Functional maturation and drug responses of human induced pluripotent stem cell-derived cortical neuronal networks in long-term culture

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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 disease mechanisms and drug responses. However, the culture time required for the full functional maturation of individual neurons and networks is uncertain. We investigated the development of spontaneous electrophysiological activity and pharmacological responses for over 1 year in culture using multi-electrode arrays (MEAs). The complete maturation of spontaneous firing, evoked responses, and modulation of activity by glutamatergic and GABAergic receptor antagonists/agonists required 20–30 weeks. At this stage, neural networks also demonstrated epileptic form synchronized burst firing (SBF) in response to pro-convulsants and SBF suppression using clinical anti-epilepsy drugs. Our results reveal the feasibility of long-term MEA measurements from hiPSC-derived neuronal networks in vitro for mechanistic analyses and drug screening. However, developmental changes in electrophysiological and pharmacological properties indicate the necessity for the international standardization of culture and evaluation procedures.

Material & Methods

Human iPSC-derived cerebral cortical neurons [AxolBioscience Inc.] (1)

・Long-term culture of hiPSC-derived neurons were performed by astrocyte co-culture method (2). We performed long-term culture over 300days.

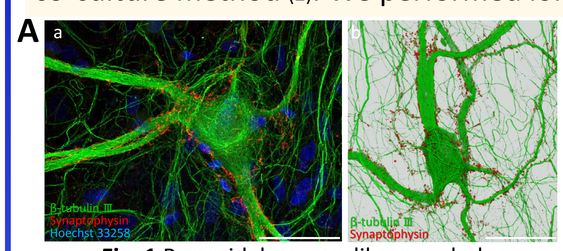
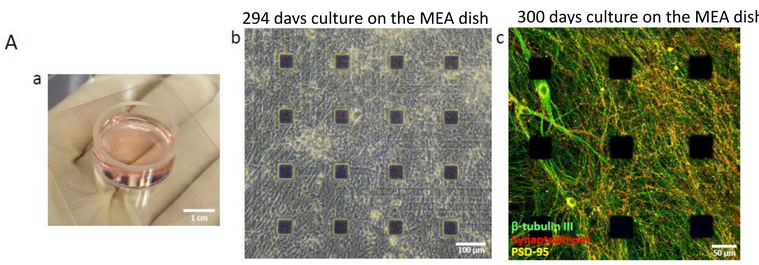


Fig. 1 Pyramidal neuron-like morphology and synaptogenesis at 300 DIV.

(A) Scale bars = 50 µm. Green: neuronal marker β-tubulin III. Red: presynaptic marker (synaptophysin). Blue: nuclear marker (Hoechst 33258) revealing underlying astrocytes. Synapses were formed around the soma and thick dendrites (B) Formation of pre- and postsynaptic structures at 300 DIV. Blue: nuclear marker Hoechst 33258. Green: neuronal marker β-tubulin III. Red: presynaptic marker synaptophysin. Yellow: postsynaptic marker PSD-95.

Multi-electrodearray system [Alpha med scientific Inc.]



・To evaluate the long-term electrophysiological characteristics and drug effects of hiPSC-derived neurons, we used a planar MEA measurement system (Alpha MEDS cientific, Japan). The MEA chips contain 64 electrodes (MED-P515A) with low impedance and high S/N ratio. Spike analyses were performed using Mobius software (Alpha MED Scientific) and MATLAB.

