Electrical stimulation of patterned neuronal networks *in vitro*

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Abstract— **The implementation of functional neuronal circuits in vitro is a challenging task but also attractive in that these may become valuable tools for studying the fundamental principles of the neural coding in neural networks. The creation and characterization of simple neuronal circuits are the first steps toward this goal. Cultures that are geometrically controlled, or patterned, constitute a starting point. These networks develop spontaneous activities after two weeks in vitro. In this study, two different patterns of neuronal networks have been produced and their network activities were studied. The evoked responses by electrical stimulation as well as spontaneous activities were detected and analyzed.**

Keywords—**patterned neuronal network, microelectrode array, electrical stimulation**

I. Introduction[1]

Cultures grown in patterns are applicable to MEA technology. It has been reported that patterned networks of hippocampal neurons can be maintained on MEAs for more than two weeks, and their spontaneous activity has been characterized [1]–[2]. After 7 DIV (days *in vitro*), both spontaneous and evoked responses are observed. Further it is claimed that directing the morphology of the network increases the frequency of spike activity and the formation of synaptic connectivity [3].

Studies on the connectivity within in vitro neuronal networks have been done by other researchers using electrical stimulation. Short trains of pulses were more effective than a single pulse to trigger networks of spinal cord neurons [4]. Networks comprised of cortical neurons showed various evoked responses in early and late stages after the stimulation [5]. Selective learning in cortical neurons were also demonstrated in [6]. Most of the work is based on the cultures that are grown without patterns.

This paper investigates network connectivity for patterned networks.

II. Methodology

A. Microelectrode Array preparation and microcontact printing

MEAs (Multi Channel Systems (MCS), Reutlingen, Germany, TiN electrodes, 10 μm or 30 μm in diameter, 200 μm spacing) were ultra-sonicated in the acetone bath for 5 min and rinsed by isopropanol (IPA) and deionized (DI) water successively.

AZ4620 (Clariant Corp., Sommerville, NJ) was used to fabricate molds for PDMS stamp. Two different patterns were used. One was a circle array pattern–100 μm circles at 200 μm spacing–that allowed cells to attach close to the electrode initially. The other was line pattern–four lines (40 μm wide, 2 mm long) spaced 400 μm apart–to overlie 30 of the 60 MEA electrodes. PDMS was poured on the mold and cured for 6hr at 90 °C in oven. Acetone and IPA were used to remove any organic contaminants on PDMS stamps. 10% SDS (sodium dodecyl sulfate) was coated on the stamp surface by soaking it for 15min. After the SDS coating, the stamp was dried carefully with nitrogen and excess SDS was removed by dipping the stamp once in DI water. Poly-D-lysine (0.1 mg/mL, mixed in DI) was loaded on the stamp for 30 min. Using a custom-built contact aligner, the stamp was aligned with the MEA and brought in contact for 3min. Printed MEAs were rinsed thoroughly with DI water and blow-dried by nitrogen gas.

B. Cell culture

Hippocampal neurons (Brain Bits, www.siumed.edu/brainbits) from 18-day gestation Sprague-Dawley rat embryos were dissected mechanically and cultured at 37°C, 5% CO_2, 9% O_2, in serum free B27/Neurobasal medium (Invitrogen, Gaithersburg, MD) with 0.5 mM glutamine and 25 μM glutamate. Cells were plated in the center of MEA (area 100 mm^2) at a density of 200 – 300 cells/mm^2. Half of the medium was changed weekly, without glutamate.

C. Electrical recording and Stimulation

For the recording and stimulation, the MEA was placed in an MEA 1060 amplifier (Gain 1200, 10 Hz – 3 kHz, MCS) and 5% CO_2, 10% O_2 gas (filled with nitrogen) was fed to the recording chamber to maintain the proper pH level. 60-channel continuous data were digitized by the MC Card (sampling rate 25 kHz, MCS) and displayed simultaneously by the MC Rack (MCS). If necessary, the digitized data were stored in a hard disk for a post analysis. For real time detection and spike sorting, we used RASPUTIN (real time acquisition systems programs for unit timing in neuroscience, Plexon Inc., TX). To use this software, the MEA 1060 amplifier was connected with MAP (Multichannel Acquisition Processor, Plexon Inc., TX). Software provided automatic spike detection by setting

