Toxicological Responses in cultured human iPSC-derived neuronal networks using high-throughput MEA system

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Introduction

The functional network of human induced pluripotent stem cell (hiPSC)-derived neurons is a potentially powerful in vitro model for evaluating drug toxicity. Epileptiform activity is one of phenomena in neuronal toxicology. To evaluate the dynamics of epileptiform activities and the effect of anti-convulsant drug in cultured hiPSC-derived neurons, we used the high-throughput multielectrode array (MEA) system, where we simultaneously record extracellular potentials for 16 channels per well across 24-well plates. We firstly confirmed the modulation of activity by typical glutamatergic receptor antagonists/agonists in spontaneous firings. Spontaneous activities and pharmacological responses against synaptic related drugs were detected with high S/N ratio using high-throughput MEA system. Next, we examined chemically evoked epileptiform activity. Electrophysiological seizes were induced by pentylentetrazole (PTZ) and 4-Aminopyridine (4-AP), the most widely used chemical convulsant in animal models to screen for new anti-epilepsy drugs. We also examined the anti-convulsant effects of common clinical anti-epilepsy drugs (AEDs), phenytoin. PTZ and 4-AP induced a rapid increase in synchronized burst firings (SBFs) in a concentration-dependent manner. Phenytoin suppressed PTZ-induced epileptiform activity. From these results, we suggest that the electrophysiological assay in cultured human iPSC-derived neuron using high-throughput MEA system is a useful to investigate the neuronal toxicity in drug screening and pharmacological effects of human neurological disease.

Material & Methods

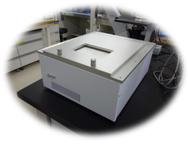
Human iPSC-derived neurons [XCell Science]

Human iPSC-derived neurons (XCell Science) were cultured 3.0×10^5 cells/cm^2 on the MEA. Human iPSC-derived mature astrocyte (XCell Science) were added 1.0×10^5 cells/well. A After 8 days culture, medium was exchanged to BrainPhys medium (Stem cell technologies).

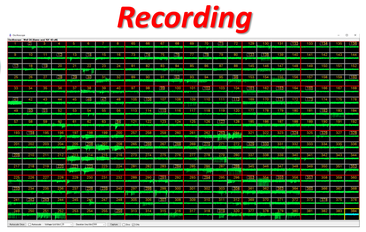
High-Througput MEA system [Alpha med scientific]

24 wells (384 electrodes)

System (Presto)



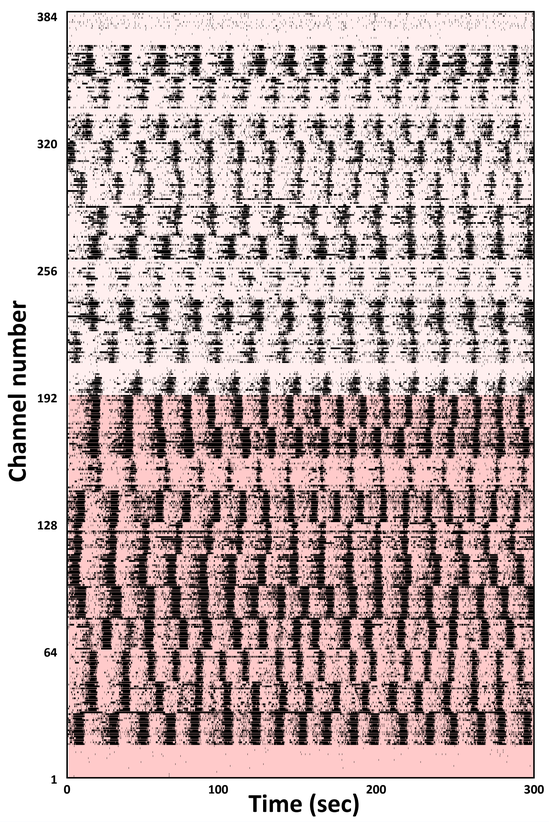
Recording



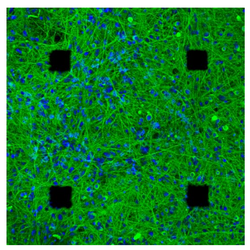
Low impedance and high sensitivity

Laster plots of spontaneous firings in hiPSC-derived neuros

Co-culture neuros with astrocytes (12 wells) Neuros(12 wells)



hiPSC-derived neurons on the MEA



Spontaneous extracellular field potentials were acquired at 37° C under a 5% CO_2 atmosphere using the high-throughput multielectrode array system, where we simultaneously record extracellular potentials for 16 channels per well across 24-well plates (Presto, Alpha Med Scientific) at a sampling rate of 20 kHz/channel. Signals were low-pass filtered at 100 Hz and stored on a personal computer. Firing analyses and spike sorting were performed using Mobius software (Alpha Med Scientific Inc.).

