Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biosensors and Bioelectronics 19 (2004) 1703–1709
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Depletion type floating gate p-channel MOS transistor for recording action potentials generated by cultured neurons

Ariel Cohen [a], Micha E. Spira [a,1], Shlomo Yitshaik [a], Gustaaf Borghs [b], Ofer Shwartzglass [a], Joseph Shappir [a,*]

[a] *Faculty of Science, School of Applied Science, The Hebrew University of Jerusalem, Givat Ram Campus, Jerusalem, Israel*
[b] *IMEC, Kapeldreef 75, B-3001 Leuven, Belgium*

Received 10 August 2003; received in revised form 8 January 2004; accepted 14 January 2004

Abstract

We report the realization of electrical coupling between neurons and depletion type floating gate (FG) p-channel MOS transistors. The devices were realized in a shortened 0.5 μm CMOS technology. Increased boron implant dose was used to form the depletion type devices. Post-CMOS processing steps were added to expose the devices sensing area. The neurons are coupled to the polycrystalline silicon (PS) FG through 420 Å thermal oxide in an area which is located over the thick field oxide away from the transistor. The combination of coupling area pad having a diameter of 10 or 15 μm and sensing transistor with *W*/*L* of 50/0.5 μm results in capacitive coupling ratio of the neuron signal of about 0.5 together with relatively large transistor transconductance. The combination of the FG structure with a depletion type device, leads to the following advantages. (a) No need for dc bias between the solution in which the neurons are cultured and the transistor with expected consequences to the neuron as well as the silicon die durability. (b) The sensing area of the neuron activity is separated from the active area of the transistor. Thus, it is possible to design the sensing area and the channel area separately. (c) The channel area, which is the most sensitive part of the transistor, can be insulated and shielded from the ionic solution in which the neurons are cultured. (d) There is an option to add a switching transistor to the FG and use the FG also for the neuron stimulation.

Keywords: Field effect transistor; Floating gate; Depletion type device; Neuron transistor coupling; *Aplysia*

1. Introduction

Since the demonstration that MOS transistor can be used to detect extracellular field potential generated by neurons by Bergveld et al. (1976) and Fromherz et al. (1991), technological progress improved significantly the quality of action potentials recordings. In parallel, significant progress was made in the understanding of the electrochemical–physical processes underlying the neuron–transistor interface (Fromherz, 2002; Fromherz et al., 1991; Fromherz and Stett, 1995; Offenhausser et al., 1995; Schatzthauer and Fromherz, 1998; Zeck and Fromherz, 2001).

The commonly used configuration for the formation of neuron–transistor hybrid (Fromherz et al., 1991; Zeck and Fromherz, 2001) is based on culturing neurons directly on top of the exposed gate dielectric, so that the neurons themselves and the solution in which they are cultured serve as the gate of the transistor. The placement of the neuron on the most delicate element of the MOS transistor involves high risk for its endurance. An alternative approach is the use of a floating gate (FG) electrode (Offenhausser et al., 1995). This approach offers two advantages: (a) isolation and protection of the thin gate oxide from the ionic solution which serves to culture the neurons; and (b) uniform modulation of the transistor channel.

In all previous studies, enhancement type transistors were used. Consequently, a bias voltage of more than 1 V had to be applied between the solution and the silicon in order to set the transistor in conduction mode. As is well known from previous studies on ion-sensitive field effect transistor (ISFET), this bias contributes to severe drift currents

* Corresponding author. Tel.: +972-2-6586188; fax: +972-2-5663878.
E-mail addresses: spira@cc.huji.ac.il (M.E. Spira), shappir@cc.huji.ac.il (J. Shappir).
[1] Present address: Department of Neurobiology, Life Sciences Institute, The Hebrew University of Jerusalem, Givat Ram Campus, Jerusalem 91904, Israel. Tel.: +972-2-6585091; fax: +972-2-5637033.

