Trial for data analysis of drug responses in primary rodent neurons and human induced pluripotent stem cell - derived neurons using MEA and deep learning

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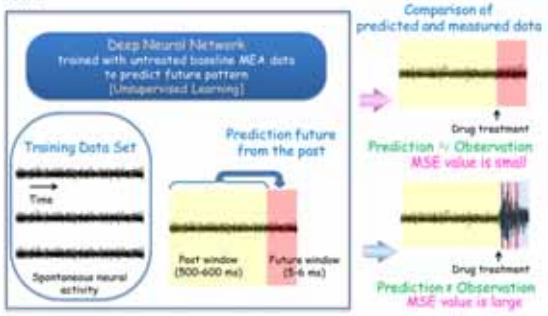
Background

Use of the multi-electrode array (MEA) system to record spontaneous electrophysiological activity generated from neuronal networks *in vitro* could be a good risk evaluation method for drug-induced seizure events in drug discovery. The electrophysiological activity is able to be obtained from primary rat neurons and human induced pluripotent stem cell (hiPSC)-derived neurons as well as rodent hippocampal slice cultures. The electrophysiological activity in neural networks consists of action potential spikes and organized patterns of action potential bursts. The synchronized burst pattern from spatially separated neurons is thought of as epileptogenicity and it is affected by seizurogenic drugs. However, the conventional method of MEA data analysis is required for a multi-step manual operation process, which requires experienced skill, and is labor-intensive and time-consuming. Furthermore, how to express the drug-induced seizurogenic indices is under discussion. Summarily, we are trying to establish a fully automated method to detect various types of drug-induced abnormalities with single index using a deep learning algorithm in MEA data analysis.

Materials and Methods

Deep Learning for Detecting Drug-induced Changes of Extracellular Field Potential Patterns in MEA Data:

The developed deep neural network was trained with baseline data (without compound applied) to predict the future window (several milliseconds) from the past window (several hundred milliseconds). After the training, prediction was performed sequentially up to the total period of time measured. The difference between predicted and measured data was quantified as mean squared error (MSE). We tested whether this MSE can or can't reflect alteration on drug-induced pharmacological effects through electrophysiological changes in MEA data.



Cell Preparation, Seeding Conditions and Culture Duration before Drug Treatment:

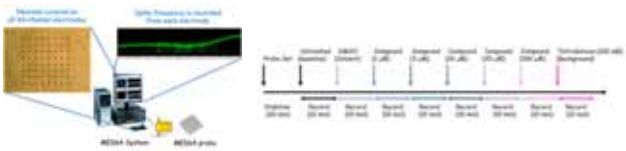
Animal experiments were approved by the Animal Care and Use Committee of Eisai. Co., Ltd, and were carried out according to the guideline for animal experimentation issued by the Japanese Association for Laboratory Animal Science.

- Rat Primary Neurons (Cortical or hippocampal neurons were obtained from 18 days post-coitum fetus of Wistar Rat, Charles River Laboratories Japan, Inc., Yokohama, Japan), using a $8x10^5$ cells/8 µL/probe. The cells were cultured for 22 to 35 days before drug treatment test.
- iCell Neurons (hiPSC-derived neurons, Cellular Dynamics International, WI, USA) and iCell Astrocytes (hiPSC-derived astrocytes, Cellular Dynamics International, WI, USA), those cells were combined $8x10^5$ cells and $2.7x10^5$ cells respectively/8 µL/probe. The cells were cultured for 106 to 139 days before drug treatment test.

Devices and analyzing software:

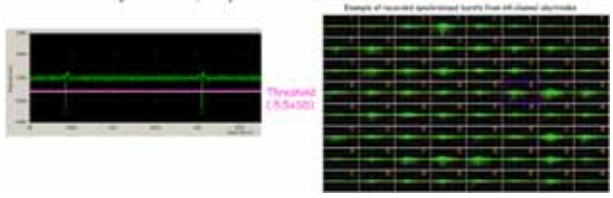
- MEA probe, MED- R545A probe (Alpha Med Scientific Inc., Osaka, Japan)
- MEA system, MED64 4 sample system (Alpha Med Scientific Inc., Osaka, Japan)
- Analyzing software, Mobius Extended and MED Burst Scope (Alpha Med Scientific Inc., Osaka, Japan)

Extracellular Recordings:

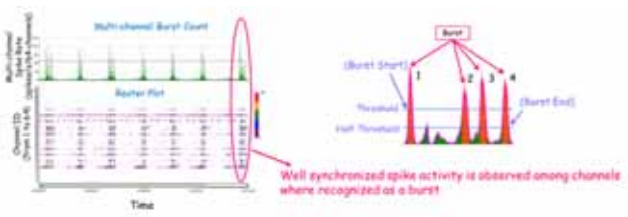


Conventional Method of Spike Recognition and Multi-channel Burst Recognition:

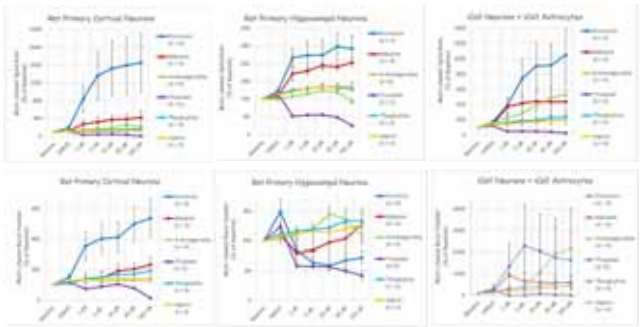
To make a raster plot and calculate the multi-channel spikes per probe in every second, the spike count threshold was determined at the minus 5.5 x SD after 100 nM tetrodotoxin treatment. When the recorded signal exceeded the threshold then returned to less than half of the threshold level immediately, the signal was recognized as a spike. Active channels were selected over 0.1 spikes/s frequency at baseline measurement.



For each channel, a Raster plot was created from the 100 msec window's spike number. The mean spike frequency of 64-channels for every 100 msec windows were calculated to make a histogram. Multi-channel burst was defined by the threshold from 200 to 500% of multi-channel spike rates at DMSO treatment. The burst was counted when the multi-channel spike histogram was over the threshold (burst start) and back to below half the value of the threshold (burst end).



1 Result -Drug Evaluation by Spike and Burst Recognition-



Multi-channel spike rate was increased by seizurogenic drugs (picrotoxin, gabazine, 4-aminopyridine) and decreased by depressant in the benzodiazepine class (triazolam). Multichannel burst number showed similar results except for rat primary hippocampal neurons in which picrotoxin and gabazine decreased number of the bursts.

2 Result -Comparison of Spike Train and MSE Train-

MSE value showed similar time course change with multi-channel spike rate in all data of examined drugs and cell types.

3 Result -Drug Evaluation by Average MSE Value-

The deep learning index, MSE was increased after treatment of 1 µM to 100 µM of picrotoxin, gabazine or 4-aminopyridine in all the type of cells. Similarly, MSE was decreased in triazolam, and it was unchanged in theophylline or aspirin in all the types of cells.

Conclusion

Different types of drug-induced spike abnormalities could be detected by MSE. The quantified index "MSE", calculated from the deep learning algorithm using MEA data, would be an informative index for judging the seizure liability of drugs. The automated MSE analysis shed light on significant throughput improvement for massive MEA data analysis without arbitrariness.

Acknowledgement

We are greatly thankful to Dr. Kohei Sawada for scientific discussion. We also sincerely thank Ms. Kathleen Vanderhoof for the English correction.