] surface, 5 to 10 ohm-cm) (Wacker-Chemie, Burghaun) at a size of 30 mm by 10 mm. On every plate six FETs were fabricated at a spacing of 2 mm.
16. The samples were successively treated with RCA (Radio Corporation of America), and silicon oxide was grown in wet O₂ at 1000°C. A mask of positive photoresist (AZ-1514, Hoechst, Frankfurt) was prepared. The source and drain regions (distance, 16 μm) were opened by ammonium fluoride solution (AF 87.5-12.5, Merck, Darmstadt).
17. The sample was evaporated from a mask written from boron nitride (Sohio Engineered Materials Company, New York) at 900°C. The base was driven in at 1120°C for 7 hours. Boron glass and silicon oxide were removed and a layer of oxide (1 μm) was grown at 1100°C. The gate was reopened (area 23 μm²) and a thin oxide layer of 20 nm was grown in dry O₂ at 1000°C. The thickness of the oxide layers was evaluated from angle-dependent light reflection. After reopening the contact sites on source, drain, and bulk, Si, we applied Al contacts by using evaporation and the lift-off technique. The chip was annealed at 350°C for 5 min. We checked the profile of the FET by gridding at an angle of 2° and scanning the pn-junction. The depth of the pn-junction was 5 μm. The distance between the pn-junctions in the gate was 6 μm.
18. The chamber was a wedge-shaped slot with width at the top 8 mm, width at the bottom 2 mm, length at the bottom 12 mm, in a piece of Perspex (thickness, 10 mm). We attached the Perspex to the Si plate using Silgard such that the contact sites of source and drain stuck out beyond the Perspex.
19. The Si surface was cleaned with hot basic hydrogen peroxide as used in RCA cleaning (30% hydrogen peroxide, 20% hydrogen peroxide).
peroxide: 25% ammonia; water = 1:1.5, 80°C). A drop of poly-l-lysine (molecular weight 15,000 to 30,600, Sigma, Heidelberg) at a concentration of 1 mg/ml in water was applied to the gate and dried. The chamber was filled with lecith Ringer (115 mM NaCl, 4 mM KCl, 1.8 mM CaCl₂, 10 mM tris-maleate, pH 7.4). A neuron was sucked several times into a wide glass pipette (tip diameter, 80 to 100 μm) to remove connective tissue and was transferred into the chamber. We attached the cell to a narrow pipette (tip diameter, 10 to 25 μm) by nacodon and pressed it gently onto the gate with a micromanipulator and a stereomicroscope. The micropins were removed. The attachment was completed within a few minutes after addition of Ringer to the chamber as the coat of poly-l-lysine faded away. Measurements with the EET were started within a minute after attachment. After tests to remove spontaneous activity, we impaled a microelectrode (tip diameter <1 μm). After completion of the measurements, the chip was cleaned with water and hot basic hydrogen peroxide and rinsed.

18. Bulk Si and source were kept at 27.2 V and the drain was kept at 0.7 V such that the source-drain voltage VDS was −2 V as marked in Fig. 3E. The current was fed into a current-voltage converter. After amplification and filtering, the signal was digitized and read into a computer. All measurements were performed in the dark to avoid photocurrents. The neuron was impaled by a glass microelectrode filled with 3 M KCl and contacted with a Ag/AgCl electrode. It was connected to a voltage follower of standard design such that the membrane potential was measured at low impedance and current could be injected through the electrode. The signal was digitized and read into the computer.

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Spectroscopic Observations of Bright and Dark Emission Features on the Night Side of Venus


Near-infrared spectra of a bright and a dark thermal emission feature on the night side of Venus have been obtained from 2.2 to 2.5 micrometers (μm) at a spectral resolution of 1200 to 1500. Both bright and dark features show numerous weak absorption bands produced by CO₂, CO, water vapor, and other gases. The bright feature (hot spot) emits more radiation than the dark feature (cold spot) throughout this spectral region, but the largest contrasts occur between 2.21 and 2.32 μm, where H₂SO₄ clouds and a weak CO₂ band provide the only known sources of extinction. The contrast decreases by 55 to 65 percent at wavelengths longer than 2.34 μm, where CO, clouds, and water vapor also absorb and scatter upwelling radiation. This contrast reduction may provide direct spectroscopic evidence for horizontal variations in the water vapor concentrations in the Venus atmosphere at levels below the cloud tops.

