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Cross-interval histogram analysis of neuronal activity on multi-electrode arrays

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Abstract- **Cross-neuron-interval histogram (CNIH) analysis has been performed in order to study correlated activity and connectivity between pairs of neurons in a spontaneously active developing cultured network of rat cortical cells. Thirty-eight histograms could be analyzed using two parameters, one for the shape and one for the average number per interval bin. The histogram shape varied gradually between flat and clearly peaked around zero interval, indicating no/abundant connectivity and direct connection pathways, respectively.**

Keywords **–multi-electrode arrays, cultured neural network, cross-neuron-interval histogram.**

I. INTRODUCTION

By using multi-electrode arrays (MEA's) and spike sorting procedures it is possible to record extracellular activity from in vitro neuronal networks. Cultured dissociated neurons develop into connected networks in a matter of days after seeding, and become spontaneously active after about one week in vitro. One of the methods to get information about the connectivity of the network is to extract and evaluate cross-neuron interval histograms [1], recorded from different MEA electrode pairs. The cross-neuron interval histogram (CNIH) will, for example, show a sharp peak around zero delay if two trains fire synchronously. If there is no correlation between the two trains, the CNIH is flat.

Cross-correlation analysis has already been developed by several research groups to investigate possible connections among neurons, lying on different electrodes, using simulation of neuronal networks [2] or recording the signal directly from lightly anesthetized animals [3] [4]. In both cases they drew quite similar conclusions: cross-correlation analysis gives some information on the effective connectivity between two neurons, but multiple pathways between neurons will not be revealed one by one.

The notion of connection can be split in two ways of interaction: direct interaction and shared input. The first one is present if a spike produced by one neuron activates a second one directly. The second possibility occurs when an external stimulus excites both analyzed neurons at the same time, producing their simultaneous firings. For example, this case applies to external stimulation in animal experiments.

By analysis with the CNIH it's possible to differentiate between the two possibilities. The shared input, indeed, produces a peaked, symmetric, histogram around zero time shift, whereas a one to one, short, connection will show a peak shifted from zero delay [4] [5].

In this analysis a classification is made into groups of CNIH –shapes, by processing the data from four different cultures. According to the criteria discussed below, two groups are found and an interpretation in terms of effective connections has been tried. Besides, two special cases were interpreted, with the aim to investigate eventual changes with time, in the connections, due to the growth of the network.

II. METHODS AND MATERIALS

Culture techniques. Cultured neuronal networks were obtained from culturing suspensions of cortical neurons in a chemically defined culture medium (R12 [6]). Two days old postnatal rats were used resulting in nine MEA's with cultured networks of which the two most active MEA's were used.

After decapitation, the cortex of the brain was prepared free and cut into small pieces. Neural dissociation occurred in R12 medium and the tissue was transferred into a conic glass tube. 750 µl trypsine/EDTA was added and incubation lasted 45 minutes at 37°C under 5% CO_2. Then the supernatant was removed and 750 µl of trypsine inhibitor, as well as 150 µl of DNAse were added to the cortical cell suspension in R12. Next, dissociation was repeated. Rinsing with 5 ml of R12 medium was followed by centrifugation at 1200 rpm for 5 minutes at room temperature. Resuspension of the cortical neurons occurred in 1 ml of R12 medium and the neuronal concentration was determined by a Burker-chamber (0.5 to $1 \cdot 10^6$ cells/ml). Cortical cell suspensions were plated on polyethylene-imine (PEI) coated MEA substrates (Fig.1).

The cells were nourished by R12 medium, containing nerve growth factor (NGF), L-Glutamine and vitamin C. The culture medium was completely replaced once a week and the cultures were stored in an incubator (37°C, 5% CO_2 and 100% humidity). Cultures could be kept alive for several months.

Electrophysiological methods. In this study substrate-embedded multi-electrode array technology was applied. The MEA consisted of a 50 mm x 50 mm glass plate with a glass ring glued on the top, with 61 platinized coated electrodes of 5 or 10 µm in diameter and a distance of 80 µm from each other (Fig.2). Electrodes, placed in the center of the glass plate were connected to the bond-taps, placed at the edges, by gold leads (31 on the left side and 30 on the right side). For measurements the MEA was placed in a mini-incubator, which was connected to a PID controller for maintaining a temperature of 37° C.