0956-5663/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2004.01.021

due to the ion migration in the device dielectric layers. In the case of the FG device, the ionic leakage under dc bias voltage conditions, may lead to charging of the FG, which will result in severe drift of the device current. In addition, the drift currents are potential source for noise, enhanced device degradation by electrochemical corrosion effects and damages to the cultured neurons. Finally, the direct coupling of the neuron to the FET by placing it over the channel (Fromherz et al., 1991) imposes a limitation on the transistor geometry, i.e. W/L is close to one with a direct impact on the device transconductance.

These shortcomings lead us to investigate the concept of using a depletion type floating gate p-channel MOS for recording from cultured neurons. Under this configuration, the neuron's coupling area is shifted aside from the transistor. This in turn enables the reduction of pick up noises and the elimination of parasitic photo effects by adding a metal shielding layer over the transistors. As a dc bias voltage between the solution and the silicon is not required, the durability of the neuron–transistor coupling is expected to increase. Finally, this configuration enables in principle the use of the FG to stimulate the neurons via a switching transistor.

2. Materials and methods

2.1. Chip design

Depletion type p-channel FG transistors with octagonal sensing area having a diameter D of 10 or 15 μm and W/L of 50/0.5 or 32/0.8 μm were designed and realized in 0.5 μm CMOS technology. The approach in this work was to separate between the sensing site and the transistor channel as illustrated in Fig. 1. This design allows for the optimization of the sensing area with respect to the neuron dimensions while realizing transistors with large transconductance (g_m). This approach has clear advantage over the previous implementation of FG (Offenhausser et al., 1995) were the FG was just over the channel with identical geometries of the two.

The poly-silicon floating electrode is placed between the gate oxide of 115 Å and top oxide of 420 Å. The use of p-channel rather than n-channel promises reduced $1/f$ noise.

Another device configuration includes a switching device connecting the FG to an external pad, is shown in Fig. 2. The fact that no dc bias is required between the ionic solution and the silicon and the fact that also the FG is at ground potential allow for the addition of the switching transistor. Under normal sense operation the switching transistor is switched off. The residual junction leakage current of 5 pA/cm^2 is too low to disturb the sensing of the neuron activity.

2.2. Device processing

Standard 0.5 μm CMOS technology with double PS layers was used. In this process, boron implant is used to adjust the p-channel threshold voltage. This boron implant dose was

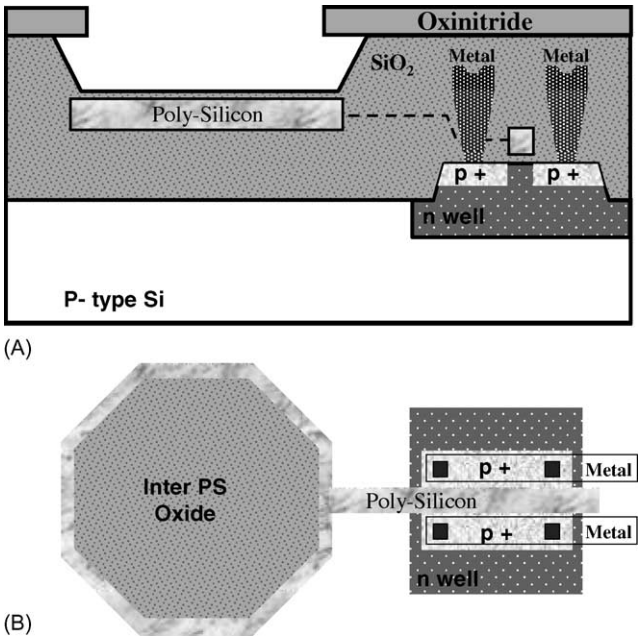

Fig. 1. (A) Cross-section of the FG transistor. The substrate is p-type Si. The p-channel transistor is located inside the n-well. The p+ indicates the source and drain regions. The gate oxide is 115 Å thick. The poly-silicon electrode is covered by thermal oxide of 420 Å. (B) Top view of the FG transistor.

increased in order to realize the depletion p-channel devices. As no exact process simulation was available, four different boron doses were attempted in order to obtain the maximum g_m and lowest drain current at gate voltage of 0 V.

