A Multichannel Waveform Generator for Spatiotemporal Stimulation of Dissociated Neuronal Network on MEA

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***Abstract*—Precision electronics which can provide multi-channel stimulation capability is important part for neurorobotic and investigation of neuronal development and plasticity. Towards this end, an electrical stimulator designed for dissociated neurons on multi-electrode arrays (MEA) is present in this paper. The stimulator is mainly designed for online application in systems requiring simulation stimulation and recording signal from dissociated neurons on MEAs. The developed stimulator includes 64 independent channels, which are able to generate the arbitrary defined biphasic voltage waveform, controlled in real time with time resolution of 3us and amplitude resolution of 12 bits. The stimulator can generate three basic waveforms (rectangle, sine and triangle wave) in default. The amplitude of the output signal produced by this stimulator can achieve a very wide range. The preliminary experiments in which neuronal activities are evoked by this stimulator have been done. Moreover, the additional analysis of neuronal activities evoked by this stimulator will be present in this paper.**

***Index Terms*—microelectrode array (MEA), electricity stimulates, stimulation generator, hippocampal neurons, evoked response**

I. Introduction

In the last 15 years, multi-electrode arrays (MEAs) have been shown proved to provide a suitable experimental framework for observing the activities in dissociated cultures [1], [2]. Development with technology, connecting neurons coupled to the MEAs and robotics, which the activities of neurons were used to control the movement of robotics, was increasingly investigated in recent year [3], [4]. In these systems, an interface was needed to provide precisely the environment of the robotics into the dissociated neuronal network in real time. The way based on the property about the activity of the neuronal network was to provide directly the electrical signal into the dissociated neuronal network. Hence, an electronics which could provide multi-channel electrical stimulation was needed to be developed for ideal closed-loop controlled neurorobotic. This electronics not only can be employed for exploration of the neurorobotic, but also for investigation of neuronal development and plasticity [5].

In order to study the application and mechanism of the neurons, many different versions of these electrical stimulators have been developed with different methods. Even, an available commercial version (multichannel systems, Germany) came into season. Although this commercial version was allowed to define a pattern of electrodes providing the identical signal or the fixed order stimulation, the stimulation signal could not be changed with activities of the neurons in real time. In addition, it was functional enough for other designs to the study where the stimulators were used. However, there was still some shortage in these designs. Some of these designs were low time resolution and amplitude resolution [6], [7], some of them could not generate the arbitrary waveform [8], [9], and some of them could not be controlled in real time [10], [11].

In this paper, we present a design of the electrical stimulator, which includes 64 independent channels, are able to generate an arbitrary defined biphase voltage waveform, controlled in real time with time resolution of 3us and amplitude resolution of 12 bits. Our design has tried to solve the problems referred to above. The stimulator will be based on the existing recording system routinely employed for recording from the neuronal network on MEA. After success in integrating the stimulator in the recording system, the preliminary test experiments with the design have been carried out, and the results demonstrated that the stimulator could evoke effectively the activities of the neuronal network cultured on MEA. Preliminary analysis about the results will be present in this paper.

II. Methodology

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A. System Architecture

The stimulator for dissociated neurons cultured on MEA is built around micro-controller which generates digital value for digital-to-analog converter (DAC) and sends necessary control signals to other circuits. As illustrated in Fig. 1, the stimulator consists functionally of six blocks, a micro-controller with peripheral IO unit, digital-to-analog converters, low-pass filter and eight 8-channels multiplexors, Universal asynchronous receiver transmitter (UART) port, DC/DC converter.

