Separation of drug effects in human iPS cell-derived neurons using MEA system

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Introduction

In vitro human iPSC-derived neurons are expected to be applied to toxicity evaluations in nonclinical studies and drug screening. Microelectrode array (MEA) measurement system is suitable to evaluate the neuronal electrophysiological responses to drugs. We have previously reported the electrophysiological responses to several convulsive compounds using MEA in cultured hiPSC-derived neurons (Elixirgen, LLC). In this study, we evaluated the responses to convulsants and anti-epilepsy drugs (AEDs) more than 10 compounds having different mechanism of actions in cultured hiPSC-derived neurons (Elixirgen, LLC). Among the 80 parameters, we identified a set of parameters that could separate the mechanism of action of the drug. Drug effects acting on GABA, Glycine, Glutamate, Dopamine, 5HT, Muscarinic receptors and Na channel, etc. were separated by principal component analysis and clustering analysis using identified parameters. It was suggested that spike data obtained by MEA measurement in cultured human iPS cell-derived neurons contained information on the efficacy of drugs having different mechanisms of action. Our analysis method of MEA data can be applied to elucidation of the mechanism of action of drugs, safety assessments of new drugs and screening.

Material & Methods

Human iPSC-derived cortical neurons [Elixirgen scientific]

Human iPSC-derived neural stem cells (Mixed Neuron from CW50065, Elixirgen scientific) were cultured 7.0×10^5 cells/cm^2 on the MEA

10 compounds (kainic acid, strychnine, phenytoin, 4-AP, pilocarpine, picrotoxin, pentylenetetrazol (PTZ), haloperidol, SKF83822, sulpiride) and dimethyl sulfoxide (DMSO) were added to neurons co-cultured with astrocytes at 3 or 5 concentrations for each compound. Each compound was cumulative administered in 14 weeks culture samples.

High-sensitivity MEA system [Alpha med scientific]

To record the electrophysiological responses to drugs, we used a planar MEA measurement system (Presto, Alpha Med scientific, Japan). The MEA chips contain 384 electrodes across 24 well plate with low impedance and high S/N ratio. Spontaneous firings in cumulative administration were recorded for 10 min per each. Spike detection were performed using Presto software (Alpha MED Scientific) . Synchronized burst firings (SBFs), major seizure-like activities, were detected using our '4-step method' (Matsuda et. al., BBRC, 2018) that can accurately detect the number of SBFs and the duration in a SBF.

Conclusion

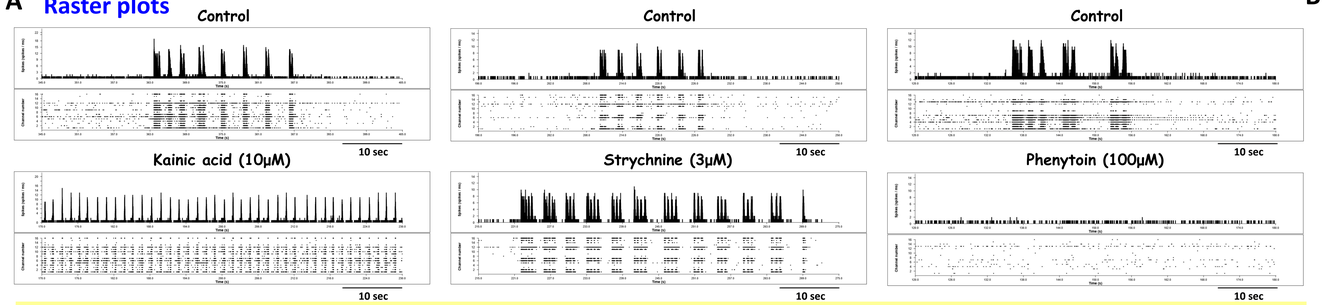
⇒ hiPSC-derived neurons (Elixirgen, LLC) responded to various convulsants, anti-epilepsy drug, antidepressant and antipsychotic and showed different responses depending on the MoA of drugs.

