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A SYSTEM FOR 256-CHANNEL *IN VITRO* RECORDING OF THE ELECTROPHYSIOLOGICAL ACTIVITY OF BRAIN TISSUE

Miroslaw Zoladz

AGH University of Science and Technology, Faculty of Electrical Engineering, Automatics, Computer Science and Biomedical Engineering, Department of Measurement and Electronics, al. A. Mickiewicza 30, 30-059 Krakow, Poland (kmet@agh.edu.pl) (✉ zoladz@agh.edu.pl, +48 12-617-32-99)

Abstract

A measurement system for 256-channel *in vitro* recordings of brain tissue electrophysiological activity is presented in the paper. The system consists of the brain tissue life support system, Microelectrode Array (MEA), conditioning Application Specific Integrated Circuits (ASIC's) for signals conditioning, Digitizer and PC application for measurement data presentation and storage. The life support system keeps brain tissue samples in appropriately saturated artificial cerebrospinal fluid at a very stable temperature. The MEA consists of two hundred and fifty-six 40 µm diameter tip-shaped electrodes. The ASIC's performs amplification and filtering of the 256-field and action potential signals. The Digitizer performs simultaneous data acquisition from 256 channels 14 kS/s sample rate and 12-bit resolution. The resulting byte stream is transmitted to the PC via USB (Universal Serial Bus). Preliminary tests confirm that the system is capable of keeping the extracted brain tissue active (hippocampal formation slices) and simultaneously to record action potentials, as well as local theta field potentials with very small amplitudes from multiple neurons.

Keywords: multichannel measurement system, neurobiological measurements, application specific integrated circuits.

1. Introduction

Modern neurobiological experiments require multichannel readout systems for simultaneous *in vitro* extracellular signal recordings from many neurons. Such tests are run in order to find the answer to fundamental questions, such as how does a complicated neuronal network encode and process information [1], or how to build a different kind of neural prosthesis in foreseeable future [2]. However, measurement systems used in these frontier neurobiological experiments must provide a high spatial resolution (in the order of tens to hundreds of µm depending on the type of neuronal network) and should consist of hundreds or even thousands of recording channels [3].

In this paper we describe the complete 256-channel recording system and its first neurobiological tests as a proof of concept. The description covers all critical elements of the system required to obtain valid neurobiological data, i.e. an appropriately selected microelectrode array dedicated to conditioning an integrated circuit, a custom-made acquisition system and a tissue life support system equipped with inspection optics.

2. Electrophysiological techniques

Electrophysiological recording methods can be divided into *in vivo* and *in vitro*. In the ***in vivo*** method, electrodes are placed in the brain of a living animal. In the ***in vitro*** method, neural activity is recorded from brain tissue samples extracted from the brain or a cultured neuronal network [4]. The advantage of the *in vivo* method is that neural networks are examined in

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their natural environment. The advantage of the *in vitro* method is that the test can focus on a small fragment of the neural network and selected chemical agents can be easily applied. Also, measuring or stimulating electrodes can be placed exactly in the desired location [5]. Electrophysiological recording methods can also be divided into intracellular and extracellular ones. The **intracellular method** measures the voltage and/or current across the membrane of an individual cell. To make such a measurement, the electrode tip has to be inserted into the cell interior. The amplitude of the signal recorded by means of such methods can reach more than 100 mV. Unfortunately, this method demands very precise positioning of the electrode if vertebrate nerve fibres have to be investigated and only few places on the cell membrane or few cells can be measured simultaneously. The **extracellular method** measures voltage in close proximity of the cell. Both Intracellular and Extracellular methods can be applied in both *in vivo* and *in vitro* manner. There are two types of extracellular signals: Action Potentials and Local Field Potentials (Fig. 1). **Action Potentials** are generated by single neurons, which are very similar to signals recorded with intracellular method. The main differences are polarity and amplitude, which, in the case of extracellular recordings, depend on a neuron type and neuron-to-electrode distance, and usually do not exceed a few mV, with average values of a few hundred μV. The action potential is a pulse consisting of two phases with opposite polarities. The initial (negative) phase lasts usually 0.5 ms whilst the second (positive) resting phase lasts usually 1.5 ms. The typical frequency of action potentials usually does not exceed 100Hz. **Local Field Potentials** (LFPs) are electric fields generated by the activity of multiple neurons in the neighbourhood of the electrode. As the result, the amplitude of the LFPs is usually higher than 1mV. The frequency of LFPs usually does not exceed 100 Hz [6,7].

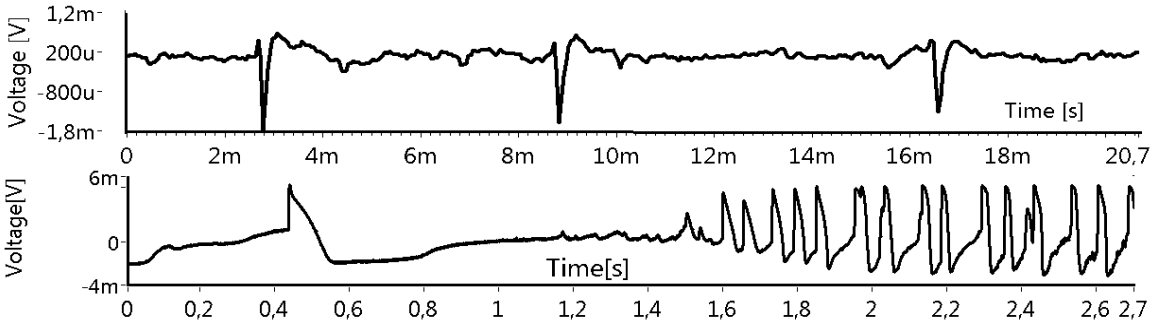

Fig. 1. Exemplary Action Potentials (top chart) and Local Field Potentials (bottom chart). (Measurements taken from the presented system).

2.1 Single-point recording

The extracellular recording setup is based on the use of a microelectrode, i.e. an electrode with a micrometre-sized tip. Two types of the microelectrode can be distinguished here: **metal electrodes** and **glass micropipettes**. Metal electrodes have the form of a thin metal wire (typically platinum or tungsten) insulated with a glass excluding electrode tip. Glass micropipettes are usually filled with a salt solution to make them conductive. Depending on the tip size, action potentials from a single cell (smaller tips) or multiple cells (bigger tips) can be recorded. They are known as single-unit and multi-unit recordings respectively [4,8]. The impedance of the electrode depends on the tip size and electrode type. The resistance of an electrode is proportional to the tip size. Metal electrodes of a given tip size have lower impedance than glass micropipettes with the same tip size (measured at 1KHz). Generally, the lower the impedance, the better the SNR (Signal-to-Noise Ratio). However, at low frequencies, metal electrodes suffer from high impedance and high noise, therefore glass micropipettes are more suitable.

