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The neuro-electronic interface: measurements and model predictions

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The functional coupling of populations of chick embryo dorsal root ganglia (DRG) neurons to planar arrays of microelectrodes has been characterized both experimentally and via computer simulations. Spontaneous electrical activity recorded with a multichannel automated workstation is compared with simulated activity, whose signal-to-noise ratio has been systematically varied by simulating changes in the strength of the neuron-to-substrate adhesion. The importance of this approach for the design of optimized neuro-electronic prostheses and for the development of new tools for *in vitro* screening tests is briefly discussed.

1. Introduction

Interfacing cultured nerve cells to recording and stimulating microelectronic planar devices is a task of major importance for two distinct objectives, namely, the design of optimized neuro-electronic prostheses and the development of new tools for *in vitro* biocompatibility and neuropharmacological tests. To accomplish the first objective, microelectronics seems to offer a very promising technology for the design of micron-sized devices to be interfaced, with a one-to-one correspondence, to selected populations of neurons inside the human body [1–3], and for integrating, on the same chip, multiple recording and stimulating electrodes with signal processing hardware/software.

Towards this end, a precise *in vitro* characterization of the transduction of neural activity operated by an extracellular microelectrode is of the utmost importance. This characterization is basic to attaining the second objective, that is, the use of this hybrid system (i.e. a network of neurons coupled to an array of microdevices) as a sophisticated tool for screening *in vitro* the electrophysiological effects of pharmaceutical agents on organized populations of neurons [4].

Experiments and computer simulations, performed to characterize the neuron-to-microtransducer coupling systematically, are described in the following, with reference to neurons extracted from the dorsal root ganglia (DRG) of chick embryos and seeded on thin-film planar arrays of microelectrodes.

2. Materials and methods

2.1. Experimental tools

Experiments were performed on primary culture neurons from the dorsal root ganglia (DRG) of 10–12-day-old chick embryos. The culture was established by the method described by Barde *et al.* [5]. About 50–60 ganglia were dissected from 2–3 embryos (thoracic–lumbar regions). During dissection, the ganglia were collected in RPMI-1640 (Sigma) culture medium, without serum, and then placed in Ca^{++}- and Mg^{++}-free phosphate-buffered saline containing 0.125% trypsin. After 20 min incubation at 37 °C, the ganglia were washed twice with a medium containing 1% heat-inactivated horse serum, and dissociated by trituration in a siliconized Pasteur pipette. The culture medium was composed of RPMI-1640, with 1% FCS, 1% horse serum, 1% antibiotics, and 10 ng/ml of NGF. The culture was maintained at 37 °C in water-saturated atmosphere with 5% CO_2. Neurons were seeded on arrays produced at the Center for Integrated Systems of the University of Stanford (USA). The microelectrode arrays were obtained by using thin-film technology; the substrate was silicon, the exposed metal was gold, and the passivation layer was silicon nitride [6]. A few experiments were also performed by using ITO (indium tin oxide) microelectrode arrays kindly supplied by Dr Akio Kawana of NTT Basic Research Laboratories, Tokyo (Japan) [7].

According to previous experience [8], the microelectrodes were coated with the adhesive natural protein laminin. Nerve growth factor (NGF) was also added to the culture to promote neuritic outgrowth. A ring of inhert plastic was glued to the array to form a culture microchamber. A population of neurons coupled to a microelectrode array is shown in Fig. 1.

For signal acquisition purposes, the microchamber was put inside a Faraday cage and connected via custom connectors to an automated workstation

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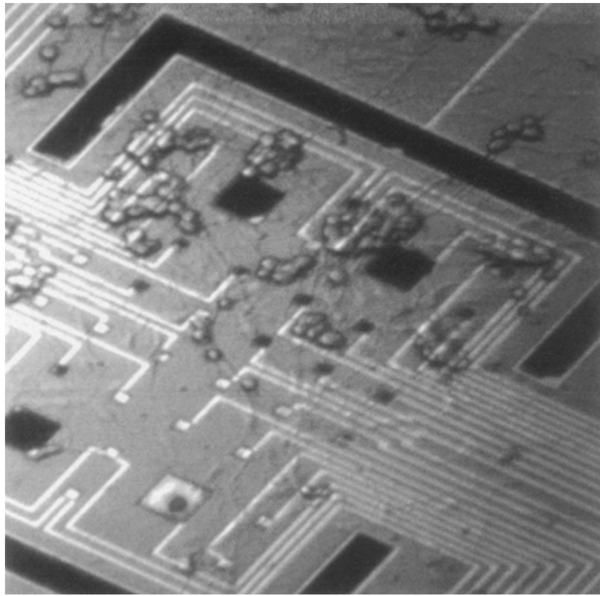

Figure 1 Low magnification view of a random population of DRG neurons growing on a 32-microelectrode array (gold on Si_3N_4).

developed for acquisition and processing of extracellularly recorded signals [6] (Fig. 2).