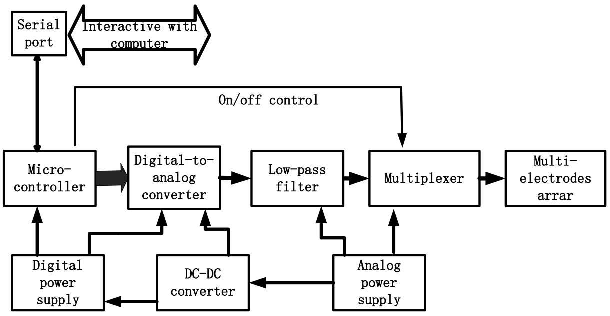

Figure 1. Function block diagram of the stimulator for multielectrode arrays. The stimulator consists functionally of six blocks. The serial port using the universal asynchronous receiver transmitter protocol was employed to communicate with the computer.

The prototype control system has been realized in both a micro-controller (STM32F207) and its associated circuitry. The micro-controller is utilized for (I) generating the digital stimulating value, (II) controlling the DAC and the multiplexors, (III) communicating with the computer. The DAC with 12-bit resolution is used as an analog interface driven by the micro-controller. Based on the in-chip programmable flash memory, the DAC received the digital value from micro-controller may provide the analog signal for stimulation patterns. The first-order low-pass filter with cut-off frequency 2.5KHz is employed to eliminate the high-frequency noise in the analog waveform. The eight 8-channel multiplexors with isolating electrically the analog signal element from successive electrodes element can follow the commands from the micro-controller to output quickly the filtered waveform to the specified electrodes. The UART port is utilized to make micro-controller interact with the computer which will define the parameter of the output waveform in real time. The DC/DC converter module which can transform the voltage 15V to 5V/3.3V and the voltage 3.3V to 1.25V at the same time is used to power all the digital chips.

B. Stimulation Generation Circle

Stimulus waveforms are generally either monophasic or biphasic in shape. A voltage-regulated source should provide biphasic outputs to prevent the neurons' hyperpolarization causing by monophasic stimulation. As shown in Fig. 2, a biphasic waveform generation circle consists of a micro-controller providing the digital value to DAC, a DAC converting the digital signal to analog signal, a biphasic regulated configuration. The micro-controller set the required digital value calculated by the following equation, which is deduced from the schematic in Fig. 2 (b).

$$V_{OUT} = V_{REF}\left(\frac{D-2048}{2048}\right) \quad (1)$$

where D denotes the value of the 12-bit bus with the range from 0 to 4096, and the absolute value of V_{REF} is the maximal amplitude that DA convertor can achieve.

The setting digital value was sent to DAC by a 12-bit bus. When enabled, the DAC and the voltage-regulated configuration start quickly work to output the waveform with the requiring phase and value. After that, this waveform is filtered by a low-pass filter with cut-off frequency 2.5KHz in order to eliminate the high-frequency noisy before reaching the specified electrodes.

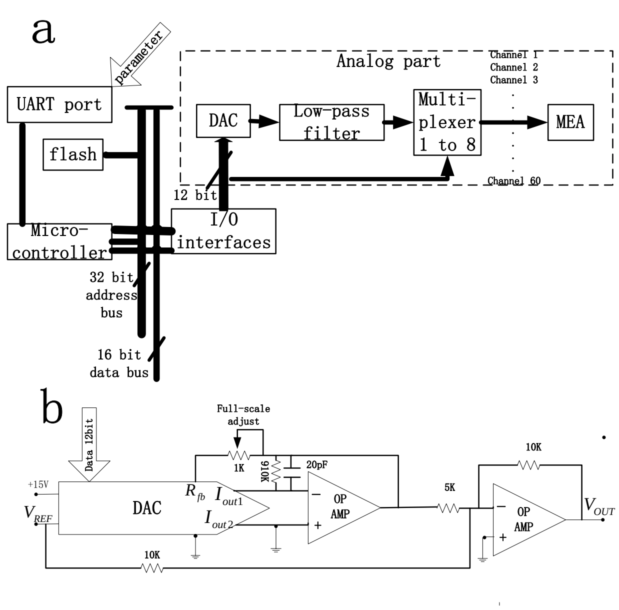

Figure 2. Block diagram of the stimulator. **a** illustrates the schematic for waveform generated by the stimulator. **b** shows the schematic for the biphasic regulation circle.