Result 1 Time course in spontaneous firings

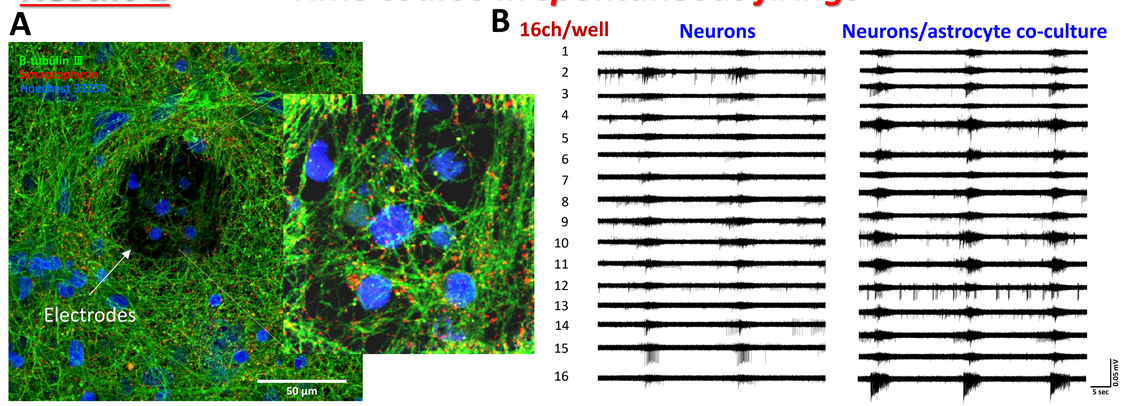


Fig. 1. (A) Immunofluorecent image. Red: synaptophysin. (B) Typical spontaneous firings in neurons and co-culture sample at 9WIV. (C) Time course in firing rate.

Synchronized burst firings by synaptic transmission were observed at 6 weeks culture both culture samples.

Result 2 Electrophysiological responses of NMDA and AMPA receptor at 6 weeks culture

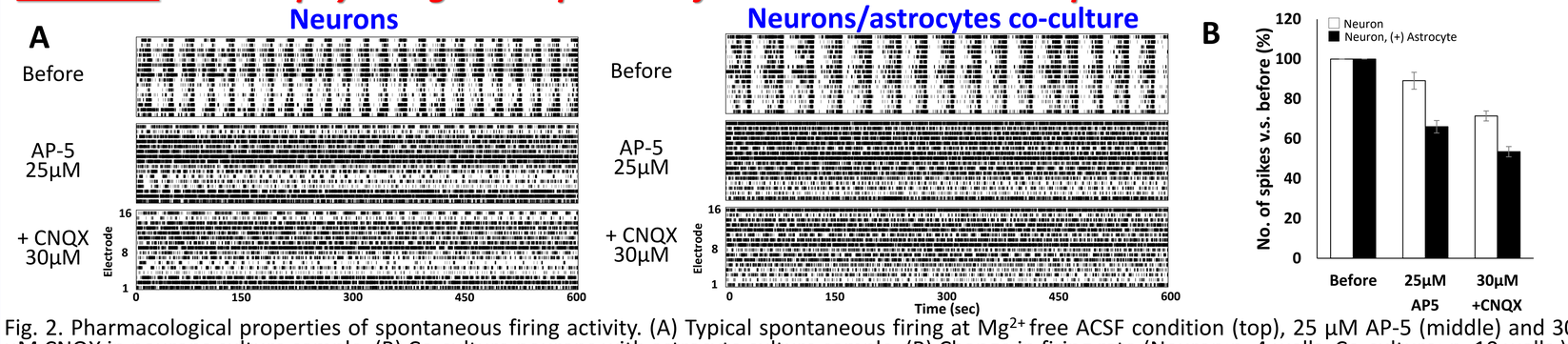


Fig. 2. Pharmacological properties of spontaneous firing activity. (A) Typical spontaneous firing at Mg^{2+} free ACSF condition (top), 25 μM AP-5 (middle) and 30 μM CNQX in neurons culture sample. (B) Co-culture neurons with astrocyte culture sample. (B) Change in firing rate.(Neuron:n=4 wells, Co-culture: n=10 wells.)

NMDA and AMPA receptors had electrophysiological function at 6 weeks culture both culture samples.