2.2. Simulation tools

A set of equations modelling a neural oscillator [9] were implemented in a computer program, written in C language, to simulate the electrical behaviour of a DRG neuron. This model is characterized by a set of five ionic conductances – three for sodium, calcium, and chloride ions and two for potassium ions. The equation for the total membrane current is:

$$C_m \frac{dV}{dt} = I_{stim} - [g_{Na}(V - V_{Na}) + (g_K + g_{KCa})(V - V_K) + g_{Ca}(V - V_{Ca}) + g_{Cl}(V - V_{Cl})] \quad (1)$$

where V is the membrane potential; C_m is the membrane capacitance; g_{Na}, g_K, g_{KCa}, g_{Ca}, and g_{Cl} are the sodium, potassium, potassium Ca^{++}-dependent, calcium, and chloride conductances, respectively. Sodium and potassium conductances are described according to the Hodgkin–Huxley formalism [10]. More details can be found in the literature [9], [11]. Signals extracellularly recorded (V_{Ext}) by using the technique described in the experimental section are primarily characterized by a capacitive component which can be defined as:

$$V_{Ext} = R_{seal} C_{me} \frac{dV}{dt} \quad (2)$$

where R_{seal} is the sealing resistance that characterizes the coupling between neuron and planar microelectrode, C_{me} is the membrane–electrolyte capacitance, and V denotes the membrane potential.

To obtain signals simulating extracellular recordings, the action potentials generated by the simulation program were filtered by using a derivative filter based on the coupling parameters (i.e. R_{seal} and C_{me}). The coupling area between neuron and microelectrode considered in the simulations was $100\,\mu m^2$. By changing the R_{seal} and C_{me} values, it was possible to characterize the neuro-electronic interface as a function of the coupling strength [12–14]. Moreover, Johnson noise was added to the simulated signals, according to the relation [15]:

$$V_{Noise}(rms) = \sqrt{4(R_{seal} + R_e)kTB} \quad (3)$$

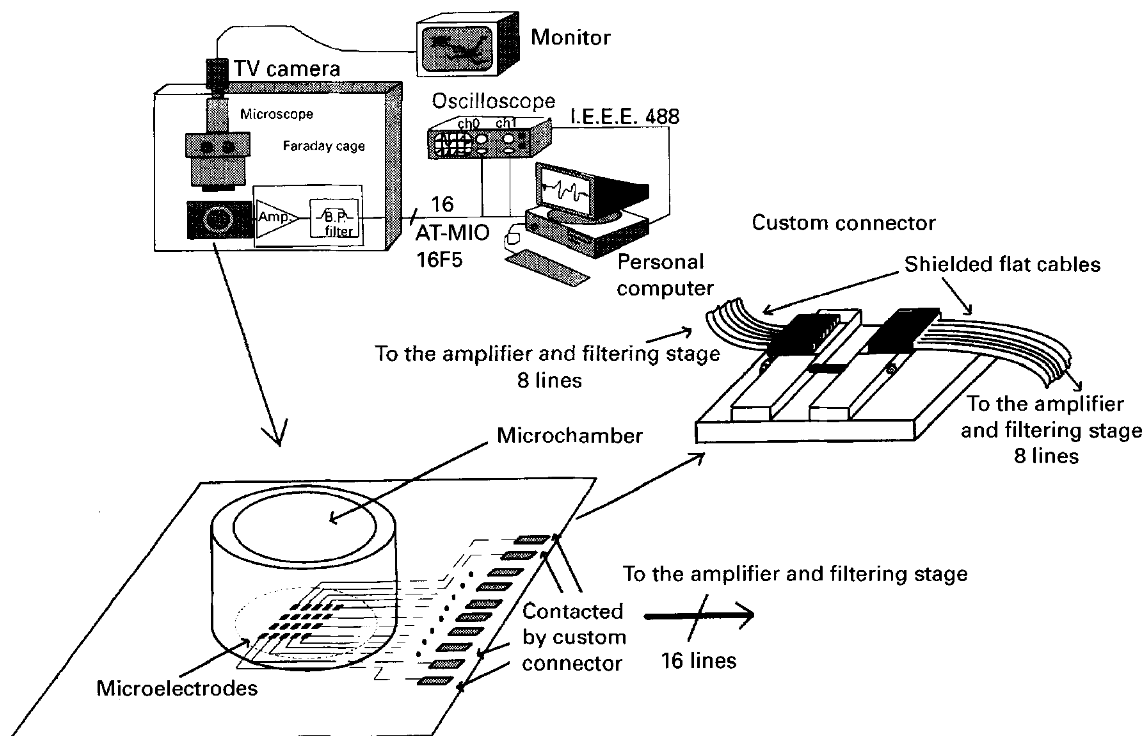

Figure 2 Schematic of the automated workstation utilized for acquisition and processing of extracellularly recorded neuronal activity.