The use of double PS CMOS process serves for the purpose of exposing the sensing area of the device were the neuron is coupled to the FG. During the last masking step, which exposes the bonding pads of the silicon die, the oxynitride passivation layer is etched also at the sensing area. At the end of this step, a residual oxide layer was left on top of the second PS layer.

Once the standard CMOS process was completed, additional processing steps were performed at the university laboratory. They included:

1. Masking step to protect the exposed aluminum bonding pads.
2. Buffer HF etching to remove the residual oxide on top of the upper PS layer.
3. KOH wet etching of the upper PS layer to expose the 420 Å high quality PS thermal oxide to be used for

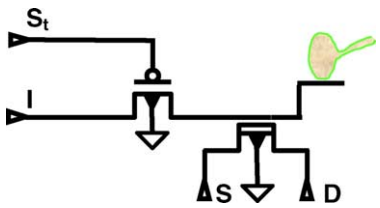

Fig. 2. Combined sensing and stimulating transistor: (S) source; (D) drain; (I) electrode for neuron stimulating pulses; (S_t) the switching control.

isolation between the bottom PS FG and the culturing medium with the neuron (Fig. 1).

2.3. Device assembly and encapsulation

Once the process was completed, the wafer was cut by a diamond saw to individual dies. The dies were packaged in dual in-line (DIL) package with 28 pins. Following formation of the gold wire bonding, epoxy was applied to protect all the exposed metal parts except for the central part of the silicon die where the active devices are located in a similar way to that of Offenhausser et al. (1997).

It was found that the plasma etching of the oxynitride above the sensing area caused uncontrolled charging of the FG. An exposure to UV radiation for several hours was used in order to remove this charge.

2.4. Cell cultures

Left upper quadrant neurons from the abdominal ganglia of *Aplysia californica* were isolated and maintained in culture as previously described (Schacher and Proshansky, 1983; Spira et al., 1996, 1999). Briefly, juvenile *A. californica* (1–10 g) were anesthetized by injection of isotonic $MgCl_2$ solution (380 mM) into the animal's body cavity. Abdominal ganglia were dissected and incubated in ms-L15 containing 1% protease (type IX, Sigma, Rehovot, Israel) at 34 °C for 1.5–2.5 h. Following the protease treatment, the ganglia were washed with L15 supplemented for marine species, pinned and desheathed. Left upper quadrant neurons were manually pulled out along with their original axon with the aid of a sharp glass microelectrode. The neurons were plated on the thermal oxide layer above the FG which was coated with poly-L-lysine (Sigma). The experiments were performed 2–5 days from plating, at room temperature (21–25 °C) after replacing the culture medium (supplemented for marine species) with artificial sea water (ASW).

2.5. Solutions

L15 supplemented for marine species (ms-L15): Leibovitz's L15 medium (Gibco-BRL, Paisley, Scotland) was supplemented for marine species according to Schacher and Proshansky (1983) by the addition of 12.5 g/l NaCl, 6.86 g/l D-(+)-glucose·H_2O, 3.15 g/l anhydrous $MgSO_4$, 344 mg/l KCl, 192 mg/l $NaHCO_3$, 5.7 g/l $MgCl_2 \cdot 6H_2O$ and 1.49 g/l $CaCl_2 \cdot 2H_2O$. Penicillin, streptomycin and amphotericin B (Biological Industries, Kibbutz Beit Haemek, Israel) were added up to final concentrations of 100 units/ml, 0.1 mg/ml and 0.25 μg/ml, respectively.

Culture medium: consists of 5% filtered hemolymph obtained from *Aplysia faciata* (specimens were collected along the Mediterranean coast) diluted in ms-L15.

Artificial sea water: 460 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 55 mM $MgCl_2$, 10 mM HEPES, adjusted to pH 7.6.