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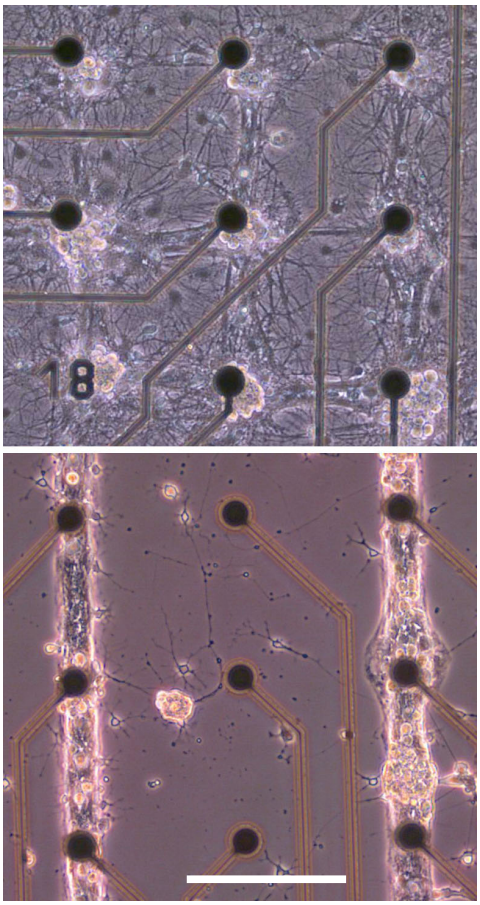

Fig. 1. Patterned neuronal circuit. (a) Circle array pattern, cell clusters on top of electrodes, glial cells at the background. (b) Line pattern. Scale bar = 200 μm

threshold at 4 times the standard deviation after collecting the peak amplitude for 12 sec. Multiple units were sorted manually using the template matching or principal component analysis (PCA). Only the time stamp data was recorded in data file.

A Stimulus Generator (STG-8, MCS) was used to deliver a desired voltage pulse to the electrode. All of the stimuli were positive-first biphasic voltage pulses. The amplitude of the stimulus was 1.0 V and the duration of each phase was 200 or 500 μs. Stimulation electrodes were configured to be either monopolar or bipolar. During stimulation, the evoked response was monitored through a real-time perievent client (PEC, Plexon Inc., TX). Channels that detected large action potentials, suggesting that neurons were positioned close to the electrodes, were selected for stimulation.

D. Data Analysis

The off-line data analysis with Neuroexplorer (NEX, Nex Technologies) operates on time stamp data. Spontaneous activity was characterized by mean firing rate. To do a quantitative study of the evoked responses, perievent histograms were calculated from –100 ms to 100 ms with respect to the stimulation time. A time bin of 1ms was used identify the early and late response within 100 ms range and a time bin of 0.1 ms was used to measure the precise time delay of the response as a function of distance in a line pattern. 100 – 500 epochs were averaged to calculate the probability for each time bin. For display purposes the histograms were rearranged to map onto the original MEA configuration (8x8). The individual perievent histograms were exported to Matlab (Mathworks, Natick, MA) for the calculation of population perievent histograms as the first principal components of the perievent correlation matrix.

III. Results

A. Patterned growth and extracellular recording

The circle array pattern was used to create a circle of cells, with the desired radius, on top of each electrode. During the first few days, the growth of neurons was confined to the circle. Soma and neural processes followed the border of the circle and seldom grow out of the circle. After 1 week, cell bodies within the pattern tended to aggregate into clusters and neurites from these clusters began to grow out to form connections. There was extensive outgrowth across the circles after 10 DIV. Since most of the somata were located on top of the electrodes, all channels detected spikes from multiple units. However, there were a many glial cells proliferating at the background at 18 DIV (Fig. 1(a)).

In case of 40 μm-line pattern, cells were evenly distributed along the line (Fig. 1(b)). The cell growth was well confined to the line. Five to six neurons were estimated to grow within a 50 μm radius of the electrode.

B. Spike detection

For the circle array pattern, all of the 60 electrodes were active at 18DIV. The average amplitude of background noise was 25 – 30 μV (peak-peak), while the signal amplitude varied from 40 – 400 μV (peak-peak). 93 units were sorted in real time from 60 channels.