2.2 Multi-point recording

Although investigations of neural networks can be carried out by means of single-point recordings, multi-point recording is a much faster and reliable method. Many hardware setups have been proposed for multi-point recording by using metal electrodes and glass micropipettes [9,10]. The key issue in such setups is the electrode number and positioning. For multiple recordings, metal electrodes or glass micropipettes are mounted on a headstage (Fig. 2). Each electrode is mounted inside a telescopic tube. This type of attachment allows

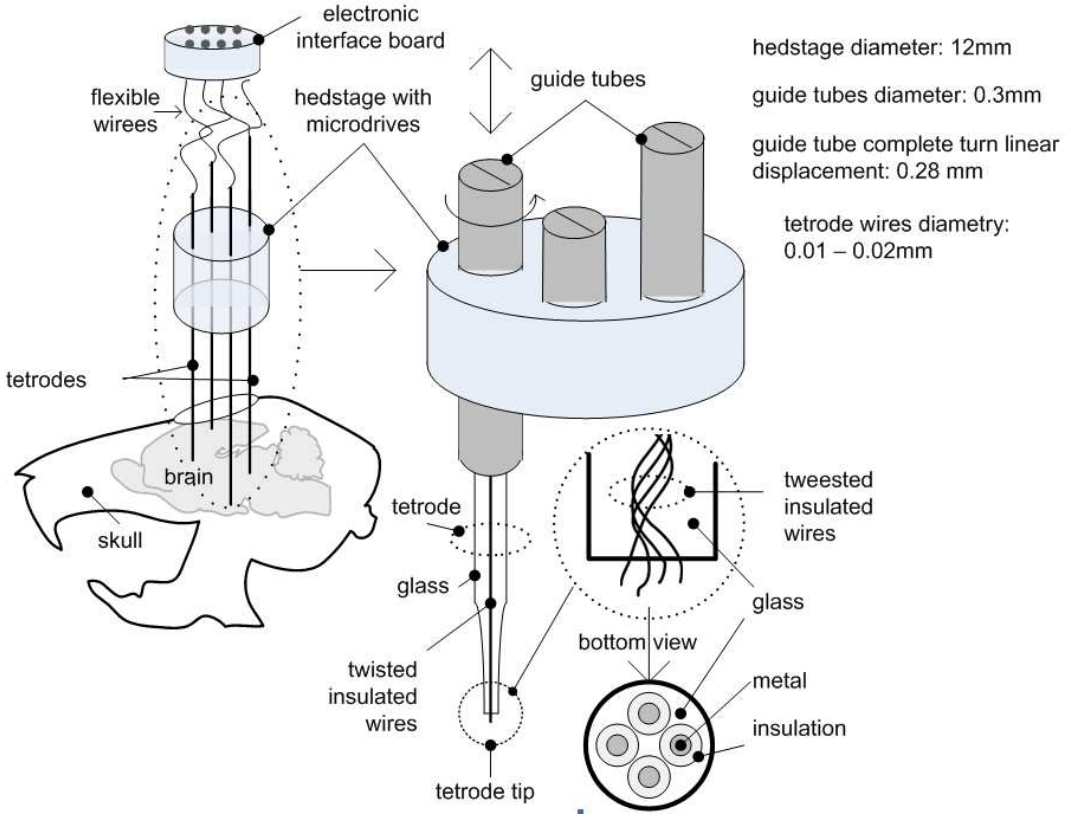

Fig. 2. Scheme of setup for *in vivo* multi-point neural activity recording by using tetrodes. Exemplary dimensions of key elements are taken form [10]. (The author's drawing).

independent positioning of each electrode. The other independent method of multipoint recording is to use multichannel electrodes called tetrodes [10] (not to be confused with multielectrode arrays). Essentially, this is the bundle of insulated wires glued together. A single wire diameter is in the order of several micrometers. The insulation on both ends of the wires is stripped. All wires record the activity of the same populations of neurons. Recordings taken from an individual neuron are different for each wire, mainly due to different wire–to-neuron distances. This phenomenon can be used to discriminate signals from selected neurons and for determining the neuron's position. In spite of the fact that many valuable recordings conducted by using the above described techniques have been reported, there are certain drawbacks of the approaches. These include: difficult implementation (especially electrode positioning), significant size of the electrode headstage, relatively large distance between electrodes and a relatively low number of simultaneously active electrodes. Multipoint recordings by using multiple electrodes are additionally hampered (for *in vitro* methods) due to the necessity of using inspection optics for the electrode positioning, which is difficult to implement (Fig. 3). Advances in microfabrication methods, especially in MEMS (Micro Electro-Mechanical Systems) technology, have contributed to the design of **microelectrode arrays** (MEAs). They comprise multiple electrodes with size and spacing of the order of tens

of micrometers. One can distinguish two classes of MEAs considering their usage. These are: MEAs for *in vivo* and *in vitro* experiments. **MEAs for *in vivo*** recordings are **penetrating electrodes**. Fig. 4 shows a photo and scheme of an exemplary penetrating MEA. They consist of one or more shanks, each containing one or more electrode sites. In penetrating MEAs, the amount of shanks, number of electrode sites, spacing, size and configuration (e.g., stereotrode/tetrode/octrode) can vary. **MEAs for *in vitro*** experiments are usually fabricated on glass substrates using standard microfabrication technologies. Fig. 5 shows an example of an MEA for *in vitro*. The shape of a single electrode site can be planar or tip shaped (Fig.5). MEAs with **planar electrodes** are used for cell/tissue culture applications.

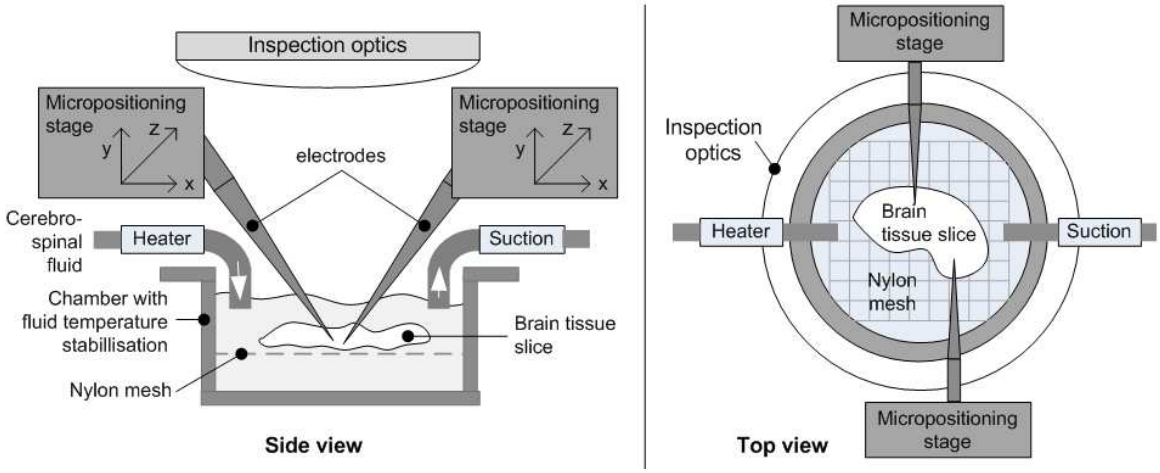

Fig. 3. Scheme of the setup for *in vitro* multipoint neural activity recording by using single electrodes. (The drawing by the author).

