The electrophysiological responses to antibacterial drugs in human iPSC-derived neurons demonstrate the classification of antibacterial drug encephalopathy in clinical.

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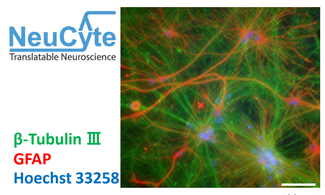
Introduction

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 convulsants using MEA in cultured hiPSC-derived neurons. In this study, we examined its application as evaluation system for the risk of antibiotic associated encephalopathy (AAE), which is a serious central nervous system disorder associated with antibiotic administration. AAE can be divided into 3 unique clinical phenotypes: encephalopathy accompanied by seizures (caused by cephalosporins and penicillin); encephalopathy characterized by psychosis (caused by quinolones, macrolides, and procaine penicillin); and encephalopathy accompanied by cerebellar signs (caused by metronidazole). We investigated whether the electrophysiological responses to antibacterial drugs in human iPS cell-derived neural networks classify into three clinically reported types. Human iPS cell-derived neurons (SynFire Co-Culture kit, Neucyte Inc.) and human iPS cell-derived astrocytes were co-cultured on MEA (maestro, AXION Biosystems). More than 10 antibiotics were cumulative administered in 5 weeks culture samples. We classified the responses to antibacterial drugs by multivariate analysis. Cephalosporins and penicillins, which are classified into type 1 AAE, increased the firing frequency and synchronized activities of the neural network. These results were consistent with seizures of type 1 AAE in clinically reported. Quinolones, macrolides, and procaine penicillin, which are classified into type 2 AAE in clinically reported, showed a different response from type 1. These results suggested that the responses to antibacterial drugs in human iPSC-derived neurons and the classification of antibacterial drug encephalopathy in clinical are correlated. MEA assay coupled with human iPSC-derived neurons and our multivariate analysis are useful for the risk prediction and risk classification of AAE.

Material & Methods

Human iPSC-derived cortical neurons [Neucyte]

Human iPSC-derived glutamatergic neuron, human iPSC-derived GABAergic neuron, and human primary astrocyte (SynFire Co-Culture kit, Neucyte Inc.) were co-cultured 8.0 × 10^5 cells/cm^2 at ratio of 7 : 3 : 3.5 on the MEA.

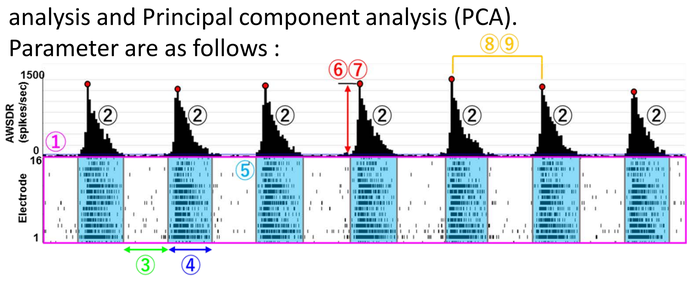


MEA system [AXION Biosystems]

To record the electrophysiological responses, we used a planar MEA measurement system. 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 AxIS Navigator software (AXION Biosystems). Synchronized burst firings (SBFs), major seizure-like activities, were detected using our '4-step method' (Matsuda et. al., BBRC, 2018) .

