In vitro electrophysiological drug testing using human induced pluripotent stem cell-derived neurons

I. Suzuki[1*], A. Odawara[1,2], Y. Shi[3], H. Jiko[4]

[1] Department of Electronics and Intelligent Systems, Tohoku Institute of Technology, Miyagi, Japan,
[2] Japan Society for the Promotion of Science, Tokyo, Japan
[3] Axol Bioscience Inc., Cambridge, United Kingdom
[4] Alpha MED Scientific Inc., Osaka, Japan

Introduction

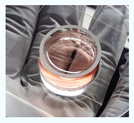
Human induced pluripotent stem cell (hiPSC)-derived neurons may be effectively used for drug discovery and cell-based therapy. We here used a multi-electrode array (MEA) system to investigate the functional characteristics of hiPSC-derived neurons on their long-term spontaneous activity and drug responsiveness over 300 days culture. We demonstrated that hiPSC-derived neurons allowed the culture to be maintained over 10 months with long-term spontaneous activity. After 70 days of culture, we observed synchronous burst firing activity due to synapse transmission within neuronal networks. Addition of the synapse agonist and antagonists kainic acid, bicuculline, CNQX and AP5 induced significant changes of the firing rate in spontaneous firings and electrical evoked responses. Furthermore, we demonstrated that epilepsy phenomenon was evoked by administration of pentylentetrazole (PTZ) and was inhibited by anti-epilepsy drug phenytoin and sodium valproate (VPA). High frequency synchronized bursts were evoked over PTZ 100 µM. These bursts were gradually decreased with the increasing the dose of anti-epilepsy drug, and disappeared over phenytoin 100µM or VPA 1 mM respectively. These results suggested that long-term electrophysiological measurements in hiPSC-derived neurons using a MEA system may be beneficial for drug screening applications.

Material & Methods

(A) Long-term electrophysiological measurement

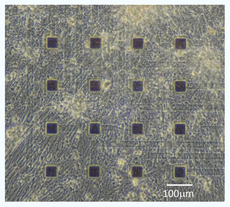
300 days on the MEA dish

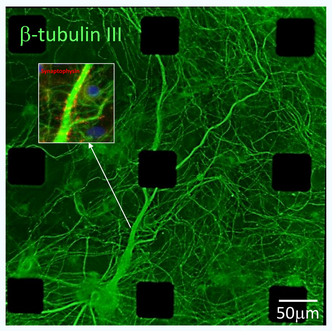
Multi-electrode array chip



Alpha Med Scientific Inc

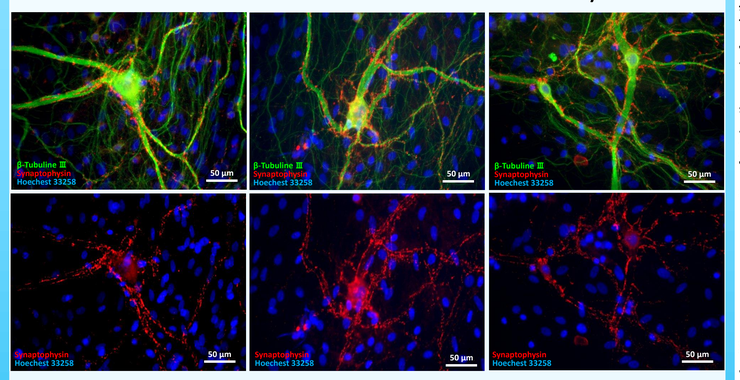
294 days culture on the MEA dish





(B) Human iPSC-derived neurons

Maturation hiPSC-derived neurons after 300 days culture



- To evaluate the long-term electrophysiological feature and drug effect of hiPSC-derived neurons, we used a planar MEA measurement system (Alpha Med Scientific, Japan). The MEA chips were 64 electrodes (MED-P515A) with low impeadance and high S/N ratio.
- hiPSC-derived neurons [Cerebral Cortical Neurons; Axol Bioscience Inc., UK] (1). were cultured on MEA chips. Long-term culture of hiPSC-derived neurons were performed by astrocyte co-culture method(2).
- Firing analyses were performed using Mobius software (Alpha Med Scientific) and MATLAB.

