Electrophysiological responses of cultured human iPSC-derived neuronal networks to convulsants or anti-convulsant drugs

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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 examined chemically evoked epileptiform activity. Epileptiform activityies were induced by 4-Aminopyridine (4-AP), pilocarpine, chlorpromazine, and pentylenetetrazole (PTZ) . The number of synchronized burst firings were increased in a concentration dependent manner at 4-AP, Pilocarpine, and Chlorpromazine administration. On the other hand, the duration and spikes in a synchronized burst were increased at PTZ administration. Phenytoin used in anti-convulsant drug suppressed electrophysiological activities. From these results, we suggest that the electrophysiological assay in cultured human iPSC-derived neuron using MEA system has the potential to investigate the neuronal toxicity in drug screening.

Material & Methods

Human iPSC-derived neurons and astrocytes

Human iPSC-derived cortical neurons (Axol Bioscience Science) were cultured 8.0×10^5 cells/cm^2 on the MEA. After 8 days culture, Human iPSC-derived mature astrocyte (Axol Bioscience) were added 1.0×10^5 cells/well. We prepared the co-culture with astrocytes sample and only neurons culture samples.

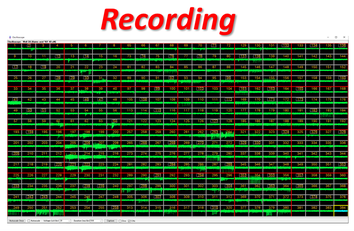
To investigate pharmacological effects, we administered 4-aminoprysine (0, 0.3, 1, 3, 10, 30 µM), pilocarpine (0, 0.3, 1, 3, 10, 30 µM), chlorpromazine (0, 0.1, 0.3, 1, 3, 10 µM), pentylentetrazole (0, 1, 10, 100, 1000 µM), isoniazid (0, 10, 30, 100, 300, 1000 µM), acetaminophen(0, 1, 3, 10, 30, 100 µM), and phenytoin (0, 1, 3, 10, 30, 100 µM). Spontaneous firings in cumulative administration were recorded for 10 min per each concentration at 12-19 weeks culture.

High-Througput MEA system

To evaluate the electrophysiological responses against drugs, we used a planar MEA measurement system (Presto, Alpha Med s cientific, Japan). The MEA chips contain 384 electrodes across 24 well plate with low impedance and high S/N ratio. Spike analyses were performed using Presto and MOT software (Alpha MED Scientific) .

24 wells (384 electrodes)

Recording



Result 1 Induction of epileptiform activities and an effect of anti-convulsant drug in co-cultured hiPSC-derived cortical neurons