2.6. Electrophysiology

Conventional intracellular recording and stimulation with single glass microelectrodes were used. The microelectrodes were pulled from 1.5/1.02 mm borosilicate glass tubes with filaments and filled with 2 M KCl whose resistance ranged between 4 and 10 MΩ. For intracellular recording and stimulation, the microelectrode tip was inserted into the cell body.

Extracellular stimulation of the neurons were done by placing the tip of a glass microelectrode filled with ASW approximately 10 μm from the cells membrane.

3. Results

3.1. Device structure and performance

SEM cross-section of the edge of the FG structure after focused ion beam etch is shown in Fig. 3. Clearly seen are the FG and the thermal oxide. Also seen is a residual ring

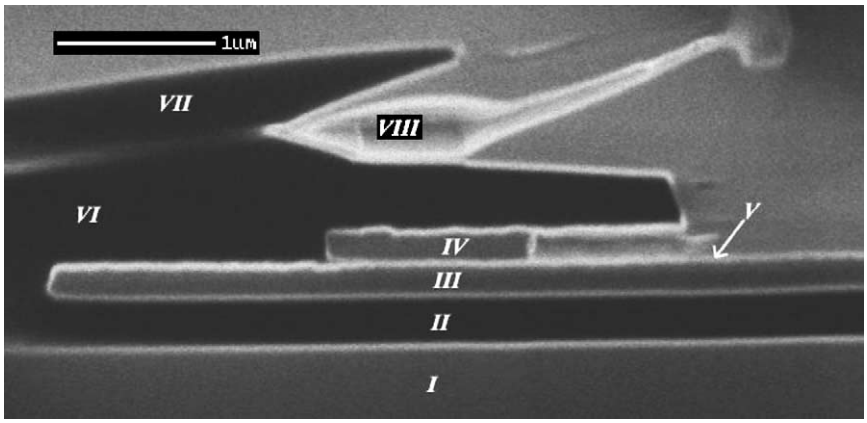

Fig. 3. Inclined cross-section SEM picture of the edge of the FG structure after focused ion beam etch. The various layers are: (I) silicon substrate; (II) 0.5 μm field oxide; (III) bottom polycrystalline silicon layer; (IV) remained upper PS layer; (V) 420 Å of thermal oxide layer between the two PS layers; (VI) PSG; (VII) oxynitride passivation layer; (VIII) artifact of the FIB platinum deposition. The bottom PS layer acts as the FG.

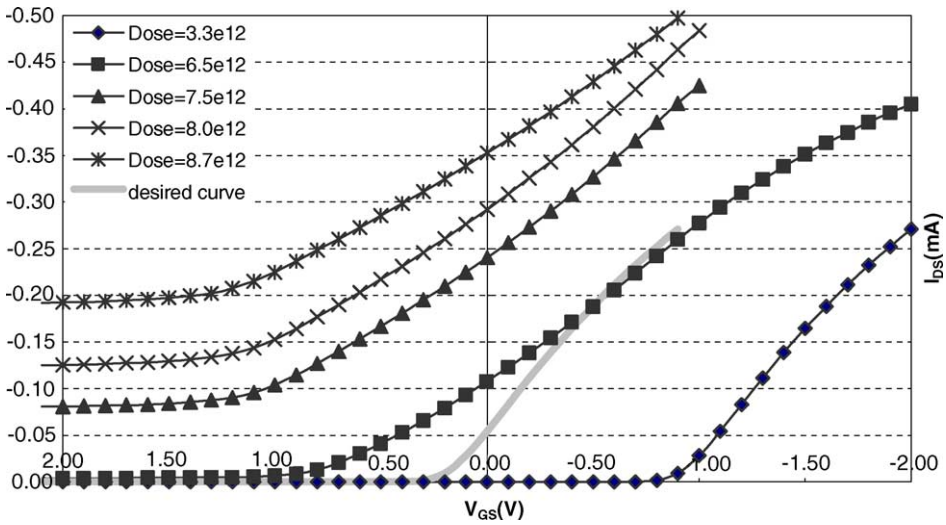

Fig. 4. I_{DS} vs. V_{GS} at $V_{DS} = 0.1\,V$ and $V_{BS} = 0\,V$ for different boron ion implantation doses. The desired device curve is also shown.

of the upper PS layer. The combined dry and wet etching steps resulted in a V shape profile of the oxynitride/oxide wall at the edge of the opening (Fig. 3).