Result 1 Time course of spontaneous firings

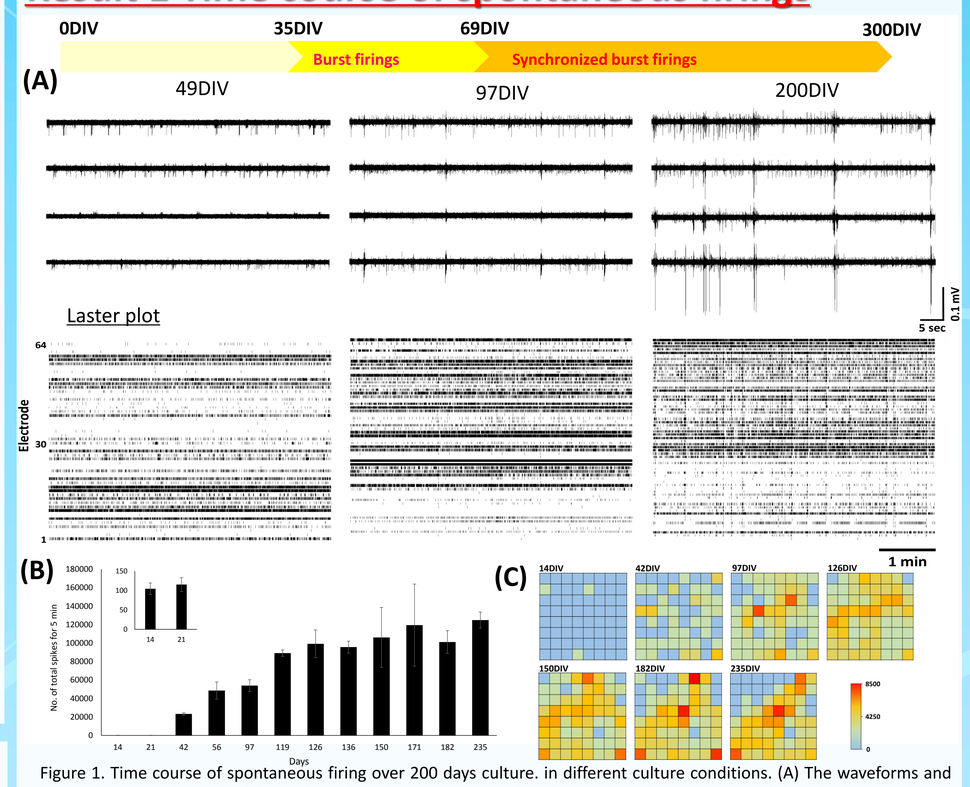


Figure 1. Time course of spontaneous firing over 200 days culture. in different culture conditions. (A) The waveforms and laster plot at 64 electrode. (B) Number of total spontaneous spikes detected vs. the time course. (C) Grids showing the 64 electrodes where colored electrodes detected signals. Electrodes that detected a higher firing frequency are shown in red.

- Synchronized burst firings were observed over 70 days culture.
- Firing rate and synchronized burst firings increased up to about 200 days

