A real-time setup for multisite signal recording and processing in living neural networks

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***Abstract*— Bioelectronic systems using MultiElectrodes Arrays (MEAs) make possible new experiments, based on long term analysis of neural system at the network level. In this paper we present an experimental platform that processes signals from a 60 electrode MEA and runs event (spike, burst, and stimulus artifact) detection and statistic in real-time. The minimum processing task (storage and monitoring) takes 25 µs and the full one takes 60 µs. The data is available for online and offline analysis: on the computer's screen, on the computer's hard disk, and on the TCP/IP layer.**

I. Introduction

The evolution of technology provides new methods for better understanding biological mechanisms. Among these innovating technologies, MEAs (MultiElectrodes Arrays) come as a solution for recording activity from large number of neurons [1]. A MEA consists in a matrix of planar electrodes (60 or even more) placed on a plan on which living cells can grow and develop for a long time (several months). The electrodes provide an extracellular access to the culture at the network level. This multisite access associated to the long lifetime of cells make possible long term experiments in large networks, and the study of plasticity and learning rules in neural networks [2-4].

But MEA advantages are associated to problems. Extracellular measurements provides signals with small amplitudes (10~100 µV) and high noise level (up to 1 mV in low frequencies). The multisite acquisition is also associated to high data flow.

To deal with this low signal-to-noise ratio, amplifying and filtering operations are critical. Real-time data processing is also necessary to avoid data loss at least for data storage. So, expansion of MEA-based experiments is strongly dependent on efficacy of the associated electronics.

The most resource demanding experiment explores the closed-loop hybrid neural network [5-10], in which real-time processing functions are mandatory, in addition to the data storage. In such an experiment, the neural network is composed by two parts, one living and one artificial. The living part is constituted by neuronal and glial cells cultured on the MEA. The artificial part can be a software or a hardware system. These two parts communicate bidirectionally in a closed-loop, and interactions only have a sense as a true network, if the responses from the artificial part are given in biological real-time. This implies that raw data acquired from the culture must be processed in real-time. Depending on the kind of study, this processing can be based on event detection (like spike, burst, and stimulation artifacts) or statistic data from these events.

In this paper, we present our system for the data acquisition and processing of neural networks (Fig. 1). Designed to process MEA extracellular data, it also deals with the low signal to noise ratio and the high data flow. The analog signal coming from the culture is amplified, filtered, digitized, and event detections and statistics are available in real-time on the computer screen, in the hard disk and for TCP/IP (Transmission Control Protocol/Internet Protocol) communication to another program. The system is fast enough to enable resource demanding experiments, like the exploration of hybrid neural networks. The system is composed by three parts – bioware, hardware, and software – that are detailed in the next chapter. We detail in the following sections the hardware and the software, the result timing issues and performances of the system.

II. Acquisition Chain Setup

A. Bioware

The first component of the system is the biological material that provides the signal for acquisition. It is mainly composed by glial and neuronal cells ($\sim 10^5$). Spontaneous activity

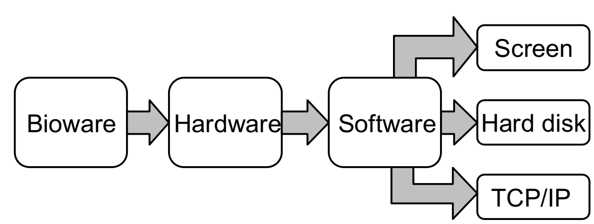

Figure 1. The elements and the acquisition data flow of our system.

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generally starts about two weeks after plating the dissociated culture, and the cells are routinely kept healthy for more than 3 months [11].

B. Hardware

The hardware unit measures signals from the cultures and conveys them to the software. Each incoming analog signal has low amplitude (10~100μV) and high noise level (up to 1 mV in lower frequencies, about 10 μV_{rms} in the 100 Hz – 10 kHz band). Output signal are digital, with a 12 bit resolution and 40 kHz sampling frequency. The hardware is composed by: *MCS suite*, *Acqboards*, *Digiboards* and a *PCIboard.* All hardware items are more detailed in [7]. With the exception of the MCS suite, all elements are custom made and assembled in a customized rack. This rack controls analog and digital signals, and its power supply and electrical references are independent from culture's and from computer's ones.

1) MCS suite: The bioware is plated on a multielectrode array, MEA200-30 from MultiChannelsSystems (MCS). This MEA is inserted in the MEA1060 preamplifier and in the BBMEA breakout box, also from MCS. This system provides an easy access to the 60 recording analog channels [12].

