ACTIVITY PATTERNS OF CULTURED NEURAL NETWORKS ON MICRO ELECTRODE ARRAYS

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Abstract-**A hybrid neuro-electronic interface is a cell-cultured micro electrode array, acting as a neural information transducer for stimulation and/or recording of neural activity in the brain or the spinal cord (ventral motor region or dorsal sensory region). It consists of an array of micro electrodes on a planar substrate, the electrodes being covered by a network of cultured neurons. The cultured neuron network layer acts as a natural host for in vivo neural connections. Besides this function, live neural networks can become spontaneously active and have the capability of information processing, as 'minibrains'. One may envisage future applications of these intermediary networks as 'front-end' signal processors.**
The paper presents results on spatio-temporal activity patterns and their characterization in neural networks, cultured from dissociated rat visual cortex. Cultures lasted 43 days in vitro on multi electrode plates with 60 electrode sites and started activity after about seven days. Firing rates increase with time thereafter. Typical 'pacemaker'-and-burst firing patterns are seen, the time characteristics of which change over days, typically.
***Keywords* – neural interface, cultured neural networks, network activity**

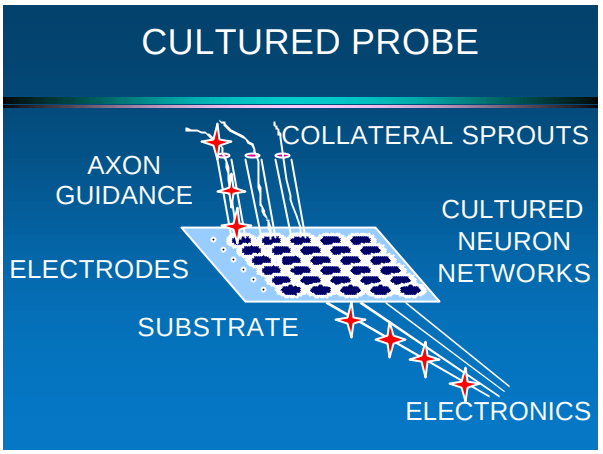

Fig. 1. Schematic impression of a 'cultured probe'-type of neural information transducer/prosthetic device.

I. INTRODUCTION

Efficient and selective electrical stimulation and recording of neural activity in peripheral, spinal or central pathways requires multielectrode arrays at micrometer or nanometer scale. At present, wire-arrays in brain, flexible linear arrays in the cochlea and cuff-arrays around nerve trunks are in experimental and/or clinical use. Two- and three-dimensional brush-like micro arrays and 'sieves', with around hundred electrode sites, have been proposed, fabricated in microtechnology and/or tested in a number of labs.
As there are no 'blueprints' for the exact positions of neurons, an insertable multielectrode has to be designed in a redundant way. Even then, the efficiency of a multielectrode will be less than 100%, as not every electrode will contact a neural axon or soma.
Therefore, 'cultured probe' devices are being developed, i.e. cell-cultured planar MEA's (Multi Electrode Arrays). They may enhance efficiency and selectivity because neural cells have been grown over and around each electrode site as electrode-specific local networks. If, after implantation, collateral sprouts branch from a motor fibre (ventral horn area) and if they can be guided and contacted to each 'host' network, a very selective and efficient stimulatory interface will result (Fig. 1).

Developing neural networks will exhibit spontaneous activity after about one week in vitro. To study this we have developed quantitative techniques for long-term, longitudinal recording of both activity levels and neurite outgrowth in individual neurons within a developing network. It has been shown that the patterns resemble those seen in vivo (see Corner and Ramakers, 1992). Recording of the firing activity of individual neurons can now be achieved by culturing neurons on multi-electrode arrays which, since the pioneering work of Gross and Pine (e.g., Gross, 1979; Pine, 1980), have become established as a useful technique (see also Potter, 2000), as is also recently shown by Jimbo et al. (1999) in a study of activity-dependent plasticity at the synaptic level. The present paper reports some results of long-term longitudinal recording of firing activity of individual neurons during network development.