Result 2 Drug effect in spontaneous firings

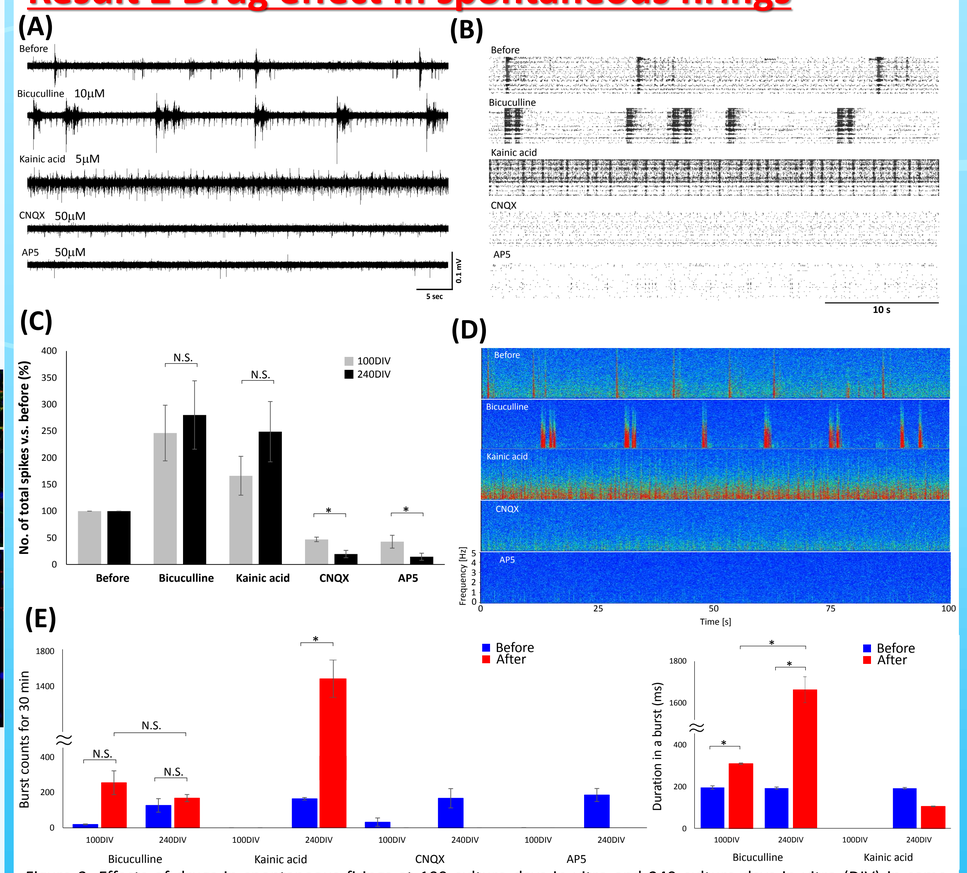


Figure 3. Effects of drugs in spontaneous firings at 100 culture days in vitro and 240 culture days in vitro (DIV) in same samples. (A) The waveforms represent typical changes in spontaneous firings after Bicuculline 10µM, Kainic acid 5µM, CNQX 50µM, and AP5 50µM administration at 240 DIV. (B) Laster plot at 64 electrode after drug administration. (C) Percentage of number of total spikes v.s. before (n=3 MEA dishes, *P<0.05). (D) Fast Fourier Transform(FFT) of spontaneous firing after different drug administration. (E) (a) Syncronized burst counts of before and after different drug administration at 100 DIV and 240 DIV (*P<0.05). (b) Duration of synchronized burst before and after Bicuculline and Kainic acid administration (*P<0.05).

- Bicuculline increased in duration of synchronized burst firings. Kainic acid increased the number of synchronized burst firings. After CNQX and AP5 administration, burst firings were disappeared.
- Functional ion channel response at 240 DIV was better than the responses at 100 DIV.
- Bicuculline induced high frequency firings. Firings frequency was different by type of synaptic's drug.

