

Pharmacological Responses in Cultured Human iPSC Derived Cortical Neurons Using Multi-Electrode Array

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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 epileptiform 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 [Axol Bioscience Ltd.] ⁽¹⁾

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

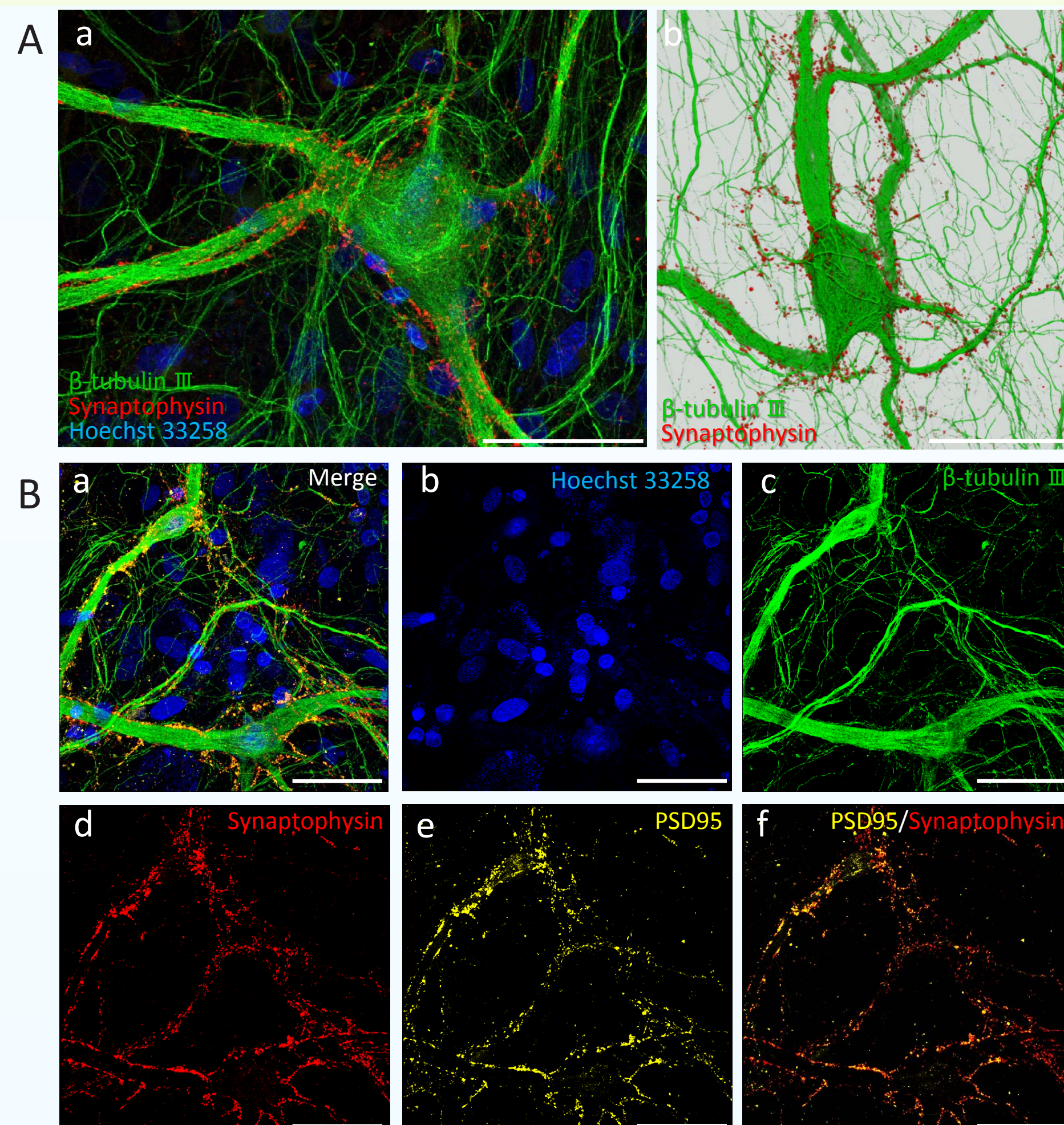