II. METHODOLOGY

Cell cultures

The cortices of E18 Wistar rat fetuses were removed and dissociated by trituration following enzymatic treatment with trypsin. The dissociated neurons were plated on the multi-electrode array (MEA) substrate coated with

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Abstract

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polyethylene-imine (PEI, Fluka, 10 mg/ml), using glass rings (inner-diameter = 7 mm). In total 150.000 cells (50 ul cell suspension) were plated. After 1 hour the rings were removed and an additional 10^6 cells in 1 ml of cell suspension were added to the culture chamber on the MEP (inner diameter 30 mm). For the inner area this resulted in a monolayer of cells in such a density that a surface area of on the average 200 um^2 (corresponding to a 16 um circle diameter) was available for each cell. At time of plating, the cells themselves had a diameter of about 5 um. Neurons were cultured and recorded in 2 ml of glia conditioned medium (GCM) +0.2 % BSA containing 1.3 mM Ca^{2+} and 0.7 mM Mg^{2+}

The culture chambers were sealed with a glass cover in order to prevent evaporation of the medium during the long-term recording period. Once a week, 200 ul of the medium was sucked out and 300 ul fresh medium added to the culture, but no further handling was applied during the entire recording period. After each recording, MEP's were cleaned by carefully rinsing and sterilized for 4 hrs at 120^o C for reuse.

MEA plates.

The MEA's consist of 5 x 5 cm glass plates onto which a pattern of 61 electrically conductive lanes were etched, running from two sides of the plate towards a central area where they ended in a hexagonal pattern with a mutual spacing of 70 um and a lane width of 10 um.

MEA's were produced with either transparent indium tin oxide (ITO: see Gross et al., 1985) or with gold as the electrically conductive medium (ITO glass plates were obtained from Philips, Heerlen, The Netherlands).

The MEA's were covered with an insulation layer consisting of a "sandwich" of silicon oxide, silicon nitride and silicon oxide layers (ONO, total thickness 800 nm).

The electrode pattern was etched from the indium tin oxide (ITO) layer using positive photoresist photolithographic techniques. On glass plates containing a 100 nm thick layer of ITO, a layer of Microposit S1818 photoresist (Microposit, Coventry, England) was spun and cured for 20 min at 90^0 C. The photoresist was illuminated through a mask and developed with Microposit S1813 (Microposit). Developed photoresist was etched for 9 min. at 45^0 C with a $HCl/H_2O/HNO_3$, (50:50:1, v/v/v) solution. Subsequently, a "sandwich" of silicon oxide, silicon nitride and silicon oxide layers was deposited.

These layers were created by plasma enhanced chemical vapor deposition (PECVD). The silicon oxide layers were deposited for 3 min. under gaseous

SiH_4, 2% N_2 and N_2O conditions. The silicon nitride layer was deposited for 36 min under gaseous SiH_4, 2%

N_2 and NH_3 conditions. Finally, the electrode tips were de-insulated (diameter 12 um) using reactive ion etching (RIE) with gaseous SF_6 and O_2 for 6 min. The electrode tips were then platinized in order to reduce their impedance to less than 1 MΩ (at 1 kHz, Buitenweg et al., 1998).

Multi-electrode recording setup

A culture chamber was created by fixing a glass cylinder with an inner diameter of 30 mm on the MEP, using a two-component resin HYSOL or a silicone elastomere, Dow Corning 3140 RTV coating. MEP's were placed in a closed incubation chamber, mounted on an inverted microscope which was kept at a constant temperature of 36^0.

The sealing impedance between the cell and the electrode, as well as the impedance of the electrode itself, are important factors in recording activity from a cell (Regehr et al., 1989; Buitenweg et al., 1998). The sealing impedance in our setup was estimated at 5 MΩ (Buitenweg et al., 1998). During the experiment, noise levels were routinely measured as RMS values ; typically noise levels are 20 uV-50 uV RMS.