The I_D–V_{GS} curves as function of the different implant doses including that of the standard enhancement type are shown in Fig. 4. Already the lowest implant dose of $6.5 \times 10^{12}\,cm^{-2}$ produced a threshold voltage of 0.75 V with reduction in g_m by a factor of two with respect to that of the enhancement type device. The optimal device I_D–V_{GS} curve is also shown in Fig. 4. Nevertheless, as shown later these devices are still capable of sensing neuron activity.

The capacitive coupling of the signals applied to the ionic solution as compared to the commonly used exposed gate oxide device (Fromherz et al., 1991), are calculated using the following equation:

$$V_{FG} = V_{SIG}\frac{C_S}{C_S + C_F + C_{FG}}, \quad (1)$$

where V_{FG} is the FG voltage, V_{SIG} the applied voltage, C_S the capacitance between the solution and the bottom poly-silicon (FG), C_F the field capacitance and C_{FG} the FG to channel capacitance. The effect of the residual ring of the upper PS layer was not included in the model.

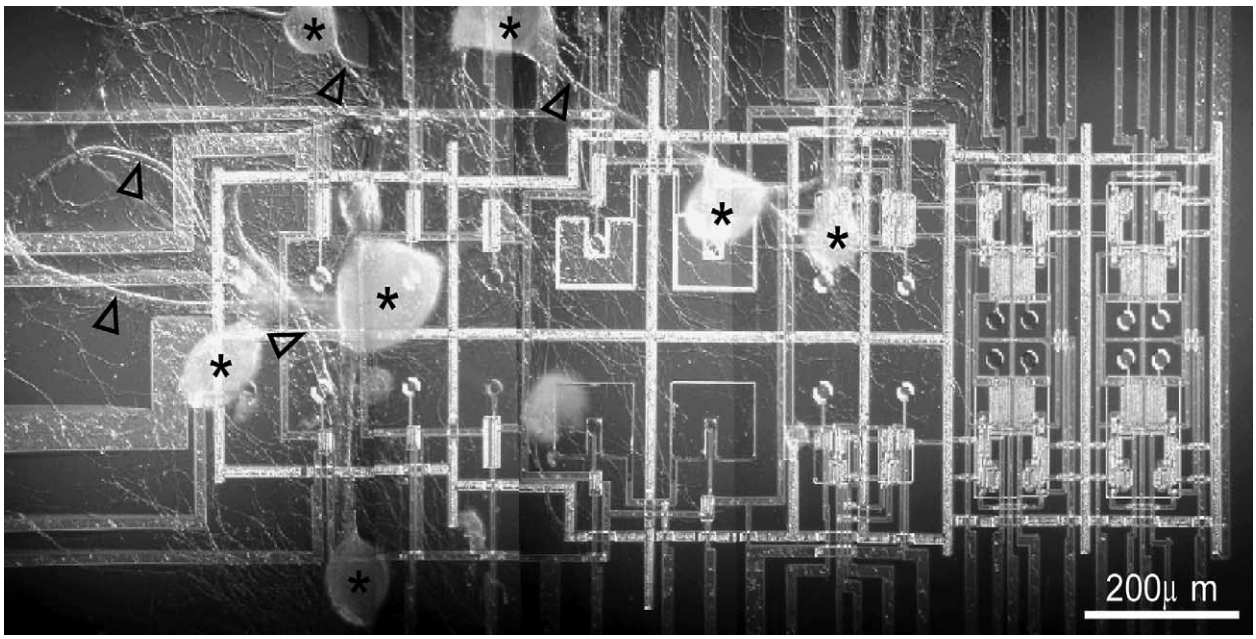

Fig. 5. Cultured *Aplysia* neurons on the FG transistors. The FG electrodes are the octagons at the center of the chip and their transistors are located on their side. The cell bodies of the neurons (asterisks) extended a main axon (arrow heads) which branch up to form many neurites. The recordings shown in Figs. 6 and 7 were made from neurons positioned over the sensing area of the FETs.