2) Acqboard: These boards are in charge of amplifying with a tunable gain (homogenizing the amplitude among channels), filtering and electrical isolation of the analog signal.

3) Digiboard: Once the amplitude among channels is homogenized and the analog levels of signals are sufficient, signals are digitalized. It is realized by the Digiboards, with a resolution of 12 bits and a sampling frequency of 40 kHz (the minimal frequency is about 10 kHz, as explained in section III). This sampling rate is specified to ensure a high quality reconstruction of the neurons dynamics for offline processing. The 60 digital signals are conveyed to the PCIboard.

4) PCIboard: this board is the bridge between the rack and computer's PCI (Peripheral Component Interconnect) bus. The data transfer rate of the 60 channel data, with a 12 bit, 40 kHz sampling, is approximately 5 MiB/s, well below the 33 MiB/s (33.2^{20} bytes per second) of the PCI transfer protocol. The PCI driver module is written in C++ and runs on Windows XP™.

TABLE I
COMPARISON BETWEEN OUR SYTEM AND THE LATEST MCS SYSTEM [12]

	MCS	Our system
PCI Connector	MC_CARD	Digi Board
Analog Input	128	16/Board
Digital Input	16	*
Digital Output	16	*
Digital Input/Output	0	18*4
Data Resolution	12	12
Sampling frequency	50 kHz	40 kHz
Programmable Amplifier	PGA Amplifier	ACQ Board
Number of Input Channels	16,32,64	4/Board
Input Voltage	+/- 0.3 V	+/-1V
Gain	10 to 5000	1 to 127000
Noise density at 1kHz	25 nV/$\sqrt{Hz}$	13 nV/$\sqrt{Hz}$
Input Impedance	$>10^{12}\Omega$ – 8pF	$>10^{10}\Omega$ – 6pF

5) Hardware performance: We compared the specifications and performances of the hardware elements with a recent commercial apparatus from MCS [12]. Results are presented in Table I. Our system is globally competitive with the MCS system in terms of static performances; individual boards process less channels, but the user can customize the experiment thanks to the modular architecture and the boards configurability [7].

The real benefit of the system lies in the real-time features of the whole system (including the software).

C. Software

At the output of the hardware, the neural signal are available for the software processing, realized by the Real-Time Application (*ReTA*). Its functions are:

1) Raw signal monitoring: Data from 60 channels can be displayed in real-time on the computer screen (Fig. 2a). A zoomed view also can be selected for a channel (Fig. 2b).

2) Events detection: Three events are extracted from the raw neural data: spikes, bursts, and stimulus artifacts.

A spike is a short electrical depolarization of a cell membrane. Spikes often reach amplitudes of 50 μV eil (equivalent input level). After the hardware processing, noise has amplitudes about 15-20 μV eil. Thanks to this level difference between spikes and noise, spikes can be detected by thresholding the signal.

ReTA presents two techniques to set the threshold. The first is to define it as a fixed voltage value, set by the user that takes the monitored signal into account. The second is to define the threshold as a multiple (n) of the standard deviation (SD) of the signal. To have less than 1 ‰ of false positive decisions (the system interprets noise as a spike), "n" is set bigger than 3. The maximum value is 5, after what the false negative decisions (true spikes are not detected) are too frequents [11]. SD is continuously updated.

Bursts have many definitions in the literature. A burst for our application is a series of N spikes on the same channel in a temporal window (W). For example, if a channel has three or more spikes (N = 3) in less than 10 ms (W = 10), this event is considered as a burst. Both values, N and W, are set by the user before the experiment.

At the start of the experiment, a circular buffer is created for each channel where a burst detection is required. Taking into account the sampling frequency (f) of the acquisition, the number of elements of a buffer is (W.f). After each acquisition sampling, the buffer is updated; depending on the values of the first element and the new element, the total of spikes (S) is changed; the first element is overwritten by the new element; the pointers of last and first elements are increased. If the total of spikes in the buffer reaches the number of spikes in a burst ($S \geq N$), a burst is validated for the current timestamp.

Stimulus artifacts are detected also by threshold. Biologically effective stimulations generate artifacts that saturate the acquisition channel. Consequently, the threshold can be fixed before the experiment.