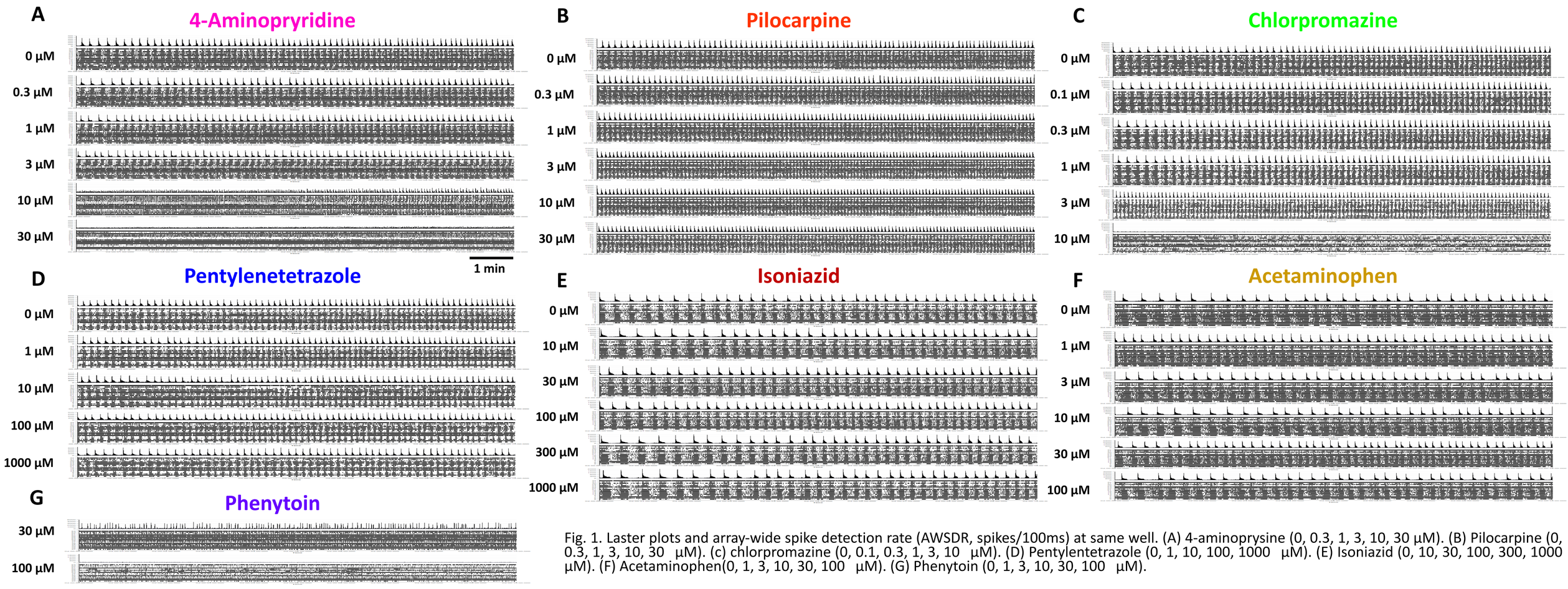


Fig. 1. Laster plots and array-wide spike detection rate (AWSDR, spikes/100ms) at same well. (A) 4-aminoprysine (0, 0.3, 1, 3, 10, 30 µM). (B) Pilocarpine (0, 0.3, 1, 3, 10, 30 µM). (c) chlorpromazine (0, 0.1, 0.3, 1, 3, 10 µM). (D) Pentylentetrazole (0, 1, 10, 100, 1000 µM). (E) Isoniazid (0, 10, 30, 100, 300, 1000 µM). (F) Acetaminophen(0, 1, 3, 10, 30, 100 µM). (G) Phenytoin (0, 1, 3, 10, 30, 100 µM).

■The number of synchronized burst firings were increased in a concentration dependent manner at 4-AP, Pilocarpine, and Chlorpromazine administration. Accordingly, the duration and spikes in a synchronized burst were decreased. ■The duration and spikes in a synchronized burst were increased at PTZ administration.
■Significant changes were not observed at isoniazid and acetaminophen administration. ■Synchronized burst firings disappeared at 100 µM phenytoin administration.

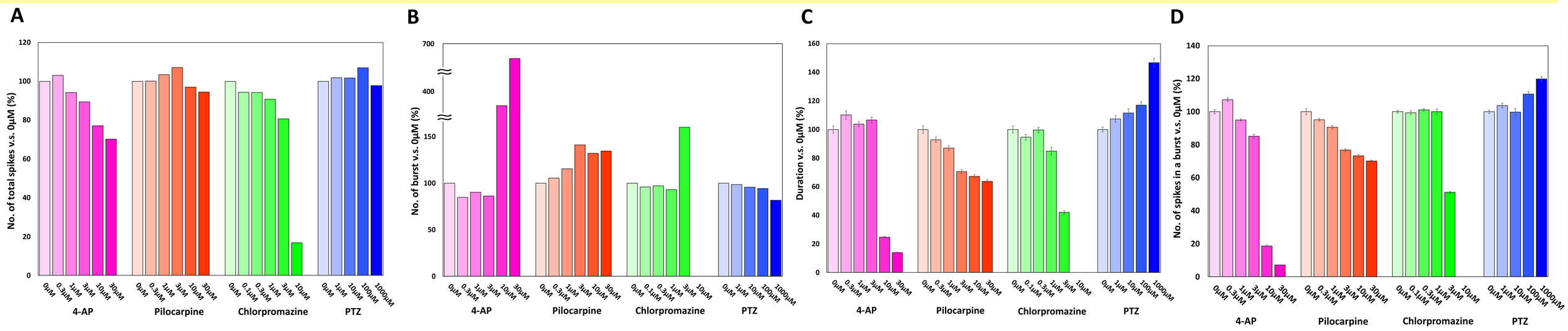


Fig. 2. The changes of firing properties in drug administration. (A) Total spikes rate v.s. 0 µM. (B) No. of synchronized burst firings v.s. 0 µM. (C) Duration in a synchronized burst v.s.s 0 µM. (D) No. of spikes in a synacronized burst v.s. 0µM.