C. Stimulation Generation Strategy

To generate the required waveform in real time for several electrodes at the same time, the parameters of the stimulation waveform are need to change at any time. As shown in Fig. 2 (a), a strategy, which the parameters required to generate the stimulation waveform is sent from the computer by a communication program to the micro-controller by UART protocol, is employed. These parameters include the amplitude, duration time to each phase, number of waveform, interval between the two adjacent waveforms, and the stimulation channels. After these parameters can precisely transmit to the micro-controller, combining with the recording system and analysis software, the stimulator can generate an arbitrary stimulation waveform on the basis of neuronal activities at any time. Based on this, the researches about not only neuronal development and plasticity, but the application of neurorobotics can be further explored.

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D. Noise Test

In order to be well application in an actual system, any electronics must have a low enough noisy level. The low-pass filter employed here could get rid of the high-frequency in the generated stimulation waveform. In addition, isolation circles in the multiplexors are used so that the stimulator can be sure to make absolutely no noisy into the electrodes when none of the stimulation is asked to provide. To test the noisy, the stimulator was combined with the existing recording system (multichannel systems, Germany) which could record the activities of the neuronal network cultured on MEA. When the stimulator powered on but provided none of the stimulus, the recording system was utilized to record the signal from the dissociated neurons (16 day in vitro) cultured on MEA for 10-15minutes. After that, the noisy level was simply calculated by the recording software.

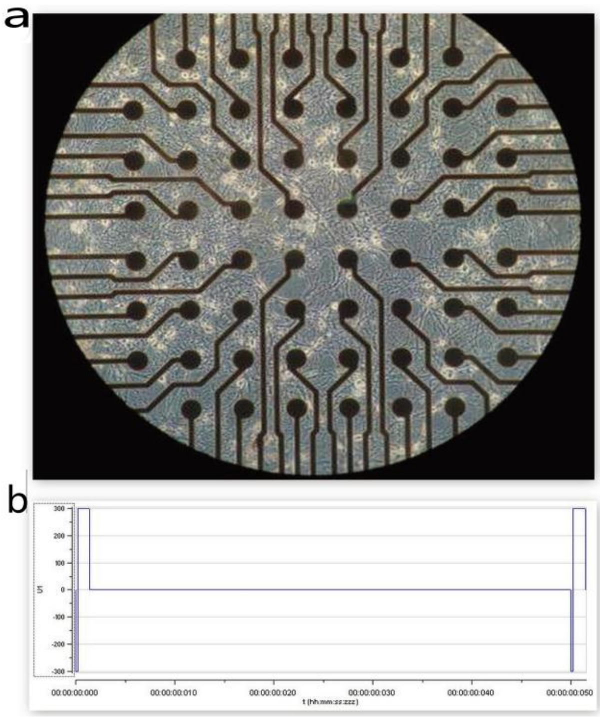

Figure 3. Neuronal network on MEA and stimulation waveform. **a** shows the dissociated neurons cultured on MEA. **b** illustrates the stimulation waveform employed in our stimulation experiment.

E. Stimulus Experiment and Spike Analysis

Firstly, the dissociated neurons cultured on MEA are needed to be prepared before the stimulus experiment. Low dense cultures of the embryonic rat hippocampus were prepared as previously described in [12] with modifications. Hippocampi were separated from embryonic day 18 (E18) rats and dissociated cells were plated on MEA chips at a density of 50-100 cells per mm2. Cells grew throughout the electrode region formed a relatively isolated island by confined polylysine coating or by PDMS rings with a circular hole. The cultures of 14 to 20 day were always used in our experiments. In experiments, in order to evoke neuronal activities, stimulus generated by the stimulator was used to provide a voltage stimulation (Fig. 3b), which had an amplitude with ±300mv (negative first), and duration of which was 1.5ms with 250us negative and 1.25ms positive. A two stimulation waveform with interval 50ms was employed to stimulate the neurons at any proper time instead of fixed time. Moreover, a Matlab toolbox called chronux was used to analysis the sorts of the spike evoked by the stimulator in each channel to estimate the effect on the stimulus waveform.