The calculated values of all the capacitances for the various device geometries and the resulting capacitive coupling are given in Table 1.

3.2. Action potential–transistor coupling

Neurons were cultured on poly-L–lysine-coated devices for period of up to 2 weeks. Fig. 5 presents an example of the FG silicon chip with the various devices and a low-density culture of *Aplysia* neurons. Under these conditions, the cell bodies of the neurons (asterisks) appear as bright circles. The main axons extend from the cell bodies over a length of few hundreds micrometers (arrow heads) and emit delicate neurites which branch to form an extensive network. The field potentials generated by spontaneously active cultured neurons (Fig. 6A) or by neurons depolarized to fire action potentials (Figs. 6B and C and 7) were recorded by single transistors with a signal to noise ratio of about 5. The shape of most often recorded field potentials were biphasic (Fig. 7). In other experiments (not shown) monophasic field potential were also recorded. The variability in the form of the field potentials recorded by the transistor was discussed in details in earlier studies and thus will not be further discussed here (Fromherz, 1999; Grattarola and Martinoia, 1993; Ingebrandt et al., 2001, 2003; Sprossler et al., 1999).

To better asses the relationships between the action potential generated by a neuron and the transistor's output, we stimulated single neurons to generate action potentials either by an external electrode placed in close proximity to the cell body's plasma membrane or by an intracellular microelectrode inserted to the cell body. Intracellular electrodes were used for both current injection and voltage recordings. Depolarizing intracellular current injection induced the firing of action potentials recorded both by the

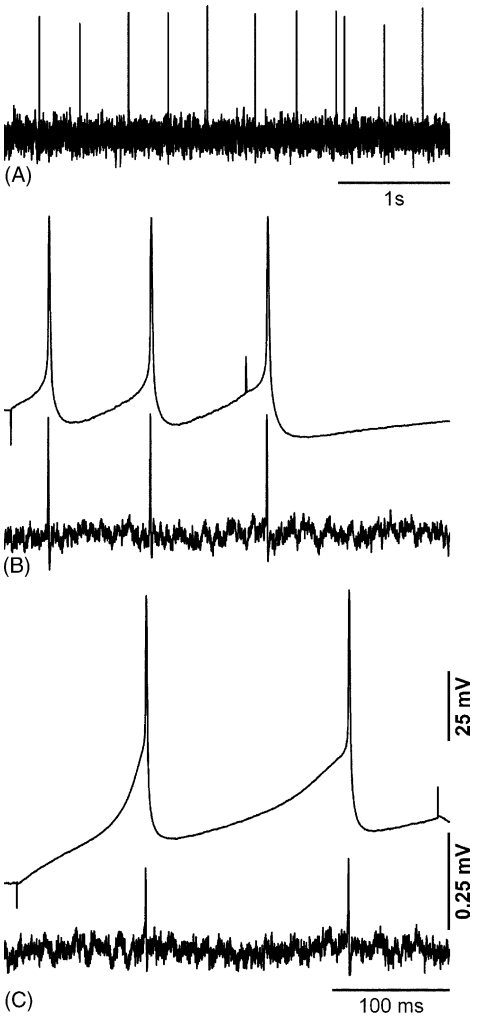

Fig. 6. Spontaneous and evoked field potential recordings by the FG transistor. A buccal neuron was cultured for 48 h on the device. (A) 3 kHz low pass filtered recordings of spontaneous action potentials generated by the neuron. (B) The neuron was impaled by a glass microelectrode for both current injection and voltage recordings. Intracellular injection of a depolarizing current pulse evoked a train of three action potentials recorded both intracellularly (upper trace) and extracellularly by the transistor (lower trace). The microelectrode was pooled out and 24 h later the neuron was reimpaled. (C) Action potentials generated by intracellular current injections 3 days after culturing (upper trace) were recorded by the transistor with somewhat smaller amplitude than those recorded on day 2.