NEAR-INFRARED (NIR) IMAGES and spectra of Venus acquired by Allen and Crawford (1) revealed that the night side of the planet was unexpectedly bright at wavelengths near 1.7 and 2.3 μm, in atmospheric windows between strong CO₂ and water vapor absorption bands. These authors concluded that this night-side radiation was produced thermally in the hot lower atmosphere of Venus. The bright-contrast and dark-contrast features were thought to be produced as this radiation passes through a higher, cooler, optically thin cloud region. Subsequent observations and theoretical analysis confirmed these conclusions. Allen (2) ratioed low-resolution spectra of bright and dark regions of the Venus night side and found that this ratio was almost independent of wavelength. This result implies that the agent that produces the dark features is a continuum absorber, such as the H₂SO₄ cloud droplets, rather than a gas. Kamp et al. (3) showed that the night side radiation could be completely accounted for by thermal emission from the pressure-broadened far wings of CO₂ and H₂O lines in the deep Venus atmosphere. Their results also showed that intensity contrasts as large as those observed on the night side could be produced by variations in the cloud optical depth of 20%. Crisp et al. (4) combined infrared images of Venus with Allen's spectra and spacecraft observations (5) and showed that the features were produced by horizontal variations in the optical depth of the middle or lower cloud deck at altitudes between 48 and 55 km. The feature rotation period at those levels was found to be about 6 ± 1 days, indicating equatorial east-west wind velocities of about 70 m/s. Bezzard et al. (6) analyzed new high-resolution spectra of a bright region on the Venus night side and showed that the thermal emission in the 2.3-μm region originates primarily at levels above 8 bars (30 km), whereas the 1.74-μm radiation is produced at deeper levels. Their spectra placed new constraints on the concentrations of several important trace gases in the lower Venus atmosphere, including H₂O, CO, HCL, and HF. They also provided an independent confirmation of the enhanced D/H ratios observed by Pioneer Venus (7) and provided the first true detection of OCS in the Venus atmosphere.

New imaging and spectroscopic observations of the Venus night side were taken from a global network of observatories during January and February of 1990 to support the Galileo spacecraft Venus flyby. On 10 February 1990 UT the Galileo Near-Infrared Mapping Spectrometer (NIMS) was the first spacecraft instrument to take NIR images and spectra of the Venus night side. These data have better spatial resolution than the ground-based data, but severe limits on the data telemetry rate and on spacecraft tape recorder storage restricted the spatial, temporal, and spectral coverage of the NIR features.

To complement the Galileo NIMS experiment we conducted daytime spectroscopic observations of Venus from the National Aeronautics and Space Administration (NASA) Infrared Telescope Facility (IRTF) at Mauna Kea Observatory during 26 to 30 January 1990. This observing period was ideal for spatially resolved studies of the night side of Venus because passage of the planet through inferior conjunction less than 2 weeks before provided a relatively large disk (60 arc seconds) and a thin sunlit crescent (α = 155°). We used the Cooled-Grating Array Spectrometer (CGAS) to provide a spectral resolution between 1200 and 1500 in the 1.7- and 2.3-μm windows (8). The CGAS has a 2.7-arc second circular entrance aperture and yielded an effective spatial resolution of 550 km on the Venus night side. Because Venus was perilously close to the sun in the morning sky, we used a special telescope mask to prevent sunlight from striking the primary mirror (9).

We coordinated our spectroscopic observation program with a concurrent NIR imaging program lead by Sinton and co-workers at the University of Hawaii Air Force 61-cm telescope on Mauna Kea (10). Their images allowed us to acquire spectra of the darkest and brightest features on the Venus night side. A 2.36-μm NIR camera image acquired on the morning of 29 January 1990 UT showed a large (~5-arc second) hot spot on the Venus night side centered at