III. Results

The dynamics of spontaneous activity can be characterized as follows: 1) activity starts to develop at 9 DIV (fig. 3) 2) long time recording is feasible (fig. 3), during which 3) firing rate increases (fig. 4) 4) networks develop clearly a great variety of patterns of repeated firing activity (fig. 5 shows burst and synchronization phenomena) , implying that 5) connectivity changes continuously in the network over time and probably increases overall with time.

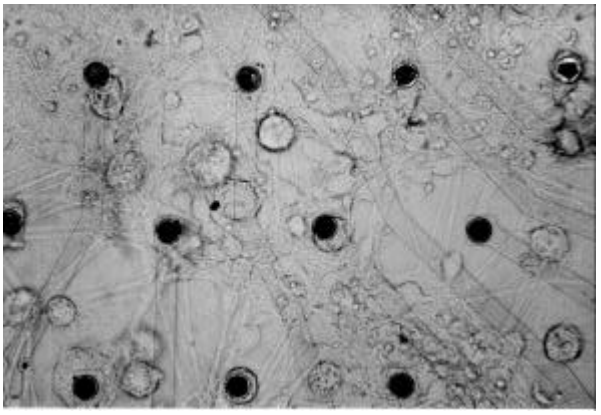

Fig. 2. Bright field microscope photo of a random network of rat visual cortex dissociated cortical neurons on a multi electrode array after 11 days in vitro. Black dots are platinized electrode (indium tin oxide, ITO) sites, diameter 12 um. ITO leads to the electrode sites are clearly visible. For adhesion of cells the surface is coated by a polyethyleneimine layer.

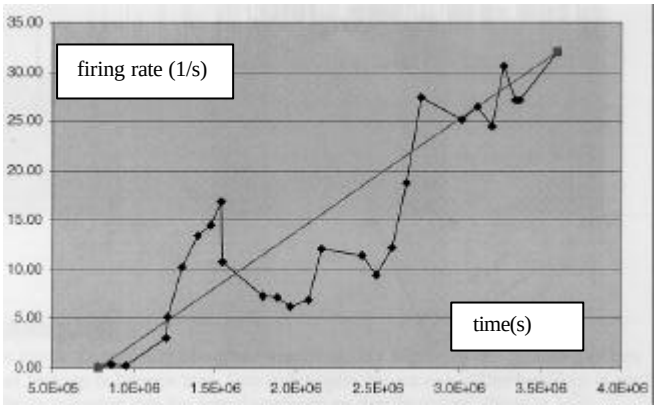

Fig.4. Firing rate (no. of spikes/s, summed over all electrodes) as a function of time (s), between 9 and 43 days in vitro (DIV).

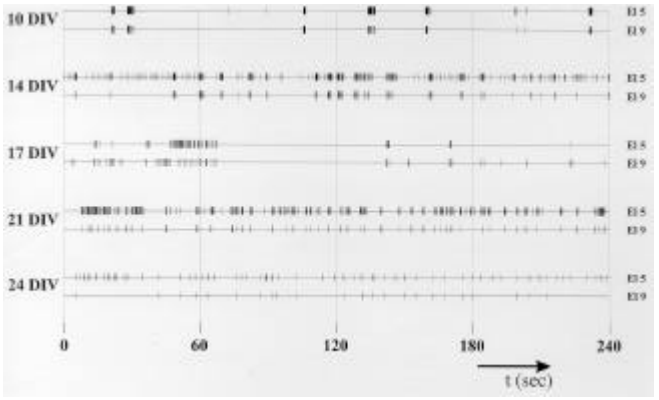

Fig. 5. Specific example of firing activity at two electrodes after 10, 14, 17, 21 and 24 days in vitro, over a time sample 240 seconds long. Action potentials are time stamped, stored and shown in the figure as events.