In case of the line pattern, 6 or 8 electrodes were overlaid on each line and the number of active sites varied between the lines despite visual appearance of roughly equal growth at 11 – 12 DIV. Average amplitude of background noise was 15 – 20 μV (peak-peak) and signal amplitude varied from 35 – 80 μV (peak-peak). On average, 3 – 4 electrodes out of 6 or 8 electrodes (55% ± 25%, S.D., N = 8 lines) detected spikes. From these active electrodes, 1.8 units ± 0.4 units (S.D., N = 8 lines) were sorted.

The average spike rates of each pattern were 2.76 Hz ± 2.80 Hz (S.D, N = 92 units, circle array pattern) and 2.11 Hz

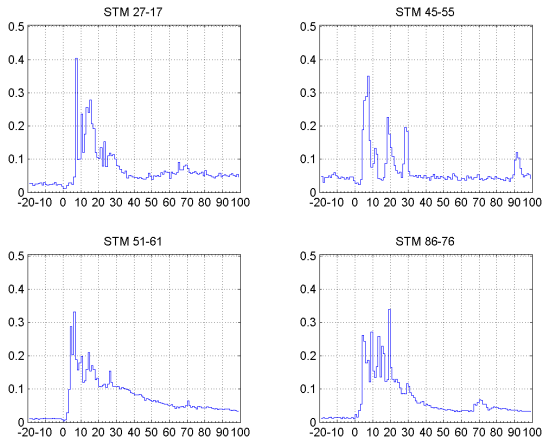

Fig.2. Population perievent histogram of 92 neurons in circle array pattern. (x-axis: ms, y-axis: probability)

± 1.75 Hz (S.D, N = 57 units, line pattern). The medians of the firing rates were 1.5 Hz and 1.3 Hz, respectively.

C. Response to the focal stimulation

Early responses were routinely detected within 10 ms. Switching the stimulation site changed the location of the most highly activated neurons. Relatively young culture at the age of 11 DIV, most of the responses appeared within 20 ms and there were no late responses appearing after this time window. However, 18 DIV old cultures had late response components after 20 ms as well as early responses. Figure 2 shows the population perievent histogram of neurons in the circle array pattern. This representation captures the variability of the responses.

Comparing the mean firing rates before, during and after the stimulation, some of the units were depressed and other units were elevated during the stimulation. Rates increased or decreased by 50 – 100% and returned to the baseline when the stimulation was ended. Trends were different between units.

D. Other observations

Both the circle array pattern and the line pattern had spontaneous bursting behavior. Cells in the circle array pattern had globally synchronized bursting pattern, while four networks formed within the line patterns had independent bursting activities. There were a few cells having high spike rates in circle array pattern, which did not appear in recording from the line pattern.

The early evoked responses described in [5] were also present in our patterned network.

IV. SUMMARY

Patterning was used to guide neurons to electrodes and to provide sufficiently high neural density for network activity to become manifest. Qualitatively the networks appear to have had more detectable activity than unpatterned networks. Considerable variation in response was seen.

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REFERENCES

[1] J. C. Chang, G. J. Brewer, and B. C. Wheeler, "Microelectrode array recordings of patterned hippocampal neurons for four weeks," *Biomedical Microdevices*, vol. 2, pp. 245–253, 2000.

[2] J. C. Chang, G. J. Brewer, and B. C. Wheeler, "Modulation of neural network activity by patterning," *Biosens. Bioelectron.*, vol. 16, pp. 527-533, 2001.

[3] Q.-Y. Liu *et al.*, "Synaptic connectivity in hippocampal neuronal networks cultured on micropatterned surfaces," *Developmental Brain Research*, vol. 120, pp. 223-231, 2000.

[4] G. W. Gross, B. K. Rhoades, D. L. Reust, and F. U. Schwalm, "Stimulation of monolayer networks in culture through thin-film indium-tin-oxide recording electrodes," *J. Neurosci. Methods*, vol. 50, pp. 131-143, 1993.

[5] Y. Jimbo, A. Kawana, P. Parodi, and V. Torre, "The dynamics of a neuronal culture of dissociated cortical neurons of neonatal rats," *Biological Cybernetics*, vol. 83, pp. 1-20, 2000.

[6] G. Shahaf and S. Marom, "Learning in networks of cortical neurons," *J. Neurosci.*, vol. 21, pp. 8782–8788, 2001.