Wave shape classification of spontaneous neuronal activity in cortical cultures on micro-electrode arrays

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Abstract- Dissociated embryonal or postnatal rat cortical cells were cultured onto multi electrode arrays (MEA's) with 61 electrode sites. They developed into networks and became spontaneously active after about one week in vitro. About 180.000 recorded action potential waveforms were sorted using several spike features and classified with a Mahalanobis distance sorting procedure. They were classified into six basic wave shapes.
Key words - Cortical neurons, MEA, cultured neuronal networks, spontaneous activity, wave shapes

I INTRODUCTION

Neuronal cultures from rat dissociated cortical cells can be cultured on Micro Electrode Arrays (MEA's, with 61 electrode sites, 12 μm diameter) on the bottom of a culture dish. The cells develop into networks which become spontaneously active after about 7 days in vitro (DIV). Our research program aims at the characterization of the spatio-temporal patterns of spontaneous spike activity and on the modification of these patterns by external stimulation and training. One part of the program is the classification of recorded waveforms. It can be expected that waveform depends on the position of a soma or axon with respect to the electrode site. Complete covering of a site by a soma is, electrically, quite different from partial coverage, or non-coverage. In this paper, we report on the classification of action potential waveforms into a spike wave shape database.

II. MATERIALS AND METHODS

A. Cortical neuron isolation and procedures:

Dissociated (Trypsin/EDTA) cortical neurons (neonatal rat cerebellum) were seeded onto patterned structures with a plating density of 5000 living cells/ mm^2 . Cells were allowed to adhere onto the surfaces during a time period of 4 hours. Samples were rinsed with NaCl (0.9%) solution to remove non-adherent cells. Neurons were cultured in R12 medium (DMEM/HAM's F12, Gibco) without serum. The cultures are stored in a CO_2 incubator with a constant temperature of 37° C, and a constant CO_2 level of 5%. The culture medium is refreshed half, 3 times a week.

B. Quantification and classification of spike waveforms

Electrode signals are amplified, filtered between 0.3 kHz and 6 kHz (first order) and captured by a 12 bit National Instruments PCI-6023E Data Acquisition PC-card. The input range as well as the sampling frequency is software con-

trolled by a Labview program. The real time data processing software reduces the data stream by rejection of data which does not contain bioelectrical activity. Artefact rejection is severe: if activity is measured at the same time in different channels, the waveforms are rejected. In each channel, the rms noise level is constantly monitored and determines the setting of a level detector to detect spike activity. The threshold is set at 6 times noise level (typically 7 μV rms).

Three spike features are the peak-to-peak amplitude Vpp, the width of the peak and the area of the peak. A fourth feature, called peak balance, distinguishes different shapes. It calculates the difference between the waveform area before and after the peak value. In literature, Vpp ranges between 50 and 350 μV , for electrode sites of 30x30 μm [1, 2].

Manual scatterplot classification

A method for classification is by plotting a selection of two or three spike features in a scatter diagram. This results in a 2- or 3-D graph with separate groups. The groups can only be assigned when there is enough spacing between the groups. Square shaped areas are drawn around the groups isolating the classes.

Minimum Mahalanobis distance classification

The classes can also be assigned using minimum Mahalanobis distance classification. First a selection of spikes must be manually classified and a set of spike features must be selected. The so-called 'training set' must be representative for all waveforms in the measurement. The training set is created by randomly selecting a number of spikes. From the manually assigned classes the mean (μ) and covariance (C) are computed. Next the spikes are classified by assigning them to the class with the smallest Mahalanobis distance. The Mahalanobis distance is calculated by:

$$D_{\text{Mahalanobis},i} = (\bar{x} - \bar{\mu}_i)' \cdot C^{-1} \cdot (\bar{x} - \bar{\mu}_i)$$

In this formula $\bar{x}$ is the spike feature vector, $\bar{\mu}_i$ the mean of the class and C the covariance matrix of the class. When the Mahalanobis distance between a spike and a class exceeds the rejection ratio threshold, this spike is not classified. So, unclassified spikes can remain after classification.

Classification finishing and checking

The still not classified spikes must be manually sorted afterwards. An unclassified group is classified afterwards with help of a manual scatterplot.

III. RESULTS

In two experiments with cultures from postnatal and embryonal rats about 180,000 spikes are measured. About half of the measurement data is further processed leading to spike classification. The classified spikes could be clustered in 6 basic waveforms or morphologies.

An example of a scatterplot after finishing the classification is shown in figure 1. This classification consisted of, first, a minimum Mahalanobis distance classification, resulting in

classes 1, 2 and 3. After this, class 4 was classified using manual scatterplot classification. The remaining spikes were classified with manual after sorting. The final step in classification is the clustering of the waveforms in 6 basic waveform groups. This clustering is per-

Figure 1. Top: Scatterplot after Mahalanobis classification based on two features, peak balance (vertical) and peak-peak amplitude (horizontal). Below: the waveforms of the four classes.

Figure 2. The 6 basic waveform groups, with the measurement number and number of spikes indicated in the lower left corner (x-axis: ms, y-axis: μV).

formed manually by the number of phases in the waveform and next by the width of the waveform. Representative examples of each of the 6 waveforms are shown in figure 2. The waveforms shown in figure 1 are clustered in waveform group 1 (class 2, 3 and 4) and waveform group 6 (class 1). In table 1 properties of the 6 classes are given: the number of measured waveforms, the peak amplitude ranges and the width range. This table shows that within each basic waveform there is a large variety in amplitude ranges and sometimes in width. This indicates that the 6 assigned basic waveforms are a rough representation of the originally classified waveforms.

Waveform	Number of spikes	1 st peak amplitude range (μV)	2 nd peak amplitude range (μV)	3 rd peak amplitude range (μV)	Width range (ms)
1	24115	-300 to -30	5 to 100		± 2
2	2242	10 to 100	-150 to -50	5 to 50	± 2
3	8998	20 to 250			1 to 1.5
4	1409	50 to 150			2.5 to 3.5
5	909	30 to 200			4 +
6	3554	40 to 100	-60 to -30		± 2

Table 1. Summary of data of 6 basic waveforms.

REFERENCES

- [1] Jimbo, Y.; Robinson, H.P.C.; Kawana, A.; 1998, Synchronised Activity by Tetanic Stimulation in Cortical Cultures, IEEE Trans on Biomed Eng; 45(11), 1297-1305.
- [2] Jimbo, Y.; Kawana, A.; Parodi, P.; Torre, V.; 2000, The dynamics of a neuronal culture of dissociated cortical neurons of neonatal rats, Biol. Cyb. 83, 1-20.