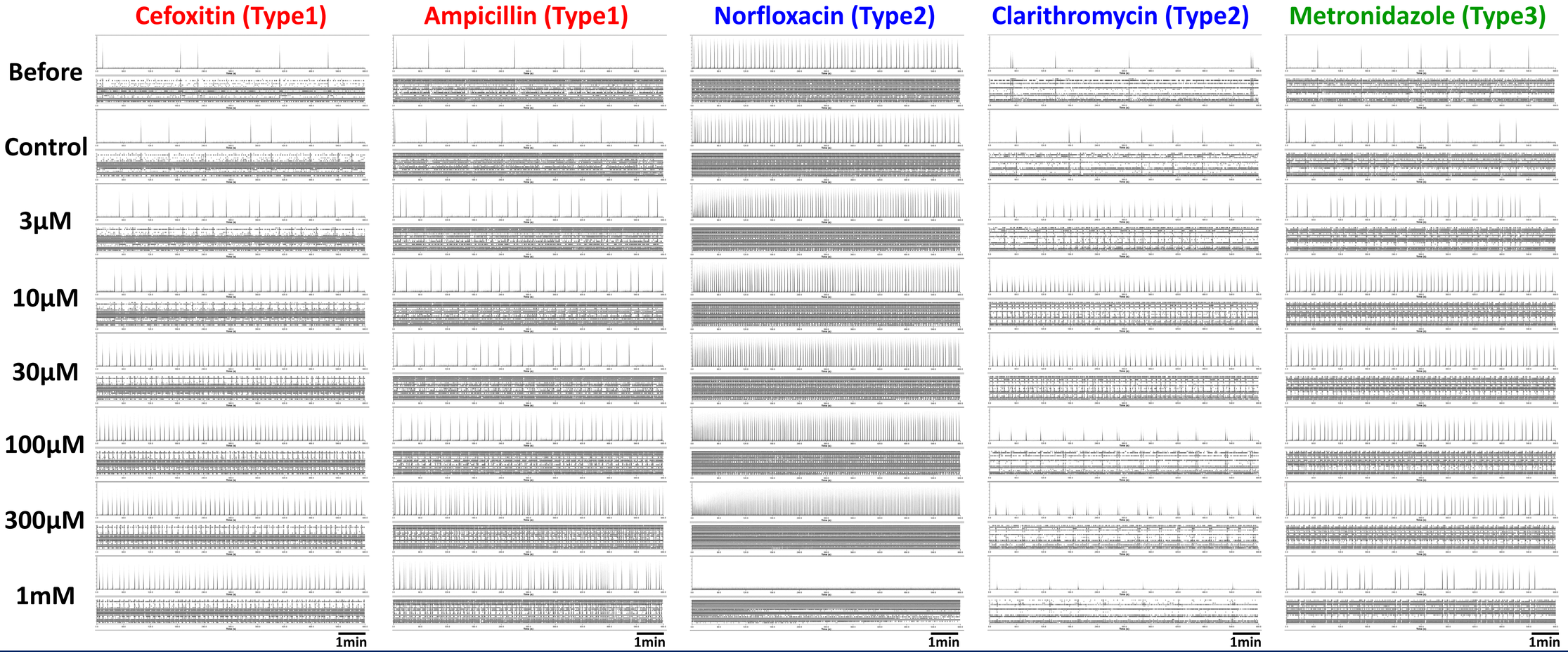
Analysis Parameters

Parameters calculated from 4-step methods were used for Burst analysis and Principal component analysis (PCA).
Parameter are as follows :



① Total Spikes (TS)
② No. of SBF
③ Inter Burst Interval (IBI)
④ Duration of SBF (Duration)
⑤ Spikes in a SBF
⑥ Max Frequency (MF)
⑦ CV of MF
⑧ Inter MF Interval (IMFI)
⑨ CV of IMFI

Results① Electrophysiological response to human iPS cell-derived neurons to antibiotic administration



■Cephalosporins and penicillins, which are classified into type 1 AAE, increased the firing frequency and synchronized activities of the neural network.

■Quinolones, macrolides, and procaine penicillin, which are classified into type 2 AAE, decreased the firing frequency and synchronized activities of the neural network at high concentration.

■Metronidazole, which is classified into type 3 AAE, increased synchronized activities at middle concentration and decreased at high concentration.

Results② Activity responses measured by 9 parameters

0% 100% 200%
vs. vehicle

Type1 AAE (Cephalosporins, Penicillin)

	Concentration (μM)	3	10	30	100	300	1000
Cefepime	Total Spikes						
	No. of SBF						
	IBI						
	Duration						
	Spikes in a SBF						
	Max Frequency						
	IMFI						
	CV of Max Frequency						
	CV of IMFI						

	Concentration (μM)	3	10	30	100	300	1000
Cefoxitin	Total Spikes						
	No. of SBF						
	IBI						
	Duration						
	Spikes in a SBF						
	Max Frequency						
	IMFI						
	CV of Max Frequency						
	CV of IMFI						

	Concentration (μM)	3	10	30	100	300	1000
Ampicillin	Total Spikes						
	No. of SBF						
	IBI						
	Duration						
	Spikes in a SBF						
	Max Frequency						
	IMFI						
	CV of Max Frequency						
	CV of IMFI						

Type2 AAE (Quinolones, Macrolides, S/T)

	Concentration (μM)	3	10	30	100	300	1000
Norfloxacin	Total Spikes						
	No. of SBF						
	IBI						
	Duration						
	Spikes in a SBF						
	Max Frequency						
	IMFI						
	CV of Max Frequency						
	CV of IMFI						

	Concentration (μM)	3	10	30	100	300	1000
Ofloxacin	Total Spikes						
	No. of SBF						
	IBI						
	Duration						
	Spikes in a SBF						
	Max Frequency						
	IMFI						
	CV of Max Frequency						
	CV of IMFI						

	Concentration (μM)	3	10	30	100	300	1000
Nalidixic acid	Total Spikes						
	No. of SBF						
	IBI						
	Duration						
	Spikes in a SBF						
	Max Frequency						
	IMFI						
	CV of Max Frequency						
	CV of IMFI						

	Concentration (μM)	3	10	30	100	300	1000
Clarithromycin	Total Spikes						
	No. of SBF						
	IBI						
	Duration						
	Spikes in a SBF						
	Max Frequency						
	IMFI						
	CV of Max Frequency						
	CV of IMFI						

	Concentration (μM)	3	10	30	100	300	1000
S/T合剤	Total Spikes						
	No. of SBF						
	IBI						
	Duration						
	Spikes in a SBF						
	Max Frequency						
	IMFI						
	CV of Max Frequency						
	CV of IMFI						

	Concentration (μM)	3	10	30	100	300	1000
Trimethoprim	Total Spikes						
	No. of SBF						
	IBI						
	Duration						
	Spikes in a SBF						
	Max Frequency						
	IMFI						
	CV of Max Frequency						
	CV of IMFI						