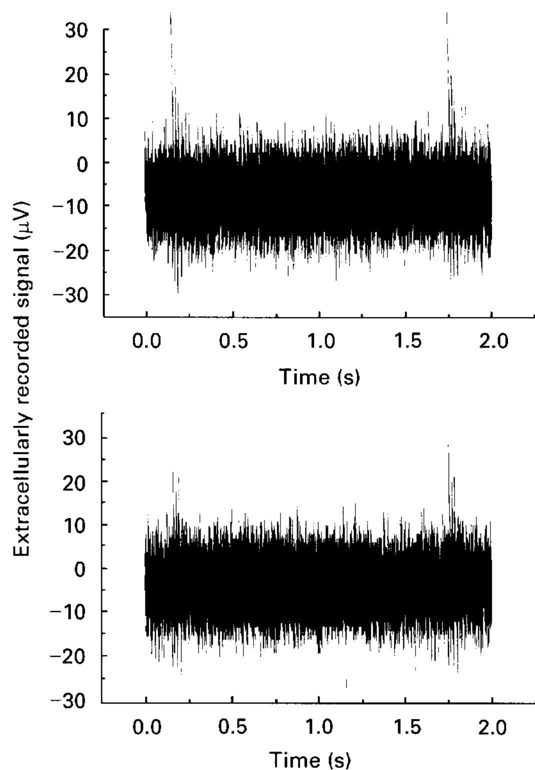

Figure 3 DRG neuron spontaneous activity extracellularly recorded by two distinct microelectrodes (gold on Si_3N_4).

where R_e is the real part of the microelectrode impedance, k is the Boltzmann constant, T is the temperature, and B is the bandwidth in hertz. In our simulations, only this kind of noise was considered.

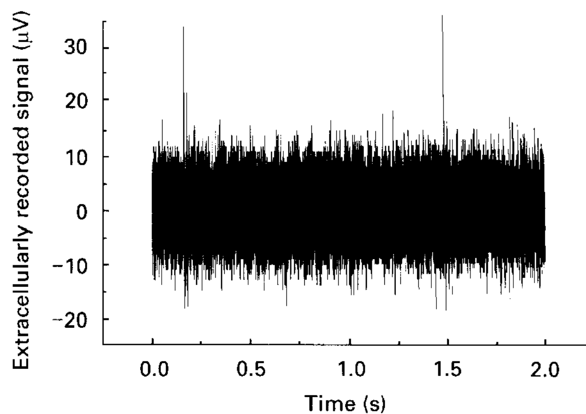

Figure 4 Simulation signals reproducing the experimental data of Fig. 3.

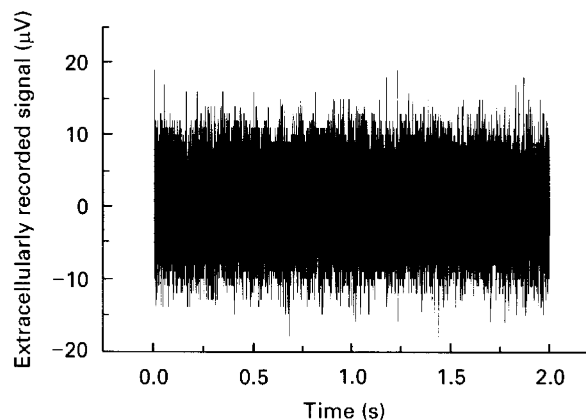

Figure 5 Simulation of recording under adverse experimental conditions (i.e., weak electrode-to-neuron coupling).

3. Results

Fig. 3 shows an example of spontaneous activity extracellularly recorded by two distinct microelectrodes (gold on silicon nitride). The signals appear in the form of a periodic burst, which was repeated roughly every 1.4 s, with an amplitude in the range 50–60 μV. The noise band is about 30 μV.

Fig. 4 shows a simulation result obtained by using the proposed model. The simulated signals and noise reproduce the experimental results quite accurately.

Fig. 5 shows a simulation result obtained by decreasing the values of the coupling parameters (i.e. by simulating the detachment of the neuron from the recording electrode). The neural electrical activity is now completely covered by noise, so the electrode fails to record it.

4. Discussion

As already mentioned, the *in vitro* coupling of nerve cells to recording/stimulating arrays of microelectrodes represents quite a recent technique with far-reaching implications, both for the design of microelectronics – based functional neuro-prostheses and for the development of sophisticated *in vitro* pharmaceutical screening tools. The electrochemical characterization of such a coupling is a preliminary, yet fundamental, step towards the achievement of these ambitious goals. As shown by our results, the coupling characterization can be utilized to predict the expected signal-to-noise ratio and to simulate the deterioration of a recorded signal. We can anticipate that this kind of simulation will become a powerful tool for the interpretation of *in vivo* experiments, where the strength of neuron-to-microelectrode coupling may turn out to be a very critical and unstable parameter.

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