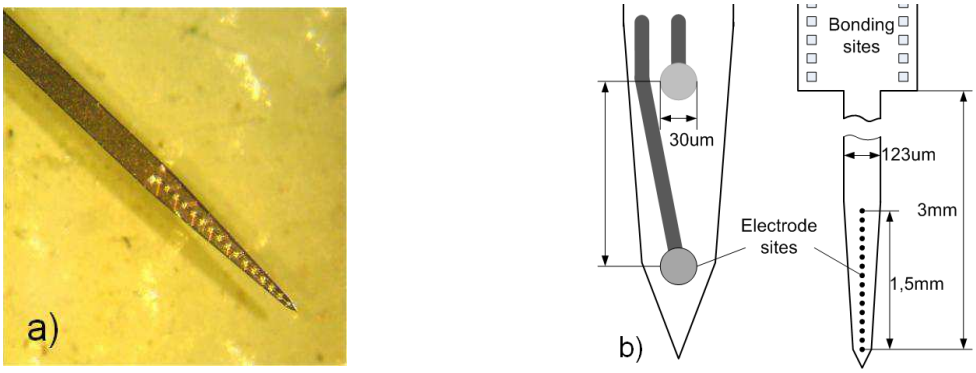

Fig. 4. Photo (a) and scheme (b) of NeuroNexus penetrating MEA with 1 shank and 16 electrode sites. (Photo and drawing by the author).

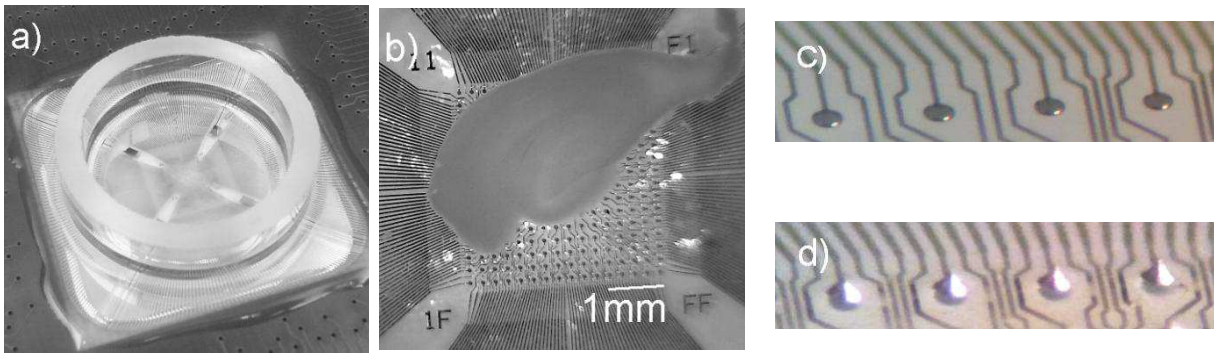

Fig. 5. Multi-electrode array for *in vitro* experiments realised on glass substrate. a) MEA mounted on PCB and surrounded by glass fringe creating a chamber for liquid. b) MEA with hippocampus tissue slice. c) planar electrodes. d) tip shaped electrodes. (Photos taken from the presented system).

Planar electrodes are not well-suited for acute experiments where a brain tissue slice is extracted from the brain and placed on the electrode array, due to the degradation of external neuron layers caused by mechanical extraction of the brain tissue slice. This increases the distance between healthy neurons and electrodes degrading the quality of electrical signal recordings. A solution to this problem is the use of **three-dimensional** non-planar (spiked) tips. These are able to penetrate the tissue slice and to collect signals from healthy neurons.

3. Measurement system

3.1 System architecture

The architecture of the system and photo of the conditioning board are presented in Fig. 6. Signals from brain slices are collected by using 16x16 MEA electrodes connected to the Conditioning Board equipped with 4 Conditioning Modules. The Conditioning Module amplifies and filters signals from 64 electrodes. It also reduces the number of signals from 64 to 1 by using an analogue multiplexer. The mentioned functionality is realized by a specially designed Application Specific Integrated Circuit (ASIC). The Supply Module provides low noise supply and reference voltages for the Conditioning Module. The Control Module generates control signals for the analogue multiplexer. Analogue-to-digital signal conversion is performed by the Digitizer Board, which also transmits data to the PC. Data acquisition, display and storage are performed by LabVIEW (National Instruments).

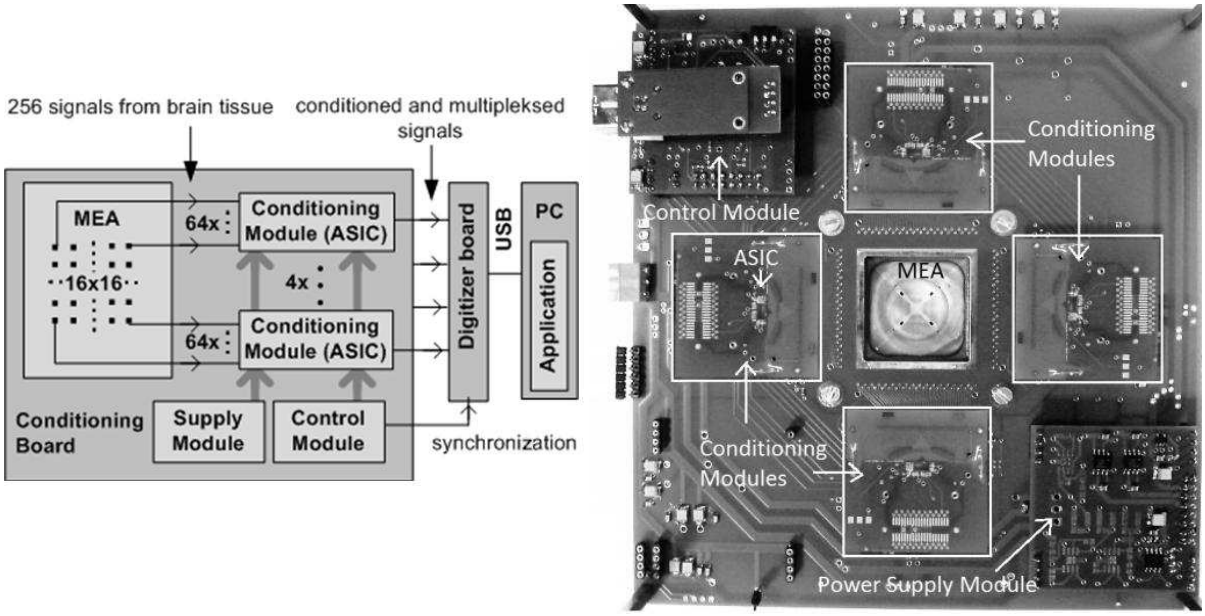

Fig. 6. Architecture of the system for *in vitro* recording and photo of the Conditioning Board.