Result 2 Induction of epileptiform activities in cultured hiPSC-derived cortical neurons

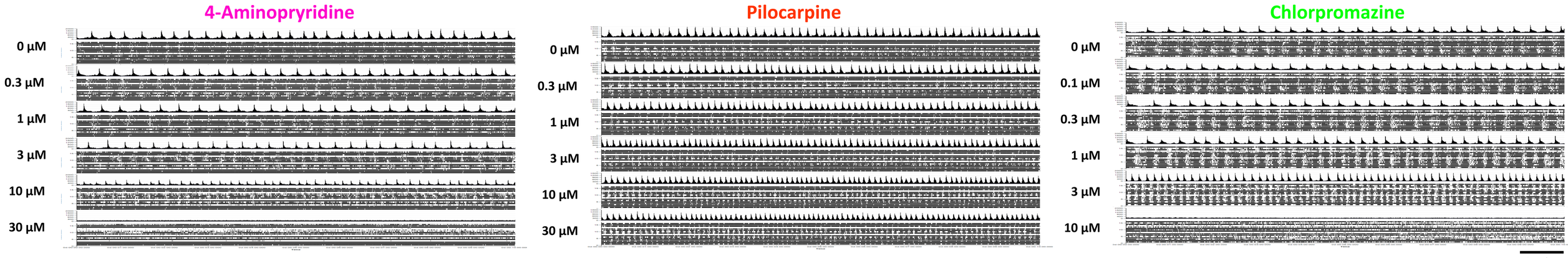


Fig. 3. Laster plots and array-wide spike detection rate (AWSDR, spikes/100ms) at same well. (A) 4-aminoprysine (0, 0.3, 1, 3, 10, 30 µM). (B) Pilocarpine (0, 0.3, 1, 3, 10, 30 µM). (c) chlorpromazine (0, 0.1, 0.3, 1, 3, 10 µM).

■Although the firing rate is different, response properties in only neurons culture sample were almost the same as co-culture sample at 4-AP, pilocarpine and chlorpromazine administration.

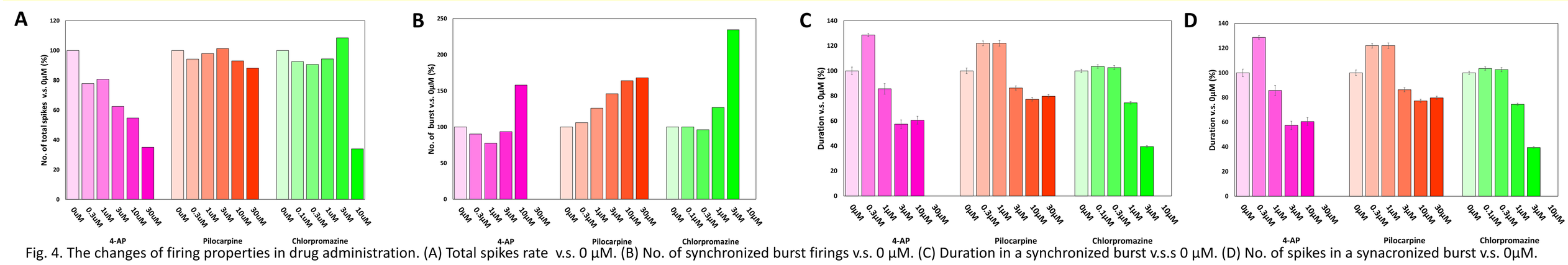


Fig. 4. The changes of firing properties in drug administration. (A) Total spikes rate v.s. 0 µM. (B) No. of synchronized burst firings v.s. 0 µM. (C) Duration in a synchronized burst v.s.s 0 µM. (D) No. of spikes in a synacronized burst v.s. 0µM.

Conclusion

In conclusion, we detected epileptiform activities using typical convulsants in cultured hiPSC-derived neuronal networks and found the differences of response properties depending on the type of convulsants. Although the firing rate is different, response properties in only neurons culture sample were almost the same as co-culture sample at 4-AP, pilocarpine and chlorpromazine administration. High-throughput MEA system in cultured hiPSC-derived neurons proved useful for toxicological assays with electrophysiological functions.

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