⇒ Principal component analysis, performed by selecting the optimal parameters, were effective in separated the mechanism of action of drugs.

⇒MEA assay coupled with hiPSC-derived neurons and our principal component analysis are useful for the prediction of seizure liability and MoA of new drugs and for drug screening.

Results① Electrophysiological responses to typical convulsants and AED in human iPSC-derived cortical neurons

A Raster plots



B

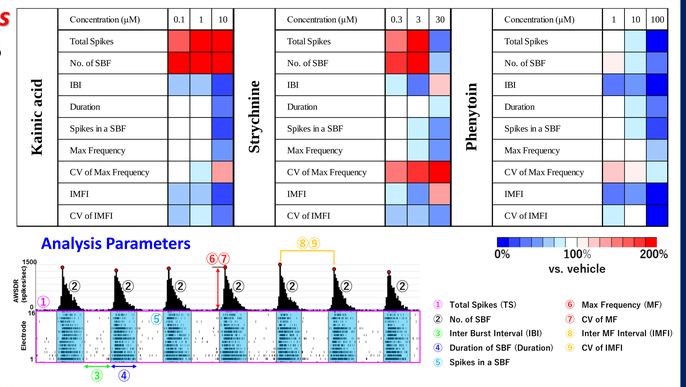
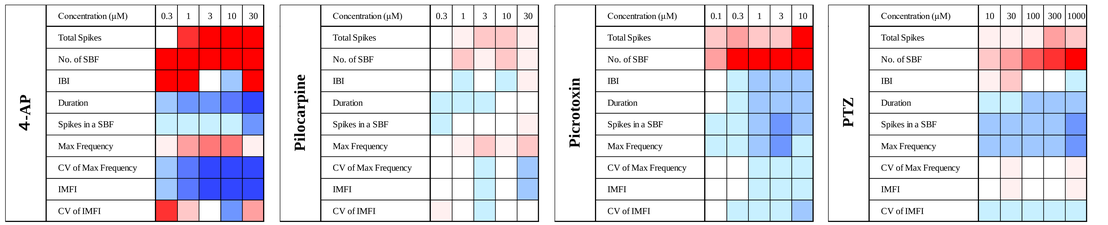


Fig. 1 Responses to typical convulsants (Kainic acid and Strychnine) and anti-epilepsy drug (Phenytoin).

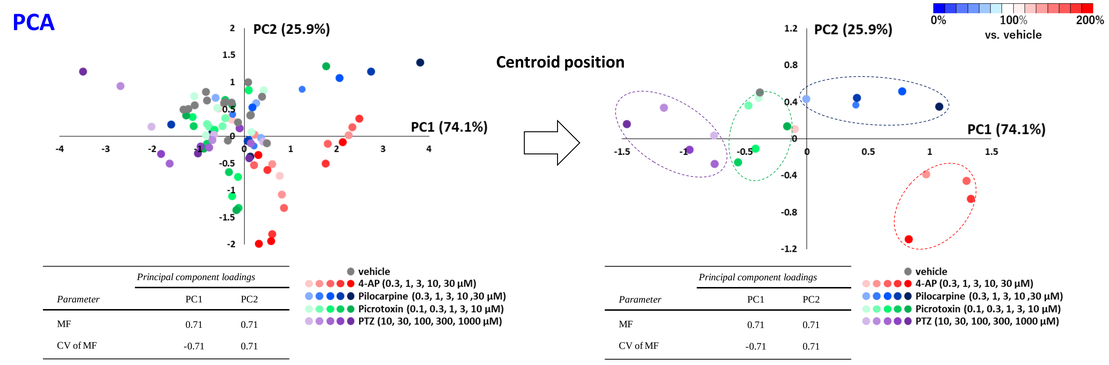
(A) Typical raster plots for 1 min with administration of kainic acid, strychnine and phenytoin . Upper: before administration. Lower: kainic acid (10 μM), strychnine (3 μM), and phenytoin (100 μM).
(B) Analysis results of 3 compounds (kainic acid, strychnine, and phenytoin). Spontaneous activities in cumulative administration for 10 minutes were analyzed. The increase or decrease of each parameter is indicated by color. Red and blue indicate the increase or decrease, respectively. Kainic acid (n = 5 wells), strychnine (n = 5 wells), phenytoin (n = 4 wells).

