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 bursting (SFB) in response to pro-consumables and SFB 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.

Materials & Methods

Human hiPSC-derived cerebral cortical neurons [Axol Bioscience Inc.] (1) •Long-term culture of hiPSC-derived neurons were performed by astrocyte co-culture method (2). We performed long-term culture over 300 days.

Result 1 Development of spontaneous firing during long-term culture

Fig. 2. (A) Changes in spontaneous firing pattern in the same long-term cultured at 7, 14, 29, and 34 weeks in vitro (WIV). (B) Typical spontaneous firing patterns. (B) Raster soft hearay-wide spike detection rate (AWSDR, spikes/s). Bin sizes 1 ms. (C) Corresponding raster plots for all 64 electrodes over 5 min. (D) 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 (± standard deviation) 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 15 min) from 13 to 34 WIV.

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 10 uM bicuculline, 5 uM kainic acid, 50 uM AP5, 50 uM 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 (SFB) due to bicuculline administration at 10–15 and 33–36 WIV. (E) Number of SBFs over the 30 min before (blue) and after (red) bicuculline administration at 10–15 and 33–36 WIV. (F) SFB duration and (G) number of spikes per SFB. (H) Number of SBFs in the 30 min before and after 5 uM kainic acid administration. (I) Change in SBFs following AP5 and CNQX administration. (J) Typical SBF waveforms before (blue) and after the administration of AP5 (red) or CNQX (red) at 33–36 WIV. SBFs disappeared after CNQX administration. SBFs were also completely abolished by AP5 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 AP5 administration. (n=3 MEA dishes, *p<0.05)

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 33–36 WIV. 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.

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 pentylenetetrazole (PTZ) and the suppressive effect of phenytoin. PTZ was added at increasing concentrations (1 uM, 10 uM, 100 uM, and 1 mM). Phenytoin was then added (1 uM, 10 uM, 100 uM, and 1 mM). The raster plots at 20 WIV (139 DIV). (B) Changes in firing rate versus before (K) and number of SBFs. (C) Effect of sodium valproate (VPA) (1 uM, 10 uM, 100 uM, and 1 mM). The raster plots at 15 WIV (990 DIV). (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).

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 networks on MEAs proved useful for neuropharmacological and neurotoxicological assays. Our results also provide an important indication for the international standardization of evaluation procedures using in vitro human neurons.