Development of a High-Throughput, Automatic Multielectrode Array (MEA) Platform for In-vitro Extracellular Electrophysiology

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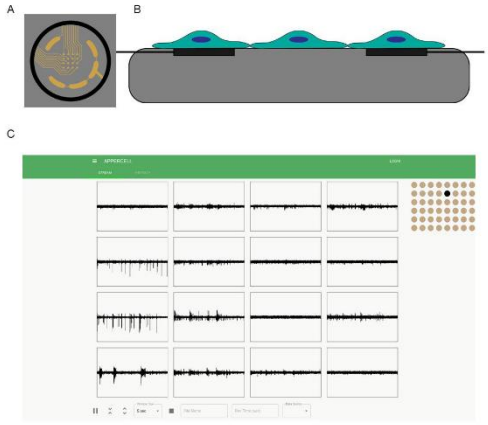
Introduction

Multielectrode Arrays (MEA) are devices that contain numerous microelectrodes through which neural extracellular electrophysiological potentials are measured with multiple measuring sites. Microelectrodes detect signals from all possible sources, as changes occur in the extracellular field caused by the current flowing from all ionic processes across the morphology of the closest neurons they detect this change. In-vitro MEA technology is widely used for various applications in the neuroscience and cardiac electrophysiology research domains. Single- and multimodal studies have proven its effectiveness in understanding single cell and network level correlates of induced pluripotent stem cell (iPSC) derived neuronal and cardiomyocyte cultures. Additionally, it can be used for slice and retinal studies. Microelectrode array technology is a useful tool for directly connecting key biological variables, like the function of ion channels or the effect of various expressed genes to cellular or network level electrophysiological correlates. Most of the current MEA solutions offer various options for low throughput data recording and stimulation. With these systems data analysis is usually manual, tedious and subjective.

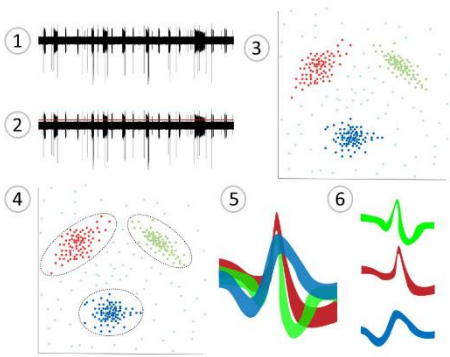
Our aim is to build a multi-device platform for high throughput recording, stimulation and automatic signal processing.



A) Electrode grid layout of a well (48 well plate). These electrodes offer interfaces with1 cultured cells.
B) Cells on the electrode grid. The electrodes detect changes in extracellular potentials.
C) Filtered voltage signals from one well (16 electrodes).

Clustering

One of the key problems occurring in today's electrophysiology is the ability to properly extract and sort different spikes. While the spike detection may be relatively straightforward the differentiation of spikes may not. In the future, after extracting various features from the recorded data, we plan to implement multiple spike clustering algorithms, like K-means, Hierarchical clustering and/or Density based scanning among others.



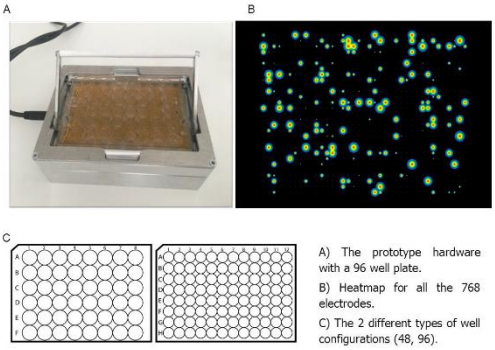
1. Filtering and signal amplification of observed extracellular activities
2. Spike detection
3. Spike alignment
4. Feature extraction
5. Clustering
6. Spike classification

Prototype Platform

The prototype platform that consists of hardware (a) and software components developed by our team demonstrated its effectiveness in recording action potentials of hundreds of primary hippocampal neurons at given developmental stages. We have also successfully demonstrated the pharmacological manipulation of neuronal activities.

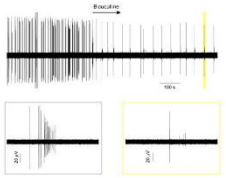
Our early results include a recording system with 768 electrodes across all plate formats, the data is recorded from 48/96 well plates (b) providing information from entire cell populations.

For the recording of both neural and cardiomyocyte signals we use 20kHz sampling rate then bandpass filtering. We also implemented online spike detection and heatmap to improve the visibility of active channels through the plate.

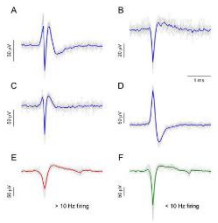


A) The prototype hardware with a 96 well plate.
B) Heatmap for all the 768 electrodes.
C) The 2 different types of well configurations (48, 96).