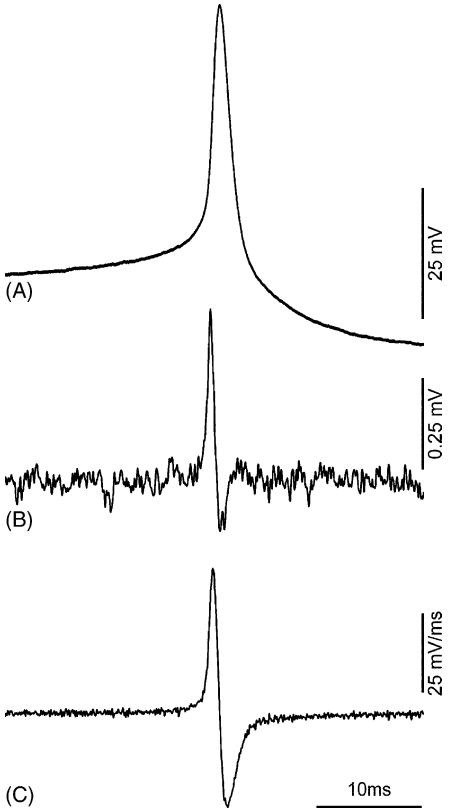

Fig. 7. The relationships between intracellular recorded action potential and the field potential recorded by a FG transistor. A buccal neuron cultured for 48 h was impaled by a glass microelectrode for both current injection and voltage recordings. (A) An intracellularly recorded action potential. (B) The field potential recorded by a FG transistor. (C) The first derivative of the action potential shown in (A). Note the similarity in the shapes of the field potential and the first derivative of the action potential voltage.

Table 1
The capacitances and the calculated capacitive coupling of the different FG transistors

Device no.	W (μm)	L (μm)	D (μm)	CS (pF)	CFG (pF)	CF (pF)	Capacitive coupling
1	50	0.5	15	153.54	59.25	14.87	0.674
2	50	0.5	10	68.24	59.25	7.03	0.507
3	32	0.8	15	153.54	60.67	14.87	0.670
4	32	0.8	10	68.24	60.67	7.03	0.502

microelectrode and by the transistor (Figs. 6B and C and 7). In many of the experiments, the biphasic shape of the transistors output corresponded well to the first derivative of the intracellularly recorded action potential (compare Fig. 7B and C). The recordings of filed potentials by the transistors was stable for several hours of continuous stimulation at low frequencies. Repeated impalement and stimulation of a single neuron revealed that the electrical coupling between the neuron and the FG transistor is quite stable over several days (for example, Fig. 6B and C).

4. Summary and conclusions

FG depletion type p-channel transistors, realized in CMOS technology were shown to be effective in recording neuron activity. This approach has certain advantages over the commonly used approach for direct coupling of the neuron to the transistor gate dielectric layer:

1. The dc bias between the biological solution and the transistor is eliminated. This has direct consequences to the neuron as well as the silicon die endurance since bias voltage induced leakage currents, which may cause damaging electrolysis phenomena are eliminated.
2. The sensing area of the neuron activity is separated from the active area of the transistor. Thus, it is possible to design the sensing area and the channel area separately, resulting in increased transistor output current for a given neuron–transistor coupling.
3. The channel area, which is the most sensitive part of the transistor, can be insolated and shielded from the ionic solution. The addition of a noble metal shield layer such as gold or platinum, over the transistor is expected to reduce electronic noise as well as optical interference. It may also add to the system durability.
4. There is an option to add a switching transistor to the FG and use the FG also for the neuron stimulation.
5. The use of standard CMOS technology with minimal process variation, paves the way to the incorporation of advanced signal processing at the vicinity of the neuron–transistor coupling. This bears advantages such as: increased signal to noise levels, sophisticated signal processing and reduced number of connections between the silicon die coupled to the neural tissue and the external world.