3.2 Conditioning module

Neural signals recorded with MEAs require specific signal conditioning. The conditioning circuit path is presented in Fig 7. The amplitude of the neural signals reaching the electrodes depends on brain tissue resistance and the distance between the electrode and the neuron (R_1,R_2), and spans from tens of μV to 1 mV. There is also a DC voltage (V_{off}) on the electrode-tissue interface of the order of mV. The individual impedance of each electrode (Z_{el}) spans from hundreds of kΩ to a few MΩ. The frequency bandwidth enabling appropriate reproduction of the original neural activity spans from 1 Hz to a few kHz. As seen from the above, the conditioning circuit has to have an amplifier with: DC offset cancellation, a minimum voltage gain of x100, input referred noise of the order of dozens of microvolts,

controllable band-pass cut-offs from the Hz to kHz range, input impedance of the order of a few MΩ and high uniformity of parameters between all channels. Such an amplifier can be easily built with discrete elements, but for a system comprising of 256 electrodes, such an approach is impractical because of the large volume occupied by electronics, lack of reliability and high cost. The alternative for a conditioning system based on discrete elements is to employ a VLSI (*Very Large Scale Integration*) process to fabricate ASICs for multichannel signal conditioning. To fulfil the above requirements, a 64-channel ASIC has been designed for neurobiological signal conditioning [11]. The chip (Fig. 8) was fabricated with 180 nm CMOS technology, occupying 11,5 mm^2 (5mm x 2,5mm) of the silicon area and consuming 15 mW power from a 1.8 V supply voltage. The ASIC consists of 64 conditioning channels and an analogue multiplexer (Fig. 9). The conditioning channel consists of an AC coupling circuit for DC offset cancellation, a low noise preamplifier with high input impedance, a band-pass filter with cut-off frequency control and a front-end amplifier for voltage gain. The **AC circuit** consists of an RC high-pass filter with a PMOS transistor (MR0) acting as a resistor. Values of the C_0 and *MR0* components have been selected to

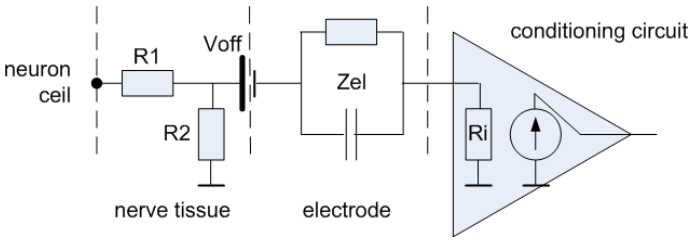

Fig. 7. Circuit model displaying the connection between the neuron and conditioning circuit.

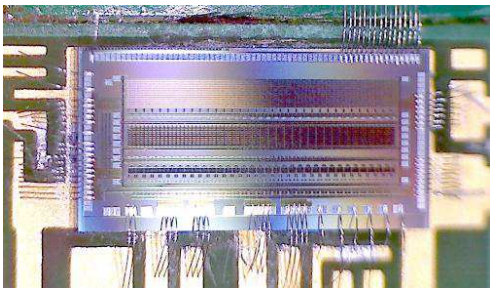

Fig. 8. Multichannel ASIC for neurobiological signal conditioning.

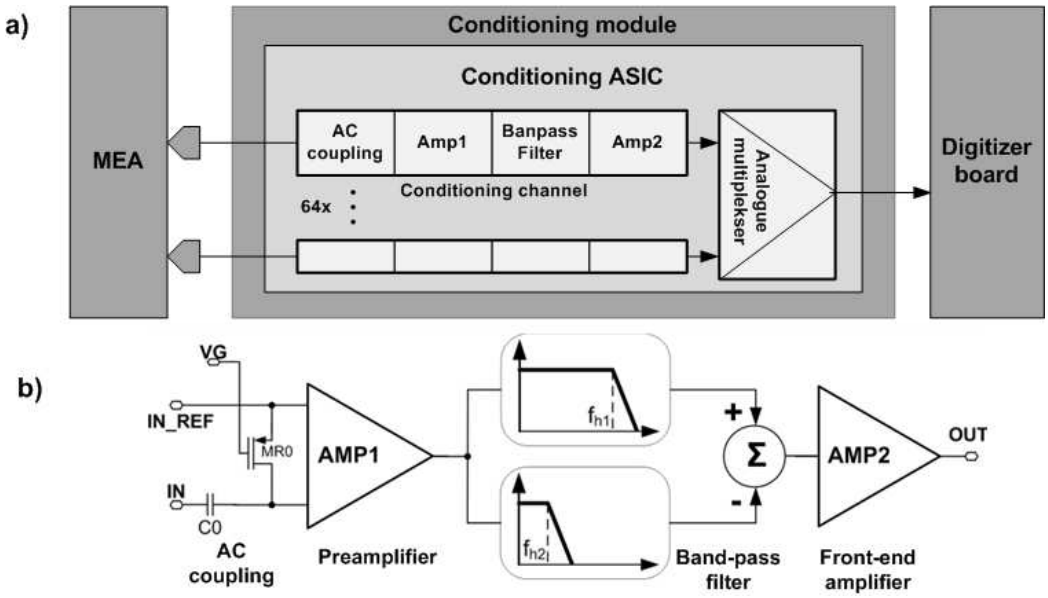

Fig. 9. Conditioning ASIC: a) architecture, b) simplified scheme of the conditioning channel.

