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

Norimasa Miyamoto[1, 2, 3*], Tetsuo Kitamura[4], Keiichi Shirakawa[5], Tomoharu Osada[6], Tadashi Kadowaki[7] and Kohei Sawada[1]

1. Biopharmaceutical Assessments Core Function Unit, Medicine Development Center, Eisai Co., Ltd., 5-1-3 Tokodai, Tsukuba, Ibaraki 300-2635, Japan
2. Consortium for Safety Assessment using Human iPS Cells (CSAHi), 3-25-22 Kawasaki, Kanagawa 210-0821, Japan
3. iPS Non-clinical Experiments for Nervous System (iNCENS), 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan
4. Safety Assessment Department, Nonclinical Center, LSI Medience Corporation, 14-1 Sayama, Kamisu, Ibaraki 314-0255, Japan
5. Alpha MED Scientific Inc., 7-7-15 Saito-Asagi, Ibaraki, Osaka 567-0085, Japan
6. Advanced Medical Business Development Deparytment, LSI Medience Corporation, 1-13-4 Uhikanda, Chiyoda-ku, Tokyo 101-8517, Japan
7. Data Science Laboratory, hhc Data Creation Center, Eisai Co., Ltd., 5-1-3 Tokodai, Tsukuba, Ibaraki 300-2635, Japan

*To whom correspondence should be addressed

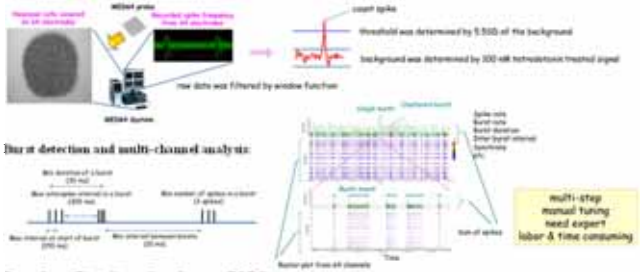
Introduction

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 week's cultures in primary rat cortex and hippocampal neurons. This spontaneous neuronal activity was difficult to obtain from human induced pluripotent stem cell (hiPSC)-derived neurons alone; but we succeeded in optimizing the experimental conditions to achieve acceleration (or enhancement) of the activity generation of the hiPSC-derived neurons by co-culture with mouse primary astrocyte conditioned medium (mACM) or by co-culture with hiPSC-derived astrocytes, as well as by co-culture with rodent primary astrocytes. The potentiated neurons showed an enhanced epileptogenic response pattern by $GABA_A$ antagonism with picrotoxin and gabazine or K_V1 antagonism 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 hiPS-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.

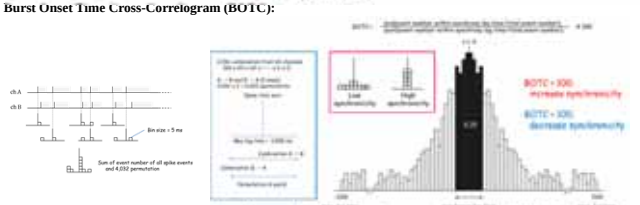
Methods

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 guideline for animal experimentation issued by the Japanese Association for Laboratory Animal Science.

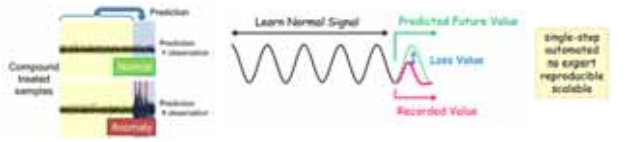
Extracellular recording and spike detection:



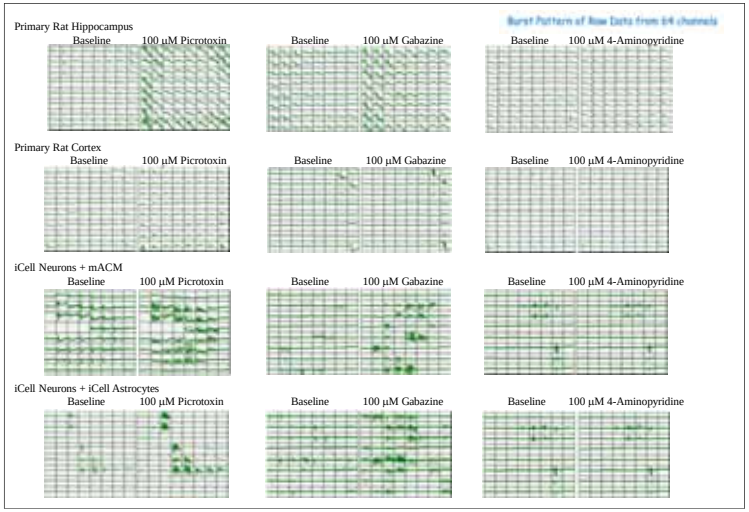
Burst Onset Time Cross-Correlogram (BOTC):