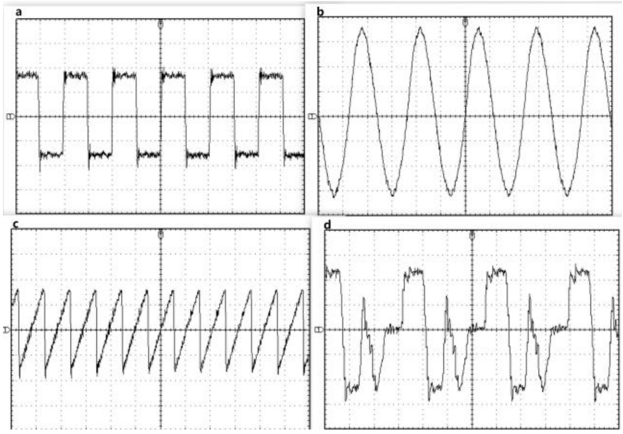

Figure 4. Waveforms generated by the stimulator system. **a** illustrates the rectangle waveforms (the amplitude was ±500mv, the period was 2ms). **b** illustrates the sine waveforms (the amplitude was ±600mv, the period was 4.8ms). **c** illustrates the triangle waveforms (the amplitude was ±500mv, the period was 1ms). **d** illustrates the generated arbitrary waveforms.

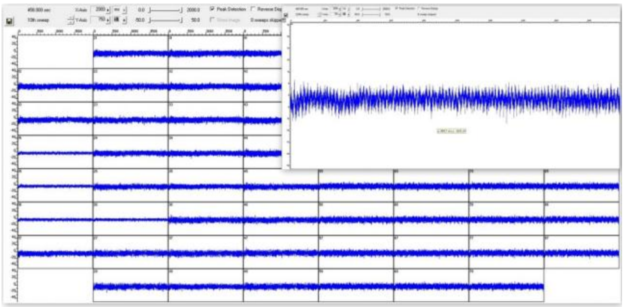

Figure 5. Noisy level without stimulation generated by the stimulator. Each plane presented the activity of a channel within 2000ms duration. The insert was the enlarged view of the channel 46. The data showed here was raw without being filtered.

III. Results

The waveforms generated by the stimulator are illustrated in Fig.4, in which the first three planes (a, b, c) show the basic waveforms (rectangle, sine, triangle) and the last one is the arbitrary waveform. As mentioned in Methodology, the UART protocol was employed such that those waveforms can be generated in real time.

The average of noisy from the waveforms generated by the stimulation was controlled under tens of millivolt (maximal one is 50 millivolt). The level of the noisy was far less than the stimulation amplitude of which hundreds of millivolt was needed for extracellular stimulation to evoke the activities of the neuronal network. As shown in Fig. 5, the noisy without stimulation waveform was tested.

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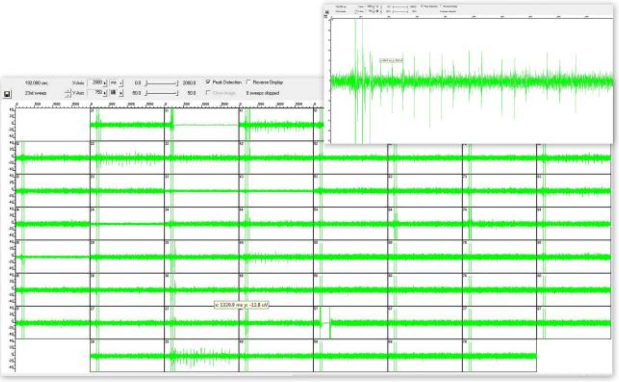

Figure 6. Activities of each channel when channel 31 was stimulated. Each plane presents the activity of a channel within 2000ms duration. The insert is the enlarged view of the channel 38. The data shows here was filtered by a ban-pass filter with the cut-off frequency 200Hz and 3KHZ.