allow recording of input signals starting from as low as 1 Hz , to minimize the noise generated by the *MR0* element, and to minimize the area occupied by the AC coupling circuit. The controllable resistance, the *MR0* transistor, works in the linear regime. It is based on six PMOS transistors connected in series (each with the channel dimensions of $W_{MR0}/L_{MR0} = 0.4\mu m/50\mu m$). The **preamplifier** is based on a differential amplifier and provides 38 dB of voltage gain. In order to limit the 1/f noise contribution of the stage, the input PMOS transistors have been selected. After the noise optimization, its channel

dimensions were set to W/L = 175 µm/0.5 µm while the current sourcing these transistors was set to 43 µA for each of the input transistors. The **Band-pass filter** is based on the architecture proposed in previous literature [12]. The band-pass frequency response of this filter is formed thanks to the subtraction of the two output signals of the low pass filters. By changing the upper cut-off frequencies of the low-pass filters, the final band can be modified. The band-pass filter consumes 20 µW of power and has a 0.7 dB voltage gain. The **front-end amplifier** is used to add a voltage gain and to form a low impedance buffer for the sample-and-hold circuits, which is the first stage of the analogue multiplexer. It adds about 6 dB of the voltage gain and consumes about 38 µW of power. The **basic ASIC parameters** are:

- globally controlled low cut-off frequency range: 1 Hz – 60 Hz,
- globally controlled high cut-off frequency range: 3.5 kHz – 15 kHz,
- average voltage gain: 110V/V,
- input referred noise 6 μV_{RMS} for 20 Hz – 6.5 kHz pass-band (spike recording)
- 11 μV_{RMS} for 1 Hz – 6.5 kHz pass-band (LFP recording),
- lower cut-off frequency spread: 4.4 %,
- high cut-off frequency spread: 1.8 %,
- voltage gain spread: 4.4 %.

3.3 Digitizer

Data flow. The Digitizer (Fig. 10) converts conditioned signals from the analogue to digital domain and sends the data to the PC. It consists of an A/D converter, CPLD (Complex Programmable Logic Device) and an FIFO/USB converter module. The CPLD synchronizes data flow between the Conditioning Module (multiplexer switching), A/D converter and FIFO/USB module. Digitizing and transmission of a single voltage sample from a single conditioning channel is done in the following steps. First, the analogue multiplexer switches to the next conditioning channel. Then, A/D conversion takes place and its result (12bits) is stored in the register. Finally, the content of the register is written into the FIFO in two steps, 4 MSB and 8 LSB sequentially and is received by the PC application.

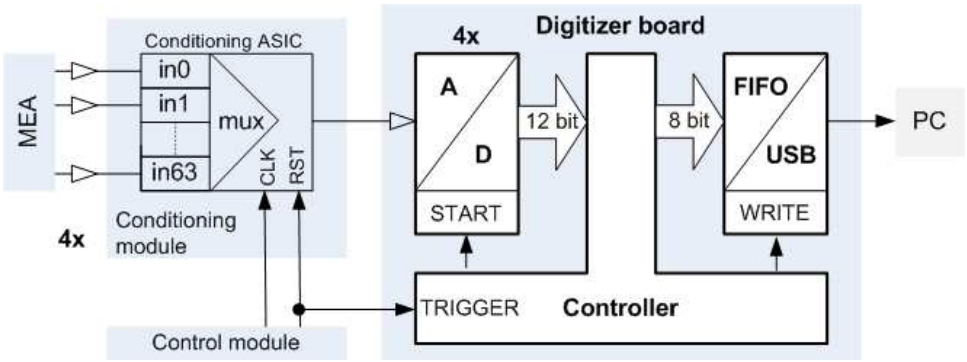

Fig. 10. Digitizer block diagram.

A/D converter sampling rate. 14 kS/s sampling rate of the voltage signal from a single conditioning channel has been assumed sufficient to reproduce the shape of action potentials. Taking into account the number of conditioning channels that have to be digitized simultaneously (64) and the 2 clock cycles necessary for multiplexer reset, a 1MHz multiplexer switching frequency and 1MS/s A/D conversion rate have been selected.

A/D converter resolution and input range. There are many action potential detection methods proposed in literature. Some of them [13,14] use the SNR to quantify the effectiveness of the methods. Accordingly, the SNR has been adopted as a determinant of the quality of the signal acquisition method. The general formula for the SNR is:

$$SNR = 10 \cdot \log_{10}\left(\frac{\sigma_s^2}{\sigma_n^2}\right), \quad (1)$$

where σ_s^2, σ_n^2 are variances of the signal and noise correspondingly. The variance of the resulting noise depends on the noise of the output of the conditioning channel (σ_c^2) and quantization noise of the A/D converter (σ_q^2). As the conditioning channel and A/D converter are independent blocks, the resulting noise variance is determined by the following equation:

$$\sigma_n^2 = \sigma_q^2 + \sigma_c^2. \quad (2)$$

Noise variance of the output of the conditioning channel depends on electrode noise variance (σ_e^2), input referred conditioning channel noise variance (σ_a^2) and conditioning channel gain (α) and is determined by the following equation:

$$\sigma_c^2 = \left(\alpha^2 \cdot (\sigma_a^2 + \sigma_e^2)\right). \quad (3)$$

Quantization noise variance depends on the A/D converter voltage resolution (q) and is determined by the following equation:

$$\sigma_q^2 = \frac{q^2}{12}. \quad (4)$$

To avoid affecting the SNR significantly, it has been assumed that the standard variation of the A/D converter quantization noise should be 10 times smaller than the standard variation of the output of the conditioning channel ($10 \cdot \sigma_q < \sigma_c$). Taking into account the above condition and equation 4, the sufficient voltage resolution of the A/D converter can be calculated. It equals 0.69 mV for the noise variance at the output of the conditioning channel equal to 2mV (value determined experimentally). The sufficient input voltage range of the A/D converter can be calculated assuming the maximum amplitude of the action and field potentials (which equal 2mV), and taking into account the conditioning channel gain, the DC level distribution and the sufficient A/D converter voltage resolution calculated earlier. It equals +/- 0.25V. Subsequently, the A/D converter resolution can be calculated taking into account sufficient input voltage range and sufficient voltage resolution. It equals 10 bits.

Selected A/D converter. In order to fulfil the above requirements, the AD7492 A/D converter has been selected for the analogue signal digitization. The most important features of the converter with respect to the requirements are: built-in sample-and-hold circuit, resolution equal to 12 bits, voltage range equal to 2.5 V, maximum conversion time equal to 880 ns, no pipeline delays, built-in internal clock generator and voltage reference.

A/D converter – analogue multiplexer synchronization is presented in Fig. 11. When conversion starts, the Sampling Capacitor (C_{sh}) is disconnected from the input signal (S_{sh} switch) (Fig. 11.a). After the conversion period (t_{conv}), C_{sh} is connected again to the input signal until the start of the next conversion. For proper conversion, C_{sh} must be charged to 0.5 LSB (least significant bit) of its final value when the conversion phase starts. Due to the impedance of the multiplexer output buffer and parasitic capacitance of the PCB path connecting the converter with the multiplexer (Fig. 11.b), a certain time is necessary to settle the new voltage value stated on the input of A/D converter (Fig. 11.c). It follows that to guarantee the settled voltage at the A/D converter input during sampling time, conversion should be started simultaneously with multiplexer switching. Fig. 12 shows the time Diagram of the conversion and transmission of a single voltage sample from a single conditioning channel no. 1.