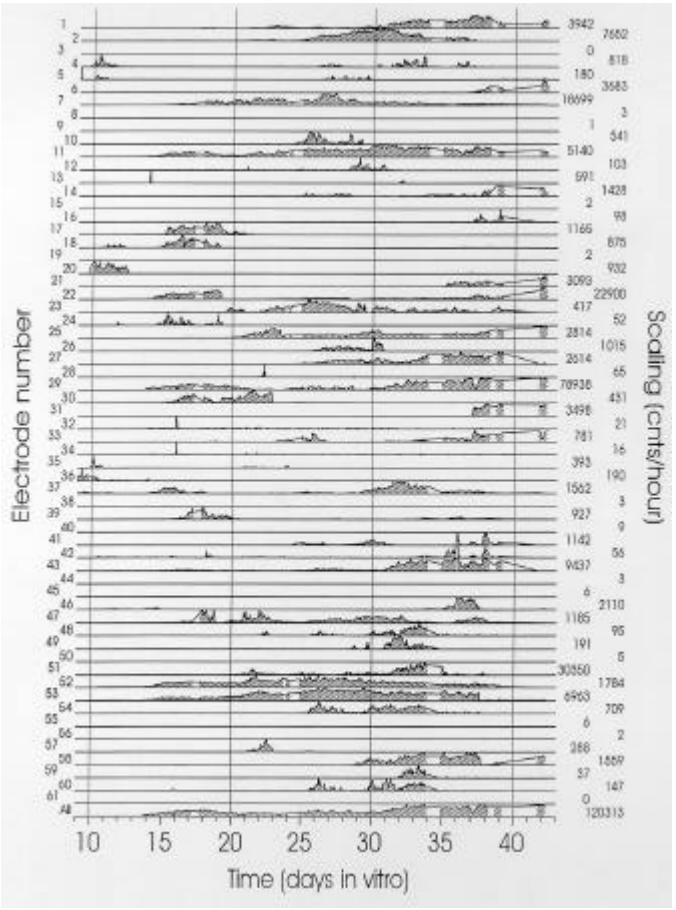

Fig. 3. Firing rates in counts per 60 minutes time bins at all 60 individual sites of the multi-electrode array for the whole period of recording from 9 to 43 DIV. The firing rate traces are individually scaled, with the maximum hourly rate indicated at the right side of the figure. The figure is composed of all the recording runs made during the experiment. The plot includes some non-dashed episodes for which no data was available, caused by earlier termination of that particular acquisition run. Traces with fewer than 10 spikes per hour at their maximum have been suppressed from plotting in order to emphasize the active traces. The traces are plotted from 9 DIV on, at which time activity was first recorded.

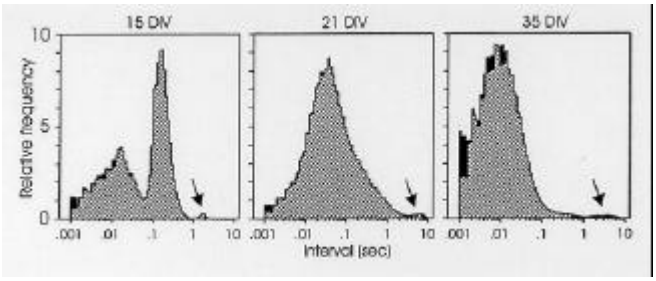

Fig. 6. Interspike interval distributions , calculated during 4-hours periods at 15, 21 and 35 days in vitro. Arrows: see text.

Main characteristics of the pattern changes are that typical gross behavior is stable over one or two days. Typically, one or two sites show regular firing, like 'pacemaker cells', interrupted at longer intervals by short (about 1 second) 'collective burst' discharges, during which activity spreads very fast as 'bursts' over many sites. After a burst, activity may be silent shortly , then the pacemakers restart and after some time a new, more or less identical activity-burst develops. Bursting is defined as sequences of relatively short intervals.

Network spike clusters include phases of uncorrelated firing at different electrode sites, and periods of intense synchronized network activity, characterized by the recruitment of multiple sites. Such synchronized network firing may be called 'network acticvity waves'.

The typical time interval between network spike clusters is 1.3 seconds. Figure 6 shows the interspike interval distribution , calculated during 4-hours periods at 15, 21 and 35 days in vitro. Each panel shows a small peak at the right side of the histogram (see arrows) originating from the time intervals between the main network spike clusters. The average location of the interval value separating this small peak from the rest of the histogram is approximately 1.3 sec.

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