■ hiPSC-derived neurons responded significantly to typical convulsants and antiepileptic drugs.
■ Kainic acid and Strychnine with different MoA showed different firing patterns.

Results② Separation of responses to convulsants having different mechanism of action (MoA)



PCA



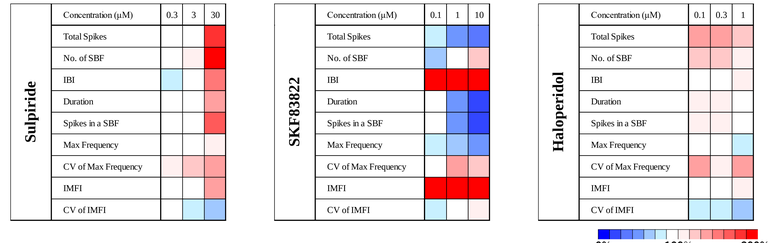
Principal component loadings

Parameter	PC1	PC2
MF	0.71	0.71
CV of MF	-0.71	0.71

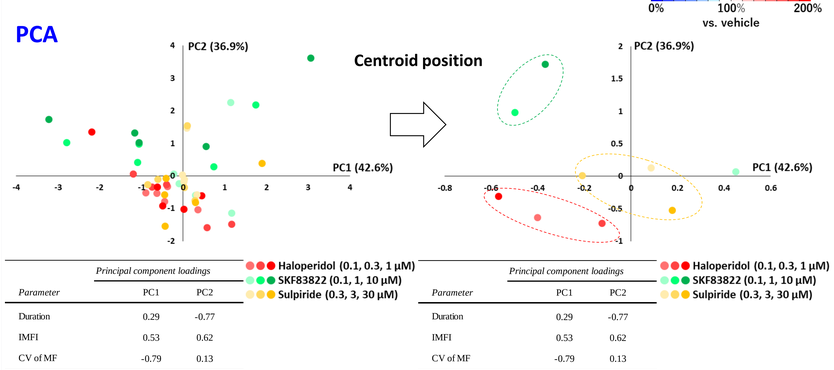
Fig. 2 Dose responses to 4 convulsants (4-AP, pilocarpine, picrotoxin, and PTZ) and principal component analysis (PCA).
Spontaneous activities in cumulative administration for 10 minutes were analyzed. The increase or decrease of each parameter is indicated by color. Red and blue indicate the increase or decrease, respectively. PCA was performed using two parameters (Maximum frequency in a burst(MF), and CV of MF). Score plot of PC1-PC2 for 4-AP (n = 4), pilocarpine (n = 3), picrotoxin (n = 5), PTZ (n = 3).

■Heat map differ depending on the MoA of convulsants.
■The compounds could be separated according to the mechanism of action by principal component analysis using MF and CV of MF.
■Picrotoxin and PTZ have smaller MaxFrequency and large variation compared with 4-AP and Pilocarpine.
■Compared with 4-AP, Pilocarpine has a large MaxFrequency and a large variation.

Results③ Dose-responses to antidepressant and antipsychotic



PCA



Principal component loadings

Parameter	PC1	PC2
Duration	0.29	-0.77
IMFI	0.53	0.62
CV of MF	-0.79	0.13

Fig. 3 Dose responses to 3 compounds (haloperidol, SKF83822, and sulpiride) and PCA.
Haloperidol (n = 5), SKF83822 (n = 5), sulpiride (n = 5).

■Heat map differ depending on the MoA of compounds. These responses reflects the difference of the subtype of dopamine receptor on which the compound acts.
■The responses to 3 compounds could be separated by principal component analysis using Duration, IMFI and CV of MF.