Result 3 Induction of epileptiform activity and effects of anti-epilepsy drugs

4-AP, Phenytoin

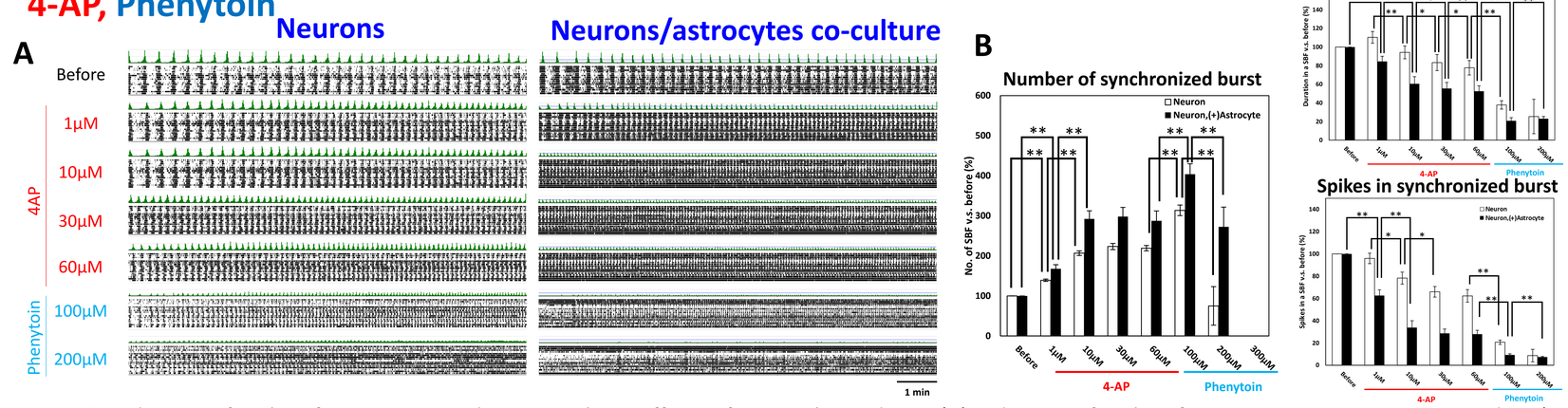


Fig. 3. Induction of epileptiform activity and anticonvulsant effects of anti-epilepsy drugs. (A) Induction of epileptiform activity using 4-Aminopyridine (4-AP) and the suppressive effect of phenytoin in neurons and co-culture neurons with astrocyte sample. (B) Changes in synchronized burst firings.

PTZ, Phenytoin

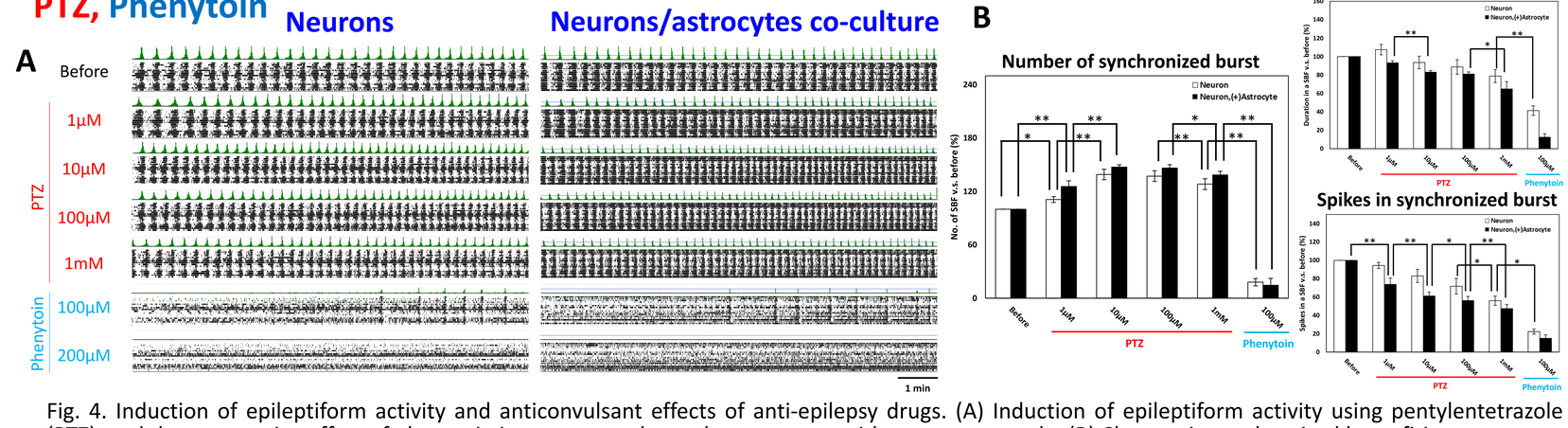


Fig. 4. Induction of epileptiform activity and anticonvulsant effects of anti-epilepsy drugs. (A) Induction of epileptiform activity using pentylentetrazole (PTZ) and the suppressive effect of phenytoin in neurons and co-culture neurons with astrocyte sample. (B) Changes in synchronized burst firings.

The induction of epileptiform activity by 4-AP and PTZ and the suppressive effects by phenytoin were observed both neurons and co-culture with astrocyte samples.

Conclusion

In conclusion, we detected epileptiform activities and effects of aniti-epilepsy drugs in cultured hiPSC-derived neuronal networks and found that functional maturation at 6 weeks culture both neuros culture sample and co-culture neurons with astrocyte sample. High-throughput MEA system in cultured hiPSC-derived neurons proved useful for neuro pharmacological and neuro toxicological assays. Our results also provide an important indication for the toxicological evaluation using in vitro human neurons.

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