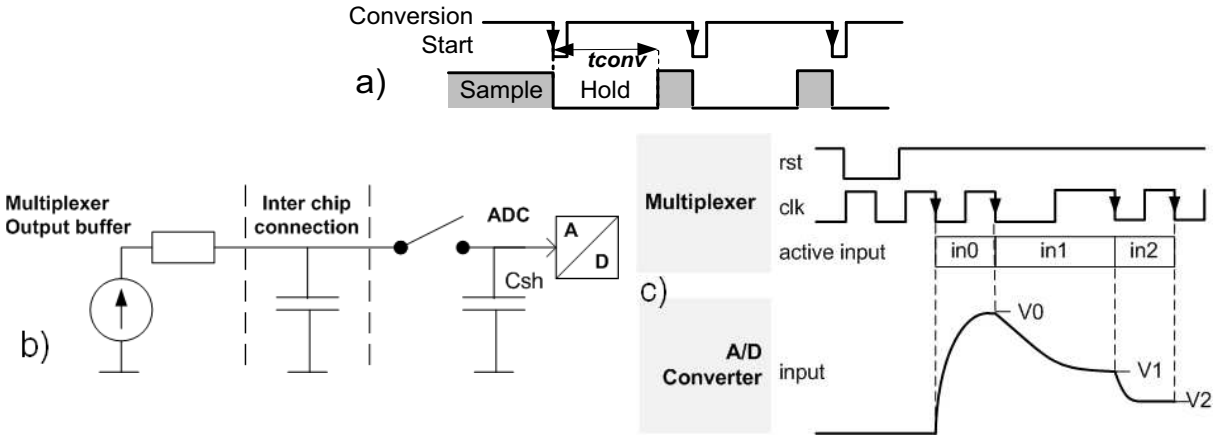

Fig. 11 a) Time diagram of A/D converter Sample & Hold circuit operation, b) Equivalent electric model of multiplexer output to A/D converter signal path, c) Time diagram of A/D converter input voltage switching.

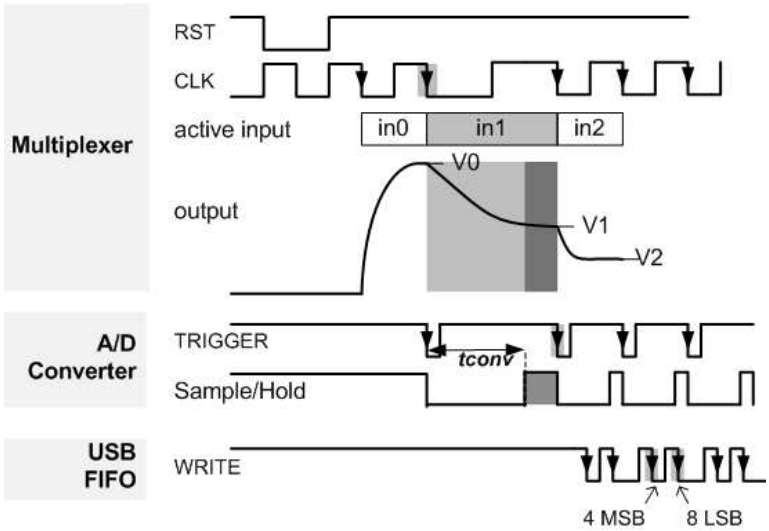

Fig. 12. Diagram of the conversion and transmission of a single voltage sample from a single conditioning channel with respect to time.

3.4 Multielecrode array

We thoroughly analyzed commercially available MEAs and details of the planned neurobiological experiments and decided to use a 256-electrode array offered by Qwane Bioscience (Fig. 5). We chose electrodes with 3D shaped tips, which have a relatively low impedance (about 300 kΩ at 1 kHz) and are very suitable for testing on brain slices. The chosen electrode pitch is 200 μm, single recording site diameter is 42 μm while its height is (50–70) μm.

4. Experimental setup

The experimental setup sketch is presented in Fig. 13. Male Wistar rats weighing (100–150) g were anaesthetized with diethyl ether and decapitated. The brains were removed and placed in oxygenated, cold (3–5^{o}C) artificial cerebrospinal fluid (ACSF). Transverse slices (500 μm) were made from both hippocampi using a tissue slicer (Stoelting Co.). Slices were preincubated in oxygenated ACSF at the temperature of 20^{o}C for 1 h after the dissection. Next, slices were transferred into the MEA and were delicately fixed in position with nylon mesh. To keep the brain slices animate, one has to provide them with artificial cerebrospinal

fluid appropriately saturated (with a proper ionic composition and nutrient content). Additionally, there is a strong demand for keeping the fluid temperature at 35°C with high precision, i.e. ±0.25°C. The fluid is delivered to the chamber via a valve (fluid flow speed control) and preheated (fluid oversaturation elimination). The bottom of the chamber which contains the MEA is made of glass, while the chamber sides and flange are made of silicone. The excess liquid is removed by suction from the chamber with a tube placed opposite to the liquid inlet side. The temperature required is maintained by heating the MEA glass substrate. The substrate is warmed up by a heating element with the use of a brass block improving temperature stabilization and homogenous heat distribution. The correct interpretation of the measured signals requires establishing their origins, i.e. the places on the tissue from which they were collected. For this purpose, a camera with proper optics is mounted on the chamber. The tissue is illuminated from below through a glass rod placed in a hole in the brass block. Dedicated connectors combine the MEA printed circuit board (PCB) and the main board PCB equipped with integrated circuit modules. Local theta field potentials accompanying a single unit activity, characteristic for the hippocampal formation maintained in the *in vitro* conditions were evoked by the perfusion of the slices with 50 μM carbachol (chilnergic receptor agonist) dissolved in the ACSF. Fig. 14 shows the photo of the experimental setup.