Next, the incubator was connected to a 16-channel amplifier by means of a selector, which enabled to register 16 out of 31or 30 electrodes. The amplifier had a first order band-pass filter with a cut-off frequency at 310 Hz and 6 kHz and an amplification factor approximately equal to 230 at 1 kHz. The amplified signals were connected to a PCI-6023E Data

Acquisition PC-card (National Instruments), were sampled at 12.5 kHz per channel and quantisied with a 12-bit resolution. The data acquisition was software controlled by specially designed virtual instruments designed in Labview.

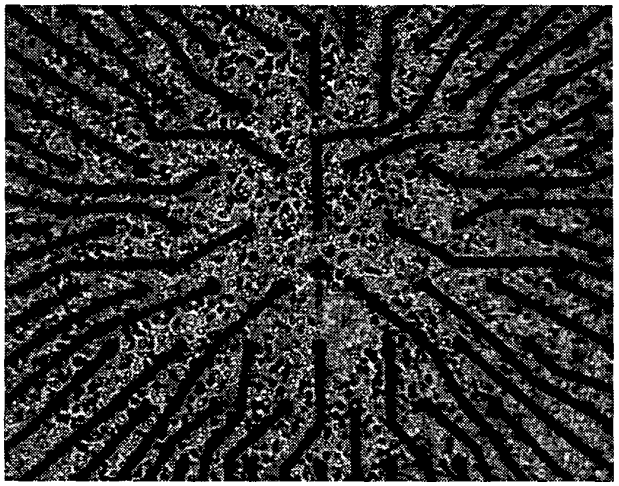

Fig.1. Picture taken from a culture 1 DIV (Day In Vitro), by using a Zeiss Axio digital camera connected to a Nikon F-301inverted microscope. For dimensions, see figure 2.

Processing spontaneous activity. The development of the spontaneous activity [7] was analyzed by evaluating the spike rate (spike/second) and the location (by the electrode number) of active channels over a period of ten minutes. A channel was considered active if the number of spikes was not less than 130 in ten minutes.

For analyzing connectivity between two electrodes the cross-electrode inter-spike-interval was calculated using forward and backward intervals [8]. This method was tested by simulated spike trains and proven correct.

III. RESULTS

The data used for the CNIH analysis were recorded from four cultures on four different MEAs with age ranges from 7 till 22 DIV (days in vitro) lying over MEAs. The distance between electrode pairs chosen was never smaller then 150 μm.

In total, 38 cross-neuron interval histograms (CNIH's) were derived. The CNIH has a bin width of 0.5 ms. For the classification two parameters were taken from a histogram: 1) the average value, named ANB (Average Number per Bin) over all bin amplitudes except the highest one 2) the ratio of maximum bin value and ANB (called AR, Amplitude Ratio).

An overview of the results is given in the scatterplot of fig. 3.

By examination of this plot and inspection of the histograms two extremes were chosen for presentation in figures 5a and 5b, as well as an intermediate examples, figure 5c.

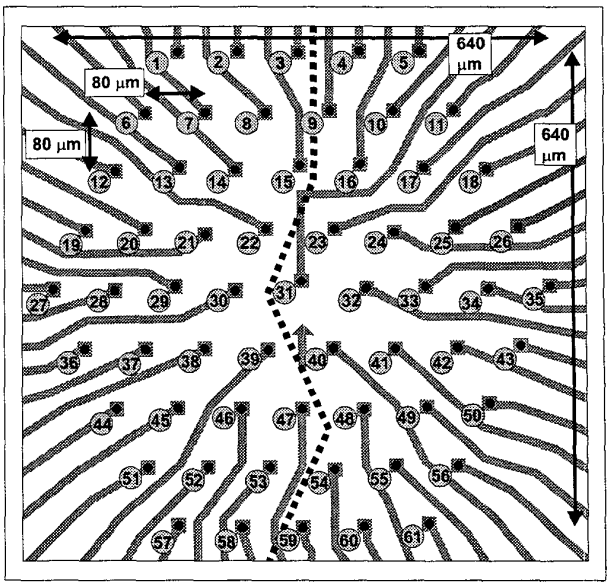

Fig. 2. MEA electrode layout. The numbering of the electrodes is indicated with gray circles, the dotted line indicates the separation of the electrodes to the left and right side connectors of the MEA

Clustering of the histogram data in figure 3, in two or more groups, is not yet possible. Nevertheless, a separation may be present at ANB=2.7 and AR=6 (two dashed lines in fig.3).