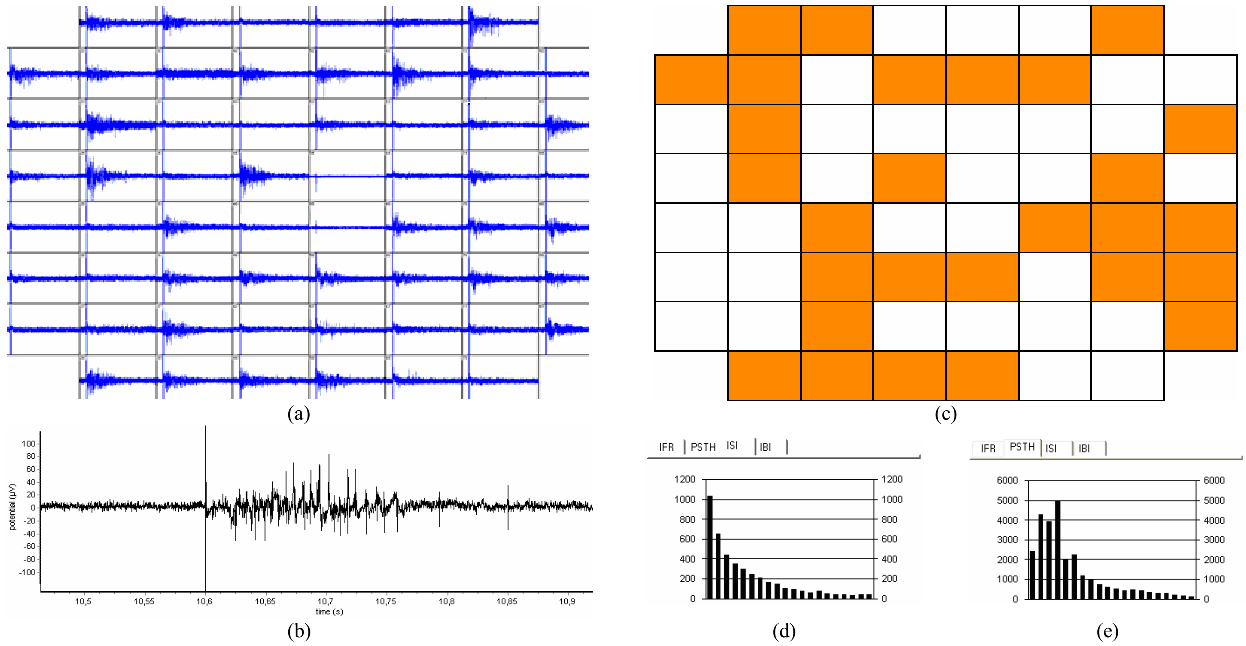

Figure 2. Real-time visualization of bursting activity induced by stimulation. (a) The raw signal of 60 channel in 1-second windows. (b) A zoomed view of one single channel. (c) Burst detection on the 60 channels; white: no burst detected; grey: burst detected within the last 0.25 s. (d) ISI and (e) PSTH histograms from a single channel.

3) Events detection monitoring: The three types of events can be monitored online. Temporal information is indicated by color persistence (Fig. 2c). Inactivated channels are white. Once the event is detected, the corresponding channel passes immediately to red, and then progressively lightens. It gives visual information about signal propagation in the culture.

4) Statistics computing: The online detected events are also used to compute statistics, like instantaneous firing rate (IFR), inter-burst interval histogram (IBI), inter-spike interval histogram (ISI), and post-stimulus-time histogram (PSTH). These statistics are commonly used in neurophysiology experiments. They are also monitored online (Fig. 2d, e).

5) Storage: All the data are stored on the hard disk for further analysis. The raw signal is stored with samples of 12 bits, and a transtyping operation is done to save space disk. Events and Statistics are stored as timestamps in a text file.

6) TCP/IP interface: In order to share the information with other programs, a TCP/IP (Transmission Control Protocol/Internet Protocol) interface is included in ReTA. The packages are configurable: they provide the selected events and statistics to the other program.

7) Channels selection: To optimize the software, the user can configure processing on each individual channel. Useless channels can be deactivated, keeping more resources for ReTA or other real-time programs running in the same machine.

III. Real-Time Performances

The real-time feature of our system is defined as: a data must be processed before the sampling of the next data on the same channel. If we apply that definition, we have to face two issues: maximize the sampling rate and minimize the data transmission delays.