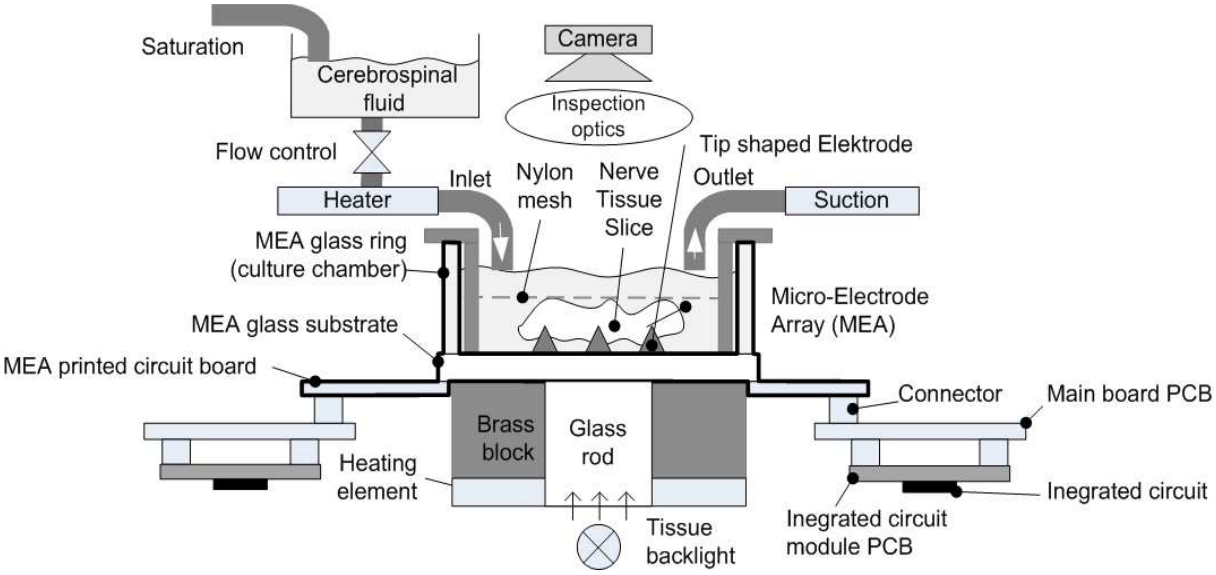

Fig. 13. Sketch of experimental setup for multichannel in vitro multichannel neural activity recording.

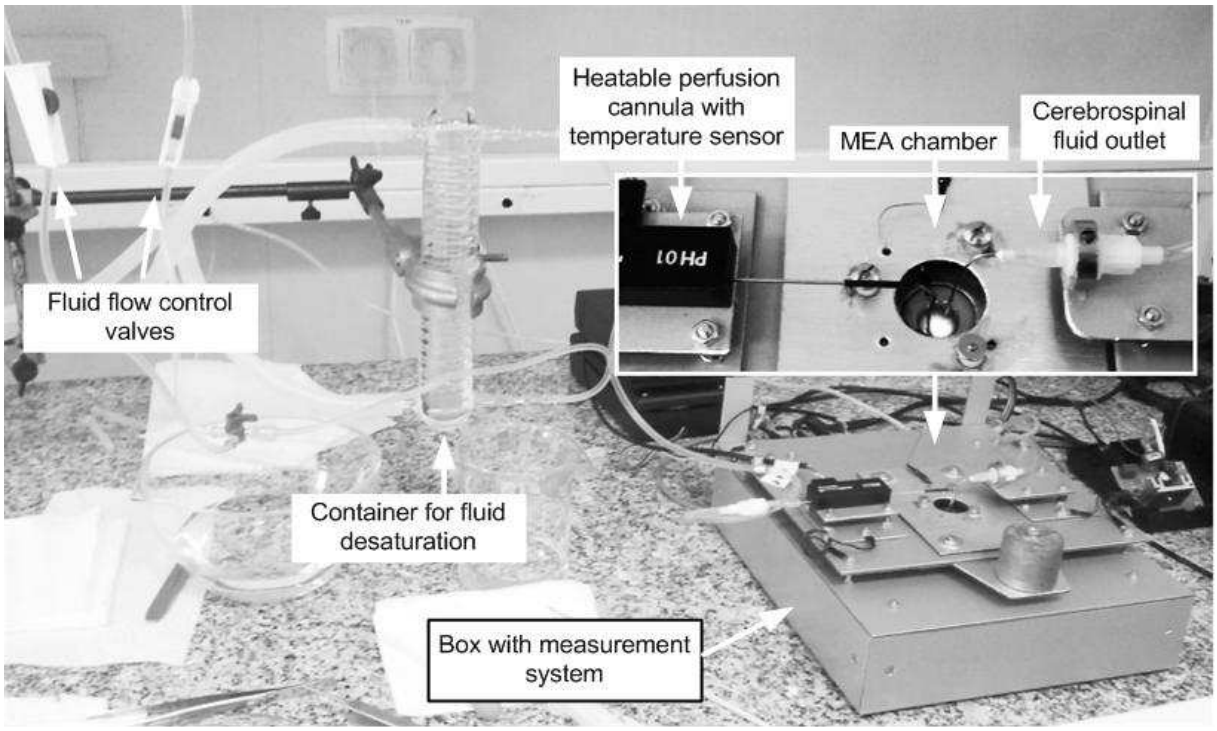

Fig. 14. Laboratory setup for multichannel *in vitro* multichannel neural activity recording using an MEA.

4. Preliminary results

Transverse hippocampal formation slice preparation has been used in order to test the experimental setup. A characteristic LFP pattern occurred spontaneously in the hippocampal formation is theta activity. *In-vitro*-recorded-theta rhythm consists of regular, rhythmic, almost sinusoidal waves in the narrow band frequency range of 8-12 Hz **[9]**. Fig. 15 shows single epileptic discharges, which are later synchronized in the theta rhythm.

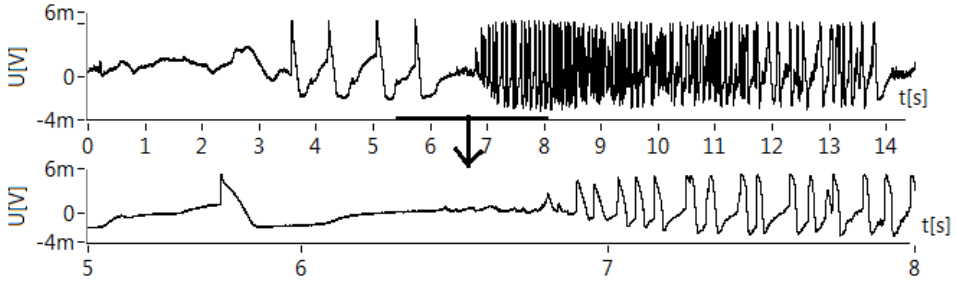

Fig. 15. Single epileptic discharges, which are later synchronized in the theta rhythm.

Such a synchronization confirms that the brain tissue is in good condition, hence it confirms that the experimental stand is capable of providing proper conditions for neurons to remain active and to generate physiological pattern of oscillations. Recordings of different local field potential activity acquired from five different electrodes (Fig. 16) confirm that the system is capable of conducting simultaneous multichannel recordings.