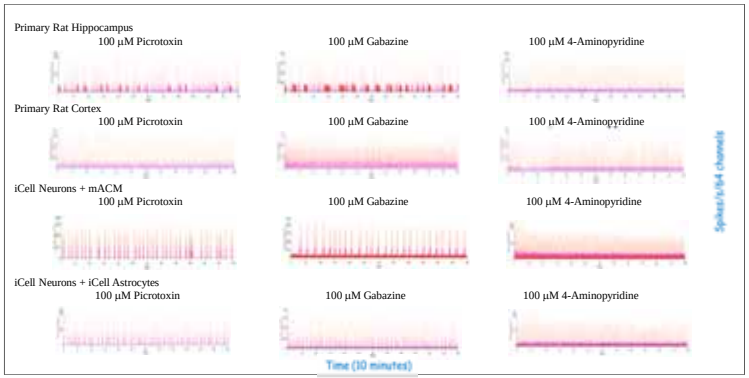
Deep Learning Algorithm for MEA Data Abnormality Detection: Drug untreated-baseline MEA data were used as for based spontaneous neuronal activity pattern for prediction with a deep learning algorithm. The deep learning algorithm predicts following section's data from previous section's data. The algorithm calculates difference between predicted value and recorded value as loss value. Drug-induced abnormality degree is shown in increased loss value.



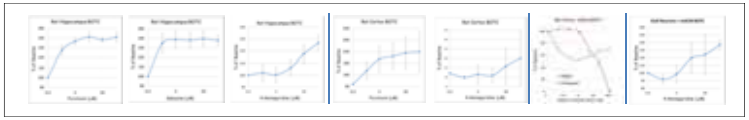
1 $GABA_A$ Receptor Antagonism, but not K_V1 Antagonism, enhanced Clustered-Burst Firing Phenotype



2 Resting Phase was observed in Maximum Dosing of $GABA_A$ Receptor Inhibition



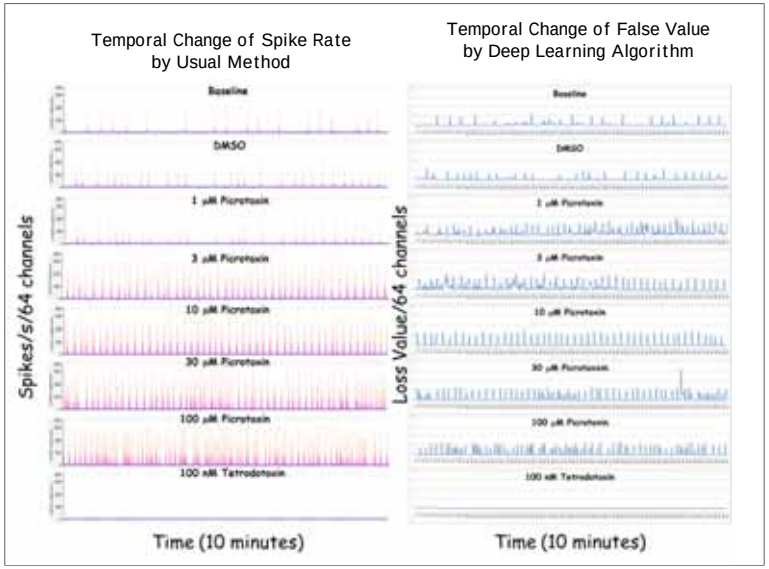
3 BOTC Values increased by Seizurogenic Compounds and decreased by AMPA Receptor Inhibition in Rodent Cells



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

	Primary Rat Cortex Neurons				Primary Rat Hippocampus Neurons			
	Gabazine	Picrotoxin	4-Aminopyridine	Triazolam	Gabazine	Picrotoxin	4-Aminopyridine	Triazolam
Baseline	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DMSO	1.03	1.02	1.00	1.03	1.05	1.03	1.00	1.03
1 µM	1.25	1.26	1.05	0.96	1.19	1.19	1.04	0.93
3 µM	1.27	1.34	1.08	1.00	1.20	1.20	1.06	0.95
10 µM	1.30	1.35	1.15	1.01	1.21	1.24	1.12	0.95
30 µM	1.32	1.38	1.28	0.96	1.22	1.26	1.18	0.95
100 µM	1.37	1.40	1.32	0.93	1.24	1.25	1.23	0.91
100 nM Tetrodotoxin	0.85	0.94	0.95	0.92	0.89	0.89	0.91	0.90

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



Conclusions

- $GABA_A$ Receptor antagonism and K_V1 antagonism showed different phenotype on burst pattern.
- The burst continuity was affected differently by drug mode of action.
- The BOTC value had potency to judge drug-induced seizurogenic risk, but data variation was large. The methodology requires refinement.
- Deep learning algorithm could detect drug-induced small change of MEA data precisely.

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