Result 1 Development of spontaneous firing during long-term culture

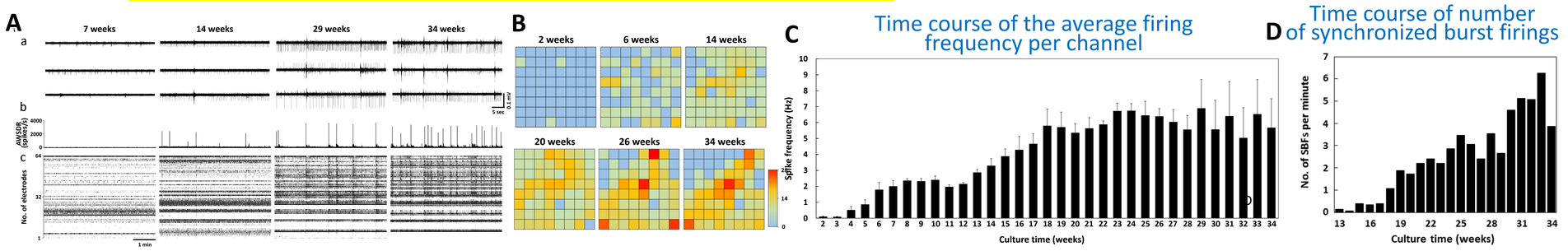


Fig.2.(A) Changes in spontaneousfiringpattern inthesamelong-term cultureat 7,14,29,and 34 weeks invitro (WIV). (a) Typical spontaneousfiring patterns.(b)Raster soft hearray-wide spike detection rate (AWSDR, spikes/s). Bin sizeis 1ms.(c)Corresponding raster plots for all 64 electrodes over 5 min. (B) Electrode grids color-coded to indicate mean spontaneous firing frequency from same culture at 2, 6, 14, 20, 26, and 34 WIV. Red indicates electrodes with higher firing frequencies. Scale bar in Hz (maximum: 28 Hz). (C) Time course of the average firing frequency per channel from 2 to 34 WIV. Firing frequency (±standarddeviation) was calculated as the average of all 64 electrodes from each of the three MEA dishes. (D) Development of spontaneous synchronized burst firings (SBFs) during long-term culture. The number of SBFs per minute (average for 15min) from 13 to 34WIV.

Firing frequency and synchronized burst firings were increasing up to approx. 20 weeks culture

Result 2 Pharmacological properties of spontaneous activity

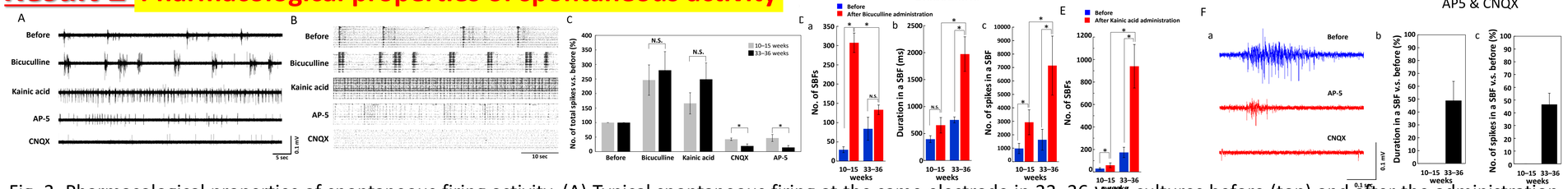


Fig. 3. Pharmacological properties of spontaneous firing activity. (A) Typical spontaneous firing at the same electrode in 33–36 WIV cultures before (top) and after the administration of 10µM bicuculline, 5µM kainicacid, 50µM AP-5, 50µM CNQX. (B) Raster plots of spontaneous firing for 1 min from all 64 electrodes before and after drug administration. (C) Total number of spikes from all 64 electrodes before (100%, baseline) and after drug administration at 10–15 and 33–36 WIV. Comparisons between 10–15 (gray) and 33–36 WIV (black) were obtained using the same cultures. (D) Changes in synchronized burst firing (SBF) due to bicuculline administrationat 10–15 and 33–36WIV. (a)Number of SBFs over the 30 min before (blue) and after (red) bicuculline administration at 10–15 and 33–36 WIV. (b) SBF duration and (c) number of spikes per SBF. (E) Number of SBFs in the 30 min before and after 5 µM kainic acid administration. (F) Change in SBFs following AP-5 and CNQX administration. (a) Typical SBF waveforms before (blue) and after the administration of AP-5 (red) or CNQX (red) at 33–36 WIV. SBFs disappeared after CNQX administration. SBFs were also completely abolished byAP-5 at 10–15 WIV but were only shorter at 33–36 WIV. (b) Change in SBF duration and (c) number of spikes per SBF following AP-5 administration. (n=3 MEA dishes, *p<0.05)

Spontaneous activity in 33–36 WIV cultures was more sensitive to GABA and glutamate receptor modulators.