	Concentration (μM)	3	10	30	100	300	1000
Penicillin G Procaine	Total Spikes						
	No. of SBF						
	IBI						
	Duration						
	Spikes in a SBF						
	Max Frequency						
	IMFI						
	CV of Max Frequency						
	CV of IMFI						

Type3 AAE (Metronidazole)

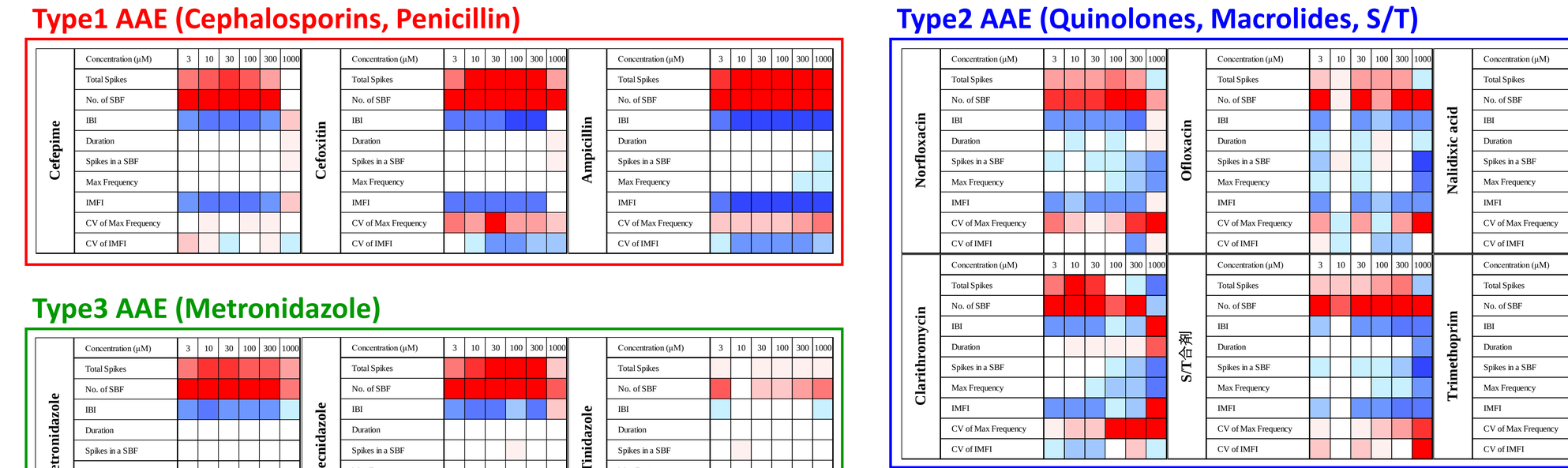
	Concentration (μM)	3	10	30	100	300	1000
Metronidazole	Total Spikes						
	No. of SBF						
	IBI						
	Duration						
	Spikes in a SBF						
	Max Frequency						
	IMFI						
	CV of Max Frequency						
	CV of IMFI						

	Concentration (μM)	3	10	30	100	300	1000
Secnidazole	Total Spikes						
	No. of SBF						
	IBI						
	Duration						
	Spikes in a SBF						
	Max Frequency						
	IMFI						
	CV of Max Frequency						
	CV of IMFI						

	Concentration (μM)	3	10	30	100	300	1000
Tinidazole	Total Spikes						
	No. of SBF						
	IBI						
	Duration						
	Spikes in a SBF						
	Max Frequency						
	IMFI						
	CV of Max Frequency						
	CV of IMFI						

■Drugs classified as type 1 AAE have an increased CV of Max Frequency.
■All types showed increase Total Spikes and No. of SBF, but drugs classified as type 2 AAE showed sharp decrease in No. of SBF at high dose.
■Drugs classified as type1 AAE and type3 AAE showed no change in Duration, Spikes in a SBF, and Max Frequency, but drugs classified as type2 AAE changed significantly.

Results④ Classification of responses to antibacterial drugs in human iPSC-derived neurons



■PCA map that allows classification of antibacterial drugs was created by principal component analysis using the No. of SBFs and spikes of SBFs.
■The drugs of type1 AAE, type2 AAE, and type3 AAE were separately distributed.

⇒The classification of antibacterial drugs by the characteristics of electrophysiological response in human iPS cell-derived neural network was consistent with the classification of antibacterial drugs by the characteristics of clinical antibacterial encephalopathy [1].

1)Bhattacharyya S, Neurology, 2016 ;86(10):963-71

Conclusion

■Human iPS cell-derived neurons showed changes in neural activity depending on the type of AAE.

■The classification of antibacterial drugs by the characteristics of electrophysiological response in human iPS cell-derived neural network was consistent with the classification of antibacterial drugs by the characteristics of clinical antibacterial encephalopathy.

■MEA assay coupled with human iPSC-derived neurons and our multivariate analysis are useful for the risk prediction and risk classification of AAE.