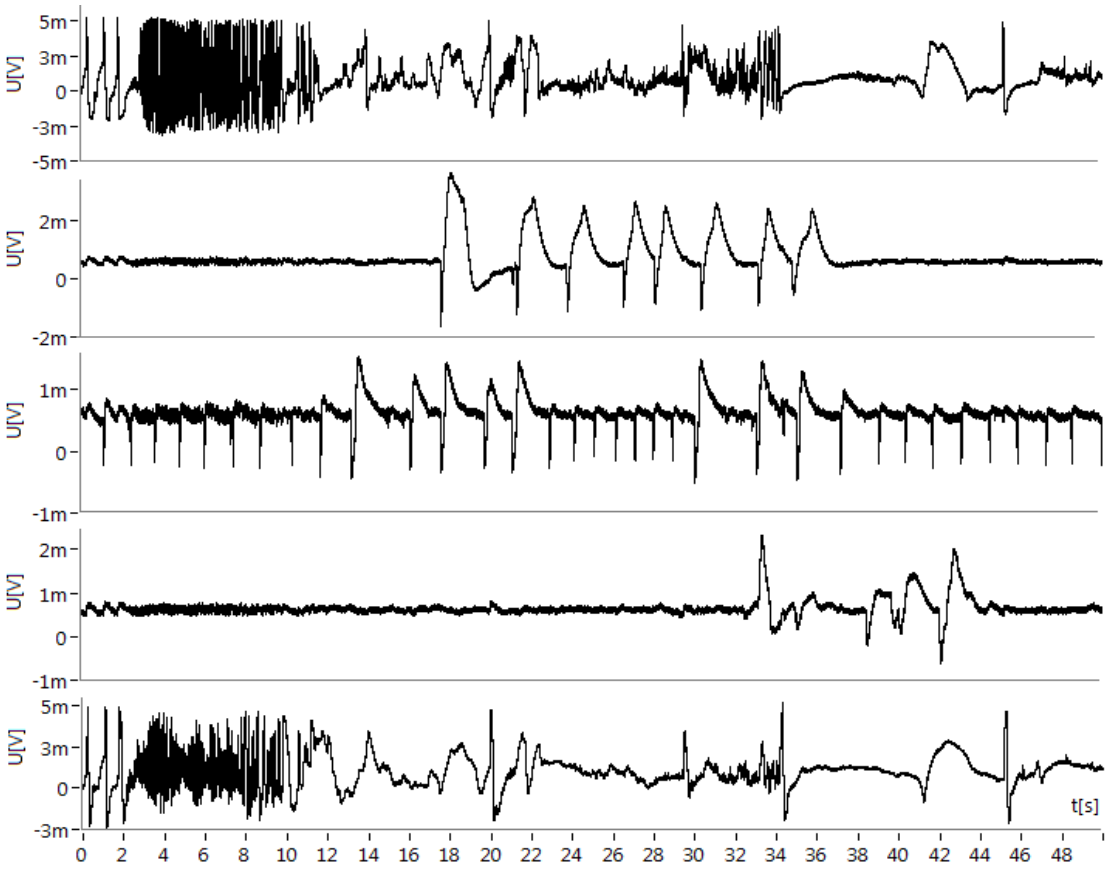

Fig. 16. Local field potential activity recorded simultaneously from five different electrodes.

Recordings of a single-unit activity superimposed on local field potential activity (Fig. 17) prove that the measurement setup is capable of recording signals within the expected range of frequencies, i.e. from single Hz to few kHz.

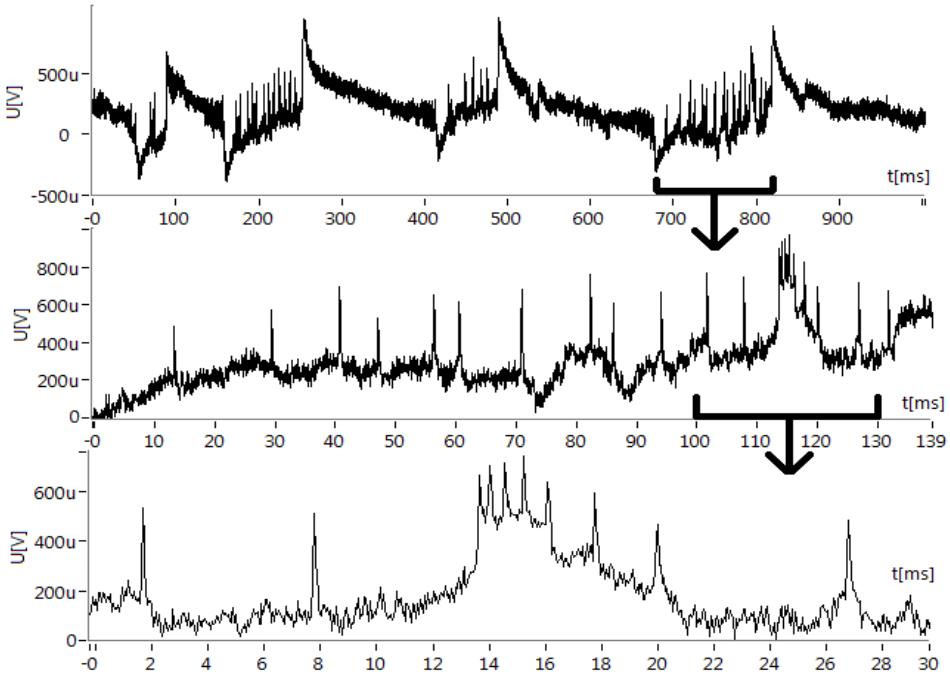

Fig. 17. Action potentials generated by a single neuron superimposed on local field potentials.

5. Summary

The paper presents the first detailed and systematic description of a complete system for recording an *in vitro* action and local field potentials from 256 electrodes. The system allows recording signals from relatively large areas of brain tissue with a resolution at the level of individual neurons. A special feature of the system is low power consumption of signal conditioning ASICs, and that is of paramount importance in the construction of systems to record neural signals from a thousand or more electrodes.
The critical elements of the system are:

- properly selected MEA (electrode shape, diameter and spacing),
- complex 64-channel ASICs with an AC coupling circuit, high impedance amplifier and band-pass filter in each channel,
- acquisition system capable of digitization and transmission of the 256 signals with 14kS/s sampling rate and 12bit resolution,
- tissue life support system equipped with temperature control and artificial cerebrospinal fluid supply,
- optical camera for tissue sample inspection, i.e. signal origin determination.

The system may be used for:

- determining the topography of theta-field potentials within the hippocampal formation and other brain structures maintained *in vitro* (e.g. entorhinal cortex, posterior hypothalamic area)
- calculating the amplitude and phase laminar profiles of hippocampal or entorhinal theta rhythmic activity
- calculating current source density profiles of hippocampal or entorhinal theta rhythm
- examining a single unit activity (action potentials) of hippocampal neurons during simultaneously recorded theta activity performed at different loci of hippocampal formation at the same time.

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