Result 4 Induction of epileptiform activity and effects of anti-epilepsy drugs

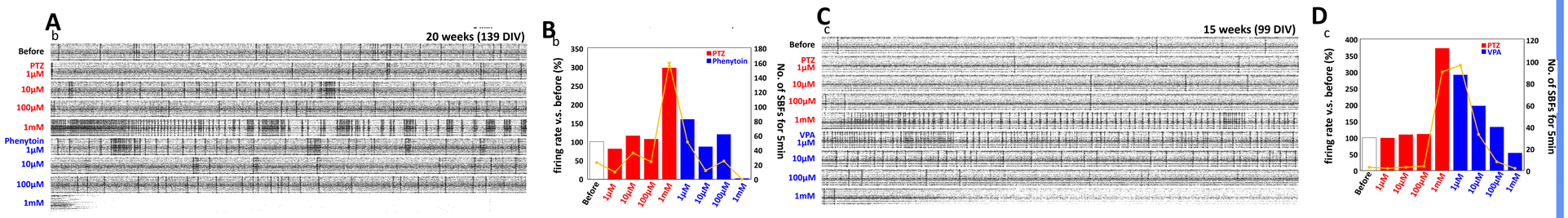


Fig. 5. Induction of epileptiform activity and anticonvulsant effects of anti-epilepsy drugs (AEDs). (A) Induction of epileptiform activity using pentylentetrazole (PTZ) and the suppressive effect of phenytoin. PTZ was added at increasing concentrations (1µM,10µM,100µM,and1mM). Phenytoin was then added (1 µM, 10 µM, 100 µM, and 1 mM). The raster plots at 20 WIV (139 DIV). (B) Changes in firing rate versus before (%) and number of SBFs Yellow). (C)Effect of sodium valproate (VPA) (1µM, 10 µM, 100 µM, 1mM, and 2mM). The raster plots at 15WIV (99DIV). (D) Changes in firing rate and number of SBFs. MEA dishes,*p<0.05).

We also observed the induction of epileptiform activity by PTZ and suppressive effects by clinical AEDs (phenytoin and VPA).

Result 3 Pharmacological properties of evoked responses

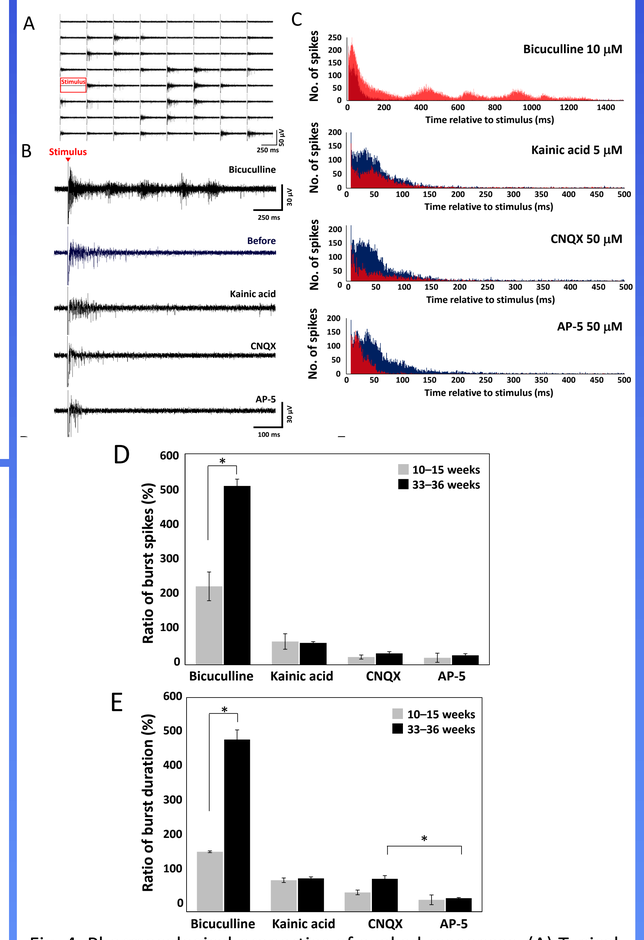


Fig. 4. Pharmacological properties of evoked responses. (A) Typical evoked responses from each of 64 electrodes following a single test stimulus at 33– 36 WIV. Red square shows stimulus site (electrode 33). (B) Typical evoked responses before (top) and after the administration of the indicated neurotransmitter receptor agonist or antagonist at 33–36WIV. Red triangle shows stimulus time and stimulus artifacts.(C)Post-stimulus time histogram (PSTH) (Sum of 10 individual responses at 64 electrodes, bin size = 1 ms) at 33–36 WIV. Blue and red indicate before and after drug administration, respectively. (D) Ratio of the number of evoked spikes after versus before drug administration at 10–15 WIV (gray) and 33–36 WIV (black) (n = 3 MEA dishes, *p<0.05). (E)The ratio of evoked burst duration after versus before drug administration. (n=3 MEA dishes,*p<0.05).

・Evoked responses at 33-36 WIV clearly shows the differences between AMPA and NMDA receptor expression and response.

Reference

Odawara A, Katoh H. Matsuda N. Suzuki I. Sci Rep. 2016, 6:26181.

Conclusion

In conclusion, we examined the electrophysiological and pharmacological properties of cultured hiPSC-derived cortical neuronal networks and found that functional maturation requires at least 20–30 weeks. Nonetheless, long-term culture of hiPSC-derived neuronal neurons on MEAs proved useful for neuro pharmacological and neuro toxicological assays. Our results also provide an important indication for the international standardization of evaluation procedures using in vitro human neurons.

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