In extracellular measurements, as is the case of MEAs, the typical data bandwidth is about 3 kHz for spike detection [13], which implies a Shannon frequency of 6 kHz. We chose to sample with a minimal frequency of 10 kHz (and then a period of 100 µs), to ensure a correct reconstruction of biological signals even in real-time. Higher sampling rate (e.g. 40 kHz) can give more information about spike shape.

Thanks to its tunable architecture, our acquisition system can provide different outputs (A to E in Fig. 3) changing its processing delay. The simplest experiment (A) necessitates 25 µs, and the complex one (E), 60 µs.

The simplest experiment (A) consists in an offline analysis. In this case, only the raw data storage and visualization are in real-time. We have a mean delay on sample acquisition of 25µs (A). In this case the sample frequency can be tuned to 40 kHz, increasing the data quality on spike shaping.

Adding other real-time processes increases the delay. The most resource demanding online detection (event detection on all channels in a 10 ms burst window) adds 15 µs (B). One statistic function requires 15 µs (C).

The delay to send data to the TCP/IP layer is 5 µs on average. Raw data is not sent because it is too resource demanding. If event detection (D) and statistics (E) are sent, the process delays are, respectively, 45 µs and 60 µs.

In the most complex experiment, all the information (event and statistic) can be sent to the TCP/IP layer with a mean delay of 60 µs. As the specification for real-time is a global delay of 100 µs (sampling rate of 10 kHz), 40 µs are still in that case for user-defined additional functions.

In the case of a hybrid network experiment, as presented in [7], only the spike detection is computed online. The whole

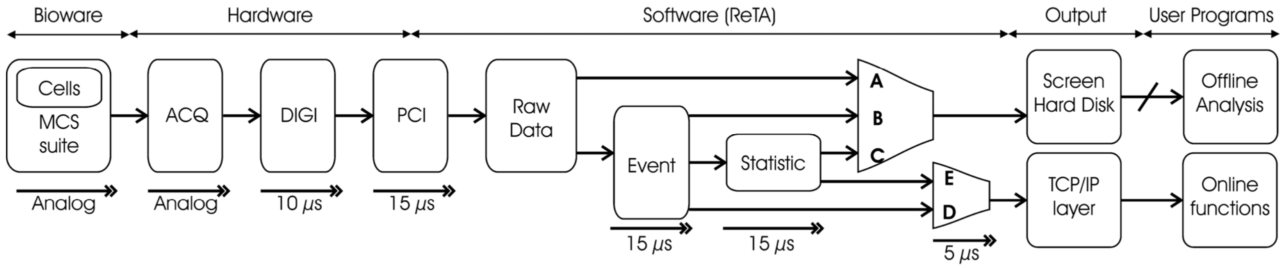

Figure 3. Data propagation delays of the acquisition chain for different experiments (A to E). The simplest experiment's delay is 25 µs for real-time raw data storage and visualization (A). The full analysis needs 60 µs (E).

closed-loop (acquisition and stimulation of the living network) delay is then 46 µs (with 30 µs for acquisition). 54 µs are left for the user program to control the feedback stimulations.

Summarizing, all the configurations (A to E, and [7]) have a mean delay lower than the period (100 µs) associated to the minimal sampling data (10 kHz). So, each data sample is effectively processed before the next one, in "real-time". Hardware timings are fixed, but software delays need a special attention as they depend on the operational system performances. To support this control, all buffers are monitored. If the delay of a task is too long, a warning error sequence is launched.

IV. Conclusion

MEA-based techniques provide new methods to better analyze the behavior of neural system at the network level. Thanks to the extracellular acquisition, the lifetime of living cells is long enough to study learning process. And thanks to the multisite acquisition, the network level study is facilitated.

These advantages are only effective if the setup is able to ensure to deal with low signal-to-noise ratio and to ensure short transmission delays.

Our system fulfills these objectives. It also proposes additional processing functions in real-time and sends data to the TCP/IP layer, where it can be fetched by user-defined programs. The tunable architecture of ReTA supports different configurations of experiments: the only storage and monitoring task in a 25 µs delay, or a complete real-time processing with a 60 µs delay. In the last case, the processing includes event (spike, burst, and stimulus artifact) detection and statistics data from these events (IFR, IBIH, ISIH, and PSTH).

Different projects based on that system are in progress. They concern exploratory studies for neuroscience using hybrid neural networks. We are in the process of integrating the hardware stage of acquisition on a single chip [14]. It will adapt the system to support high density MEAs [15]. We plan to report the whole electronic system (acquisition and stimulation) on one silicon die [16].

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