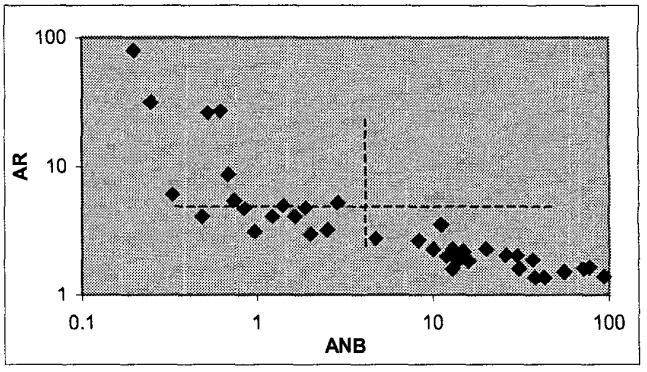

Fig.3 Scatter plot of parameters ANB and AR of CNIH's, both scales are drawn logarithmically. The two dashed lines indicate a possible separation in two groups.

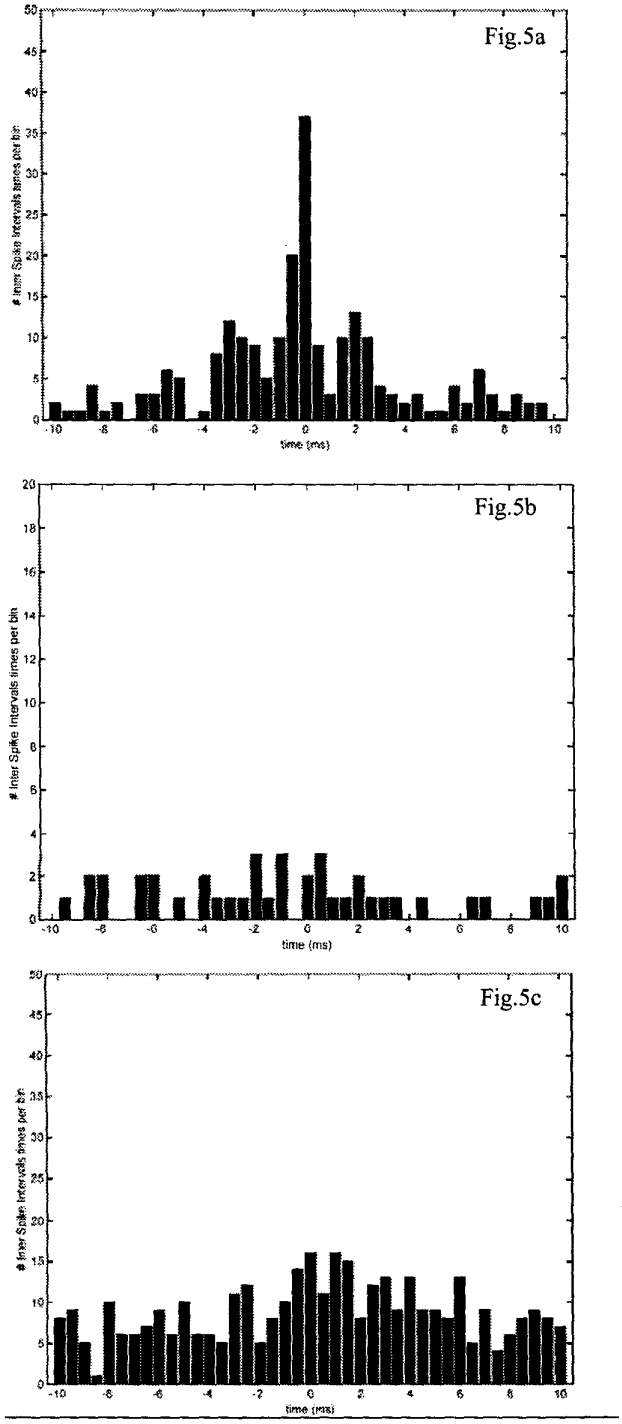

Fig.5 Histograms of cross-neuron-inter spike intervals against interval periods. Bin width is 0.5 ms. Three typical examples are shown: fig.5a) AR=7.80, ANB=4.74; b) AR=3.09, ANB=0.97; c) AR=1.84, ANB=8.69.