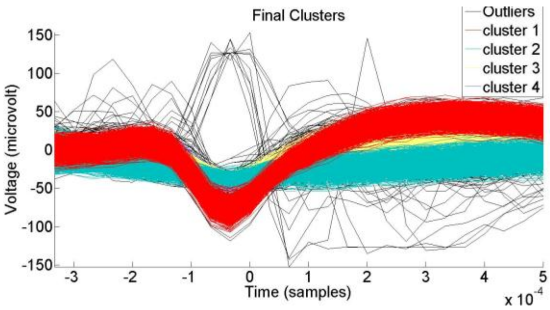

Figure 7. Spike sorting result of the channel 38. The data sets employed to calculate here come from the culture in 15th day in Vitro by stimulating the channel 31.

The average of the noisy calculated by the recording software in 60 channels was -3.03±0.86 (mean ±S.D) millivolt when using a grounded reference, and the maximal one was 3.3 millivolt. That value was far less than the value of general spike amplitude in extracellular recording. These results demonstrated that the system combining the stimulator with the existing recording device was able to provide an excellent setup for the study of neurorobotics and neuronal plasticity.

As the Fig. 6 illustrated, when the stimulation defined in subsection E of Methodology was provided into the channel 31 (channel 31 means the electrode located in column 3 and row 1 on an 8×8 electrodes array), the neurons growing around channel 12, 22, 32, 38, 41, 45, 82 and 83 could be excited and last 1 to 2 seconds. That indicated the stimulator was well able to act as a stimulation source to activate the neuronal network. There were often more than one neuron around an electrode on MEA (Fig. 3 a) so that activities of several neurons always would be recorded in experiment. Consequently, in order to estimate the effect on the stimulus waveform, the chronux which was a cluster software based on k-means algorithm was employed to sort the spikes in each active channel. Three to five sorts of spikes usually could be found in each channel. Fig. 7 shows the sort result of the channel 38, and each color indicated one kind of spike evoked by the stimulator in this channel. From the above, our design was an extremely effective stimulator for the extracellular stimulation of neurons.

Figure 8. Implementation of the stimulator system including the pre-amplifier of the existing recording system.

IV. Conclusions

A real-time controlled multi-channel stimulator for multi-electrodes array in neuron science application has been developed in this work (Fig. 8). The device is able to generate an arbitrary defined biphasic voltage waveform with time resolution of 3us and amplitude resolution of 12 bits for multi-electrodes at the same time.

In this paper, the design consisting of a micro-controller, digital-to-analog converters, low-pass filter and eight 8-channels multiplexors, Universal asynchronous receiver transmitter port, DC/DC converter, is presented for the main application of neurorobotics as the optimal compromise between functionality, size, cost, and flexibility with the following advantages:

1) Three basic and arbitrary waveform can be selectively generated with high time and amplitude resolution.
2) The stimulation waveform can be altered in real time with the change of the parameters required to generate the waveform by the interaction between the micro-controller and the computer.
3) The analog signal element is completely isolated from successive electrodes element to make sure the lower noisy level.
4) Integrated circuits with reasonable dimensions and low cost are utilized.
5) All functionalities can easily adapt to the different operation system by updating the software implementation.

According to the preliminary experiments stimulated the dissociated neurons on MEA, this design has been confirmed to be extremely effective for the extracellular stimulation of neurons. However, for these investigations of neuronal development with long-term stimulation, of the reinforcement learning method, many improvement needs to be done. Especially for neurorobotics, further work will involve: 1) coding the robot environment to

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proper electrical signal, 2) effective stimulation way for neurons learning, 3) effective communication method with the robotic system, and so on. With the development of the recording system, increasement of the stimulation channel is needed to be considered.

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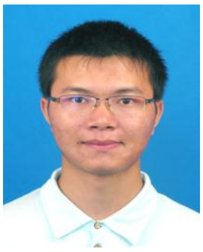

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