Field potentials were successfully recorded with signal to noise level of about 5. Moreover, the recorded signals showed stability over several days. The results prove the functionality of the new concept of depletion type floating gate devices for sensing and recording neural activity.

Acknowledgements

The project is funded by the European Union Fifth Program contract number: IST-1999-29091. We thank David Schlossberg, Noa Mazursky and Avraham Shely for their technical support, Dr. Ruth Oren for her instructions and comments, and Yaacov Agam for the electronic support.

References

Bergveld, P., Wiersma, J., Meertens, H., 1976. Extracellular potential recordings by means of a field effect transistor without gate metal, called OSFET. IEEE Trans. Biomed. Eng. 23, 136–144.

Fromherz, P., 1999. Extracellular recording with transistors and the distribution of ionic conductances in a cell membrane. Eur. Biophys. J. 28, 254–258.

Fromherz, P., 2002. Electrical interfacing of nerve cells and semiconductor chips. Chemphyschem 3, 276–284.

Fromherz, P., Offenhausser, A., Vetter, T., Weis, J., 1991. A neuron–silicon junction: a Retzius cell of the leech on an insulated-gate field-effect transistor. Science 252, 1290–1293.

Fromherz, P., Stett, A., 1995. Silicon–neuron junction: capacitive stimulation of an individual neuron on a silicon chip. Phys. Rev. Lett. 75, 1670–1673.

Grattarola, M., Martinoia, S., 1993. Modeling the neuron–microtransducer junction: from extracellular to patch recording. IEEE Trans. Biomed. Eng. 40, 35–41.

Ingebrandt, S., Yeung, C.K., Krause, M., Offenhausser, A., 2001. Cardiomyocyte–transistor hybrids for sensor application. Biosens. Bioelectron. 16, 565–570.

Ingebrandt, S., Yeung, C.K., Staab, W., Zetterer, T., Offenhausser, A., 2003. Backside contacted field effect transistor array for extracellular signal recording. Biosens. Bioelectron. 18, 429–435.

Offenhausser, A., Ruhe, J., Knoll, W., 1995. Neurnal cells cultured on modified microelectronic device surfaces. J. Vac. Sci. Techol. A 13, 2606–2612.

Offenhausser, A., Sprossler, C., Matsuzawa, M., Knoll, W., 1997. Field-effect transistor array for monitoring electrical activity from mammalian neurons in culture. Biosens. Bioelectron. 12, 819–826.

Schacher, S., Proshansky, E., 1983. Neurite regeneration by *Aplysia* neurons in dissociated cell culture: modulation by *Aplysia* hemolymph and the presence of the initial axonal segment. J. Neurosci. 3, 2403–2413.

Schatzthauer, R., Fromherz, P., 1998. Neuron–silicon junction with voltage-gated ionic currents. Eur. J. Neurosci. 10, 1956–1962.

Spira, M.E., Dormann, A., Ashery, U., Gabso, M., Gitler, D., Benbassat, D., Oren, R., Ziv, N.E., 1996. Use of *Aplysia* neurons for the study of cellular alterations and the resealing of transected axons in vitro. J. Neurosci. Methods 69, 91–102.

Spira, M.E., Gitler, D., Oren, R., Ziv, N.E., 1999. Calcium homeostasis and mechanisms of growth cone formation during neuritogenesis in cultured *Aplysia* neurons. In: Haynes, L.W. (Ed.), The Neuron in Tissue Culture. Wiley, Chichester, pp. 87–103.

Sprossler, C., Denyer, M., Britland, S., Knoll, W., Offenhausser, A., 1999. Electrical recordings from rat cardiac muscle cells using field-effect transistors. Phys. Rev. E 60 (2), 2171–2176.

Zeck, G., Fromherz, P., 2001. Noninvasive neuroelectronic interfacing with synaptically connected snail neurons immobilized on a semiconductor chip. Proc. Natl. Acad. Sci. U.S.A. 98, 10457–10462.