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Reversible transition of extracellular field potential recordings to intracellular recordings of action potentials generated by neurons grown on transistors

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Abstract

The employment of standard CMOS technology to produce semiconductor chips for recording neuronal activity or for its future use to link neurons and transistors under *in vivo* conditions, suffers from a low signal to noise ratio. Using *Aplysia* neurons cultured on CMOS floating gate field effect transistors, we report here that minor mechanical pressure applied to restricted neuronal compartment that face the sensing pad induces two independent alterations: (a) increase in the seal resistance formed between the neuron's membrane and the sensing pad, and (b) increase the conductance of the membrane patch that faces the sensing pad. These alterations (from ∼0.5 to ∼1.2 MΩ and 75 to ∼600 nS correspondingly), are sufficient to transform the low capacitive coupling between a neuron and a transistor to Ohmic coupling, which is manifested by semi-intracellular recordings of APs with amplitudes of up to 30 mV. The semi-intracellular recordings could be maintained for hours. As a number of compression and decompression cycles could be applied to a single cell without causing significant alterations in its excitable properties, we conclude that the mechanical damage inflicted to the neurons by local compression are reversible. Based on these observations, we suggest that the application of minimal local pressure or suction forces could be used to transform conventional extracellular field potential recordings into quasi-intracellular recording, and thereby dramatically improve both the signal to noise ratio and the quality of recordings from neurons cultured on CMOS semiconductors chips.
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1. Introduction

The employment of standard CMOS technology to produce semiconductor chips for recording neuronal activity, or its future use to link neurons and transistors under *in vivo* conditions, suffers from a low signal to noise ratio (Offenhausser et al., 1997; Vassanelli and Fromherz, 1997, 1998). Experimental results and theoretical considerations have revealed that the signal to noise ratio is determined by three main factors: (a) the transistor's noise level (Arnaud and Galup-Montoro, 2003; Fleetwood et al., 2002); (b) the seal resistance formed between the neuron and the flat electrode surface (R_{seal}); (c) the intensity of the current generated by the neuron's activity (Cohen et al., 2006; Fromherz, 2003).

The current flow over R_{seal} can be either capacitive or Ohmic. The model developed by Fromherz and his colleagues (Fromherz, 2003) shows that when the membrane facing the transistor's gate (the junctional membrane) expresses a negligible number of voltage-independent ion channels, the FP is proportional to the first derivative of the intracellular voltage (Fromherz et al., 1991). If on the other hand, the junctional membrane is enriched by voltage-independent ion channels, the FP shape corresponds to that of the intracellular voltage (Fromherz et al., 1993; Jenkner and Fromherz, 1997). When the capacitive current is expressed by the ionic current through the non-junctional membrane, the FP is determined by the difference between the junctional and non-junctional membrane conductances (Fromherz, 1999).

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The value of R_{seal} is determined by the planar dimensions of the neuron–transistor junction and the width of the cleft formed between the plasma membrane and the device surface (Weis and Fromherz, 1997). Using optical methods and biophysical analysis, Braun and Fromherz (1998) and Zeck and Fromherz (2003) estimated that the cleft width formed between rat astrocytes cultured on silicon dioxide coated by laminin is approximately 100 nm. Accordingly, R_{seal} was estimated to be in the range of 1 MΩ (Weis and Fromherz, 1997). Electron microscopic analysis of thin sections prepared from cultured *Aplysia* neurons grown on 2D-polyaniline-coated glass substrate revealed that the cleft width ranges between hundreds of nm in some areas to 20–40 nm in others (Oren et al., 2004). It is generally accepted that a cleft dimension of 10–20 nm is the minimum width that can be formed by cells grown on substrates coated by biocompatible molecules (Sackmann and Bruinsma, 2002). Nevertheless, in practice R_{seal} can be increased either by enlarging the contact area formed between the neuron and the transistor-gate, or by reducing the average cleft width.