MEA: Tool for In-vitro Drug Testing and Basic Research

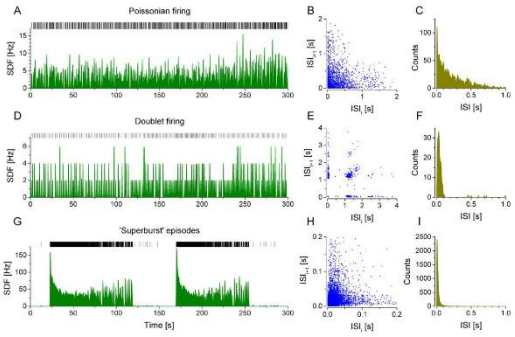


The effect of bath applied bicuculline (30 μM) on cultured hippocampal neurons is shown in the above MEA recording. Blocking GABA receptors by bicuculline paradoxically inhibits firing rate possibly due to depolarization block. Here, single spikes correspond to network events when potent network inputs depolarize the neurons to a degree when only a single spike can be emitted.



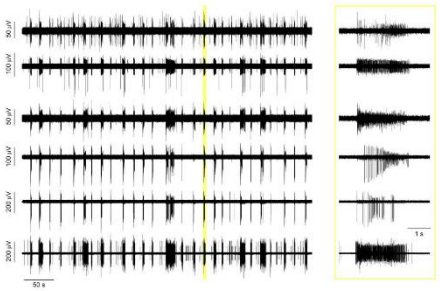
Representative extracellular spike waveforms are demonstrated above. Traces A-D are from four different unit, blue shows the average spike waveform from 20 successive sweeps. E and F show the same unit during high frequency and low frequency phase of firing.

Maturity Dependent Firing Activities of Cultured Mouse Hippocampal Neurons

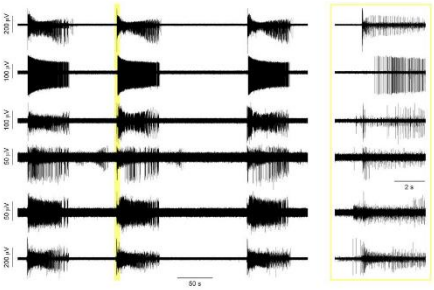


The MEA system under development will use a powerful set of analytic tools and algorithms to characterize the firing activity of cultured neurons. Above are shown a few examples of the functions and statistical descriptors the system will calculate automatically during the experiments. A, D and G show spike density functions of calculated from firing of 3 hippocampal neurons. In the young cultures (DIV 10) irregular, Poissonian type firing is often observed (A). The corresponding ISI return map and ISI histogram are shown in B and C. In more mature cultures (DIV 12) brief bursts and doublets of spikes are emitted in a regular manner (D). Superbursts are the dominant mode of activity in mature cultures (G, DIV 24).

Maturity Dependent Network Firing Activities of Cultured Mouse Hippocampal Neurons



Near regular bursting activity is demonstrated above in a culture of mouse hippocampal neurons 19 days after plating. Brief bursts of action potentials are detected at multiple electrode positions representing different neurons. The activity is tightly synchronized at the burst level. Zoomed sections of the 6 voltage traces are shown in the right panels. Spike amplitude is highly variable at most electrode positions (e.g. 4th from top).



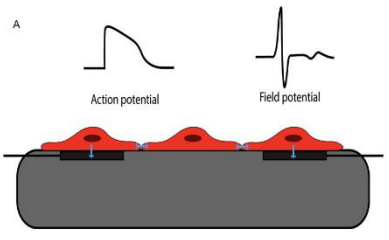
More mature cultures of mouse hippocampal neurons exhibit bursting patterns characterized by prolonged episodes of intense firing separated by silence. Here, the neurons in the 24-day old culture emitted 'superbursts' often longer than 1 min in duration and with complex intraburst firing patterns. Theta oscillations are often observed at the late phase of the superbursts.

CiPA

Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative was started by the goal to improve the specificity of proarrhythmic risk assessment so drugs cardiovascular liability can be better tested before clinical trials.

With a Cardiomyocyte Microelectrode Array (CM-MEA) one can trace drug induced changes in the cardiac field potential of beating heart cells in a dish for evaluation of proarrhythmic indicators in-vitro. From the field potential clear markers of depolarization and repolarization enables the quantification of various measures, like depolarization amplitude, beat frequency, or repolarization irregularities.

Continuous, long time recordings enables detection of rare arrhythmic events. A plethora of different drugs could be tested for their effect based on the electrophysiological markers.



A) As the cardiomyocytes generate action potentials that travels through the cells, the cardiac field potential arises. On the CM-MEA the electrodes measure this potential.

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