Trial for drug-induced epileptogenic phenotype classification in primary rodent neurons and human induced pluripotent stem cell-derived neurons using burst onset time cross correlogram and deep learning

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Introduction

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. Spontaneous electrical activity in neural networks consists of action potential spikes and organized patterns of action potential bursts. These activities are able to be observed after a couple of weeks’ cultures in primary rat cortex and hippocampal neurons. This spontaneous neuronal activity was difficult to obtain from induced pluripotent stem cell (iPSC)-derived neurons alone, but we succeeded in generating the experimental conditions to achieve acceleration (or enhancement) of the activity generation of the iPSC-derived neurons by co-culture with mouse primary astrocyte conditioned medium (mACM) or by co-culture with iPSC-derived neurons, as well as by co-culture with rodent primary astrocytes. The potentiated neurons showed an enhanced epileptogenic response pattern by GABA A antagonist with picrotoxin and gabazine or K1 antagonist with 4-aminopyridine in a dose-dependent manner. Comparative extraction feature analysis of the epileptogenic burst phenotypes was performed using 4 types of cells, rat primary cortical neurons, rat primary hippocampal neurons, mACM potentiated iPSC-derived neurons (iCell neurons), and iCell neurons and iCell astrocytes co-culture. Burst frequency and burst onset time cross-correlogram (BOTC) were examined for epileptogenic phenotype classification. We are also trying to establish a prediction method of MEA data to detect drug-induced abnormality using a deep learning algorithm.

Methods

Primary astrocyte-conditioned medium preparation and primary cortical and hippocampal neuron preparation: Animal experiments were approved by the Animal Care and Use Committee of Eisai Co., Ltd., and were carried out according to the guidelines for animal experiments issued by the Japanese Association for Laboratory Animal Science.

Extracellular recording and spike detection:

Deep Learning Algorithm for MEA Data Analysis

1. Drug untreated baseline MEA data were used as the reference for both spontaneous activity pattern for prediction with a deep learning algorithm. The deep learning algorithm predicts following section’s data from previous section’s data. The algorithm calculates difference between predicted value and recorded value as loss value. Drug-induced abnormality degree is shown in increased loss value.

2. Burst Onset Time Cross-Correlogram (BOTC): BOTC values increased by seizurogenic compounds and decreased by AMPA receptor inhibition in rodent cells.

3. BOTC Values Increased by Seizurogenic Compounds and decreased by AMPA Receptor Inhibition in Rodent Cells

4. Loss Value by Deep Learning Algorithm predicted Drug Effect on MEA Data

5. Loss Value showed Similar Pattern with Temporal Change of Spike Rate

Conclusions

- GABA A Receptor antagonism and K1 antagonism showed different phenotypes on burst pattern.
- The burst pattern was affected differently by drug mode of action.
- The BOTC value but potential to judge drug-induced seizure risk, but data variation was large. The methodology requires refinement.
- Deep learning algorithm could detect drug-induced small change of MEA data precisely.

Acknowledgements

This research is partially supported by the grant for IPS Non-clinical Experiments for Nervous System (iNCENS) project in Research Grants on Regulatory Science of Pharmaceuticals and Medical Devices from the Japan Agency for Medical Research and Development, AMED.