Consistent with the above, Jenkner and Fromherz (1997) reported that downward displacement of leech neurons cultured over transistors gate leads to an estimated increase in the seal resistance by a factor of ∼1.7 (from 2 to 3.5 MΩ). The vertical displacement of the neuron was also associated with a discrete increase in the junctional membrane conductance by approximately one order of magnitude (from 0.36 to 2.7 mS/cm^2). This switch, in concert with the increased seal resistance, led to a transformation of the field potential (FP) from a biphasic extracellular FP (proportional to the first derivative of the intracellular voltage), to a monophasic FP which resembles in shape intracellularly recorded action potential (Jenkner and Fromherz, 1997). The amplitudes of both the biphasic and monophasic FPs were increased by a factor of 2–5 (in the range of 1–5 mV). The discrete switch between the two modes of neuron–transistor coupling was attributed to alterations in the junctional membrane conductance imposed by the mechanical deformation of the cell body and its cytoskeleton which in turn affected ion channel conductances (Jenkner and Fromherz, 1997). The mechanical manipulation of cultured leech neurons, in respect to the sensing gate, inflicted mechanical damage to the neuron, and thus, repeated manipulations were rare. To the best of our knowledge, it is for this reason that this form of experimental manipulation was not reported again in the literature.

Using cultured *Aplysia* neurons grown on floating gate (FG) depletion type transistors, we extend here the pioneering studies of Jenkner and Fromherz (1997). We found that application of mechanical pressure on the cell body or axon of cultured *Aplysia* neurons gradually increased the seal resistance by increasing the contact area between the neuron and the gate surface and by reducing the average cleft width. As expected, this was associated with a gradual increase in the amplitude of the FP but was not associated with changes in its shape. With increased mechanical pressure, the capacitive coupling was transformed into an Ohmic coupling between the neuron and the transistor. Whereas the transformation appeared to be an all-or-none event, the mode of Ohmic coupling was stable for hours and the transformation could be repeated a number of times.

We conclude that high quality and hour-long recordings of action potentials can be obtained by pressing neurons against the flat sensing transistors pads. The principles described in the present study can be adopted to largely improve the quality of neuro-electronic coupling.

2. Materials and methods

2.1. The recording device

Depletion type P-channel floating gate MOS transistors with an octagonal sensing area of a diameter of 10 or 15 μm and W/L of 50/0.5 or 32/0.8 μm were designed and realized in a 0.5 μm CMOS technology, as described in an earlier study (Cohen et al., 2004). The geometrical separation between the octagonal sensing area and the transistor channel, allows for an optimal sensing area with respect to the neuron's dimensions, while realizing transistors with large transconductance (g_m). The poly-silicon floating electrode is placed between the gate oxide of 115 Å and the top oxide of 420 Å. The alteration in the FG electrode voltage modulates the transistor's drain-source current. This current is amplified by an external electronic system that filters the signal by a high-pass filter.

2.2. Materials

A marine species L-15 solution (msL-15): Leibovitz's L-15 Medium (Gibco-BRL, Paisley, Scotland) was supplemented according to Schacher and Proshansky (1983). 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.

The culture medium consists of 5% filtered hemolymph, obtained from *Aplysia fasciata* (specimens collected along the Mediterranean coast) diluted in msL-15. Sulforhodamine 101 (SR101, Kodak) was prepared as a stock solution of 10 mM in double-distilled water (DDW), and further diluted before use in ASW to a final concentration of 40 μM.

2.3. Culture procedure

Left upper quadrant neurons (LUQ) 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). The neurons were plated on the chip's thermal oxide layer above the FG, which was coated with poly-L-lysine (Sigma, Rehovot, Israel). The experiments were performed 2–5 days after plating at room temperature (21–25 °C), and after replacing the culture medium with artificial sea water (ASW).

2.4. Confocal microscopy imaging

The system used for confocal imaging consisted of an Olympus microscope IX70 and a Bio-Rad Radiance 2000/AGR-3 confocal imaging system. The objective used was an Olympus planApo 60× 1.4 NA oil objective. In the experiment of Fig. 1,

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