Result 3 Drug effect in evoked responses

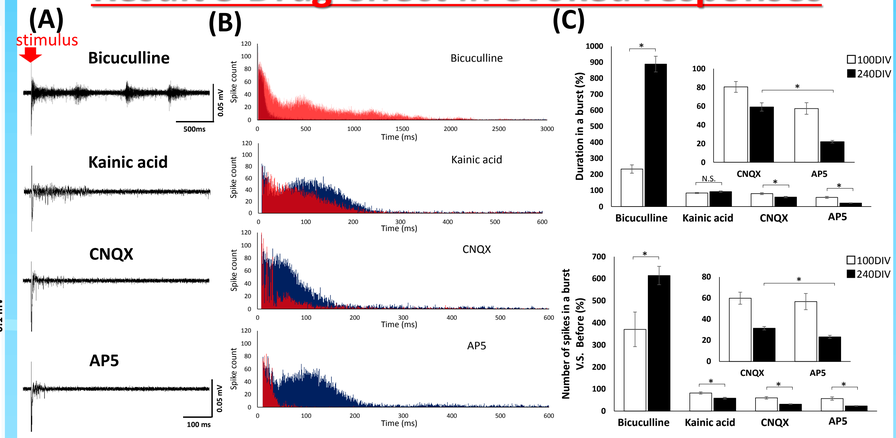


Figure 3. Effects of drugs in electrical evoked response. (A) The waveforms represent typical changes in the evoked responses after drug administration at 240 culture days in vitro (DIV). (B) Peri-stimulus time histogram (PSTH) of 120 trace data at an electrode. (C) Percentage of duration in a burst and number of spikes in a burst before and after drug administration at 100 DIV and 240 DIV (*P < 0.01).

- Effects of synaptic's drug were detected in evoked responses by electrical stimulation.
- Duration and number of spikes in a burst were different by the type of synaptic's drug.
- Change of evoked responses were significant compared with evoked responses at 100 DIV.
- AP5 significantly decreased the duration of burst. This results suggest functioning NMDA receptors.

Result 4 Epilepsy phenomenon and drug effects

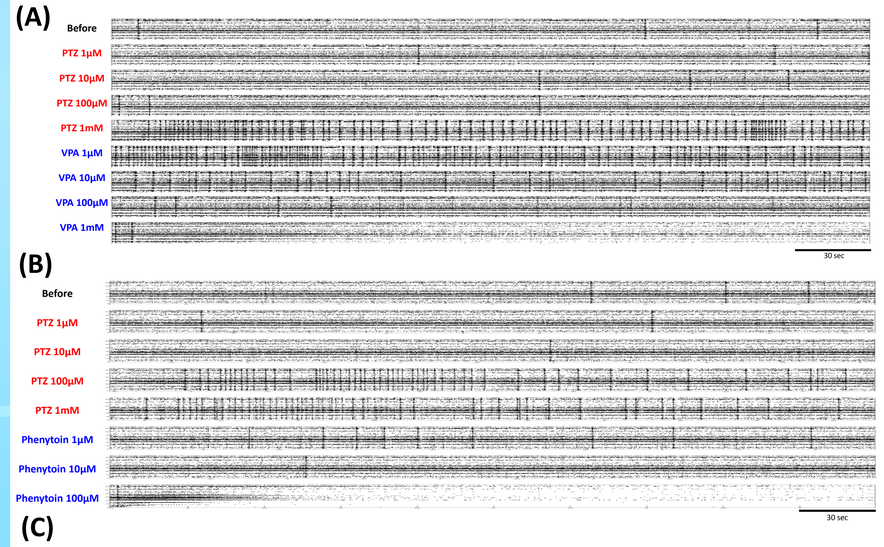


Figure 4. Evoked epilepsy phenomenon and the effects of anti-epilepsy drugs. (A)Later plot in PTZ and VPA administration. (B) Laster plot in PTZ and Phenytoin administration.(C) Changes in firing rate and burst count

- Epilepsy phenomenon was evoked by administration of pentylentetrazole (PTZ) and was inhibited by anti-epilepsy drug phenytoin and sodium valproate (VPA).

Conclusion

- The hiPSC-derived neurons allowed the long time culture for more than 300 days and the functional matulation using co-culture with astrocyte.
- We have succeeded in the detection of the long-term electrophysiological features and of synaptic's drugs effects in cultured hiPSC-derived neurons.
- hiPS-derived neurons at 240 days culture were more functional compared with 100 days culture.
- Evoked responses by electrical stimulation are useful to evaluate the effects of drugs.
- Detection of epilepsy phenomenon and effects of anti-epilepsy drug suggest human epilepsy model for drug screening.
- Long-term electrophysiological measurement using multi-electrode arrays enabled drug screening and toxicological assay.

Reference

(1) Shi Y, Kirwan P, Livesey FJ. Nature Protcol 2012, 7: 1836-1846.
(2) Odawara A, Sitoh Y. Gotoh M. Suzuki I. Biochem Biophys Res Commun 2014, 443: 1176-1181

Contact information: i-suzuki@tohtech.ac.jp

International Society For Stem Cell Research, 13th Annual Meeting, June 24-27, 2015