A special case, from the same culture, recorded after 7 DIV and 14 DIV, from electrodes 17 and 49 of MEA 195 (Fig2), are shown in figures 6a and 6b. It can be observed that the average peak, situated at 1 ms interval at DIV 7 (fig. 6a) has shifted to 1.5 ms at 14 DIV (fig. 6b).

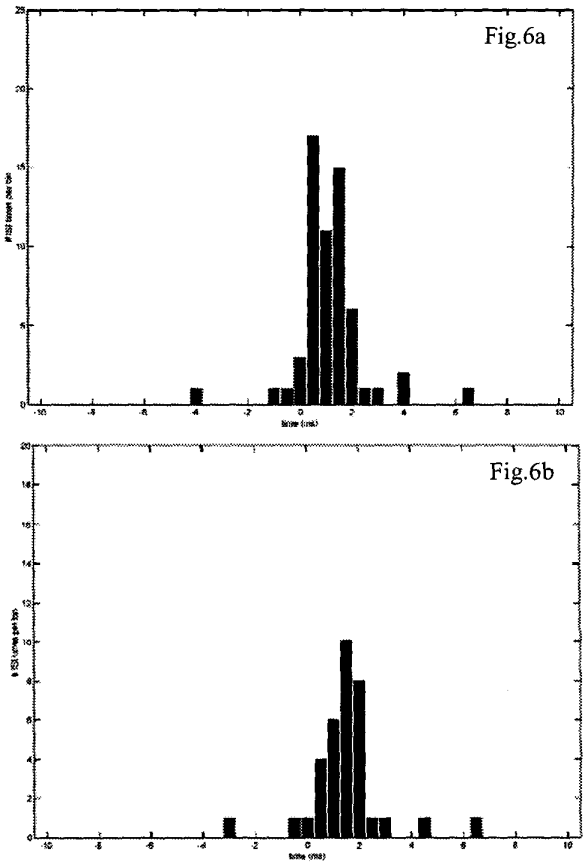

Fig.6 A special case: a) CNIH of electrodes 17 and 49 in a culture after 7 DIV, it has a clear average peak centered at 1 ms; b) CNIH relative to the same electrodes but after 14 DIV. The average peak is clearly shifted to 1.5 ms.

IV. Discussion

The histograms clustered to the right of the separation line at ANB=2.7 show a more or less flat shape that, combined with the low values of the ratio AR, may correspond to either non-connectivity between analyzed electrodes, or a very high number of pathways (many peaked histograms overlap and smooth out to one flat histogram). This result is similar to the conclusions drawn in literature for cross-correlation function analysis in the case of in–vivo recording in animal brain, and of simulation studies [2] [3] [4] [5] [6].

The presence of more or less sharply peaked histograms in the other group, left of ANB=2.7, indicates that this CNIH method can be used for the analysis of effective connectivity.
In the special case of fig.6a, b the data can be used to interpret increased traveling time of spikes between the two electrodes. Assuming constant propagating speed v, the distance versus travel time relationship is simply given by s=v*t, in which the shortest possible connection between the electrodes is equal to their distance (372 μm). Without considering any additional synaptic transition in this pathway it's possible to conclude that the minimum conduction velocities derived from the data is 0.74 m/s. Such a value is within the typical range of velocities for action potentials traveling through in unmyelinated fibers (0.5-2 m/s [9]).
The shift of 0.5 ms to the right for the peak in fig.6b could be a consequence of the formation of new pathways between the electrodes or because of the extension of the length of the axons, due to growth; in this case 0.5 ms corresponds with about 0.5 mm increase. This value is well within the range, as neurons may grow fast, they can reach average values of 1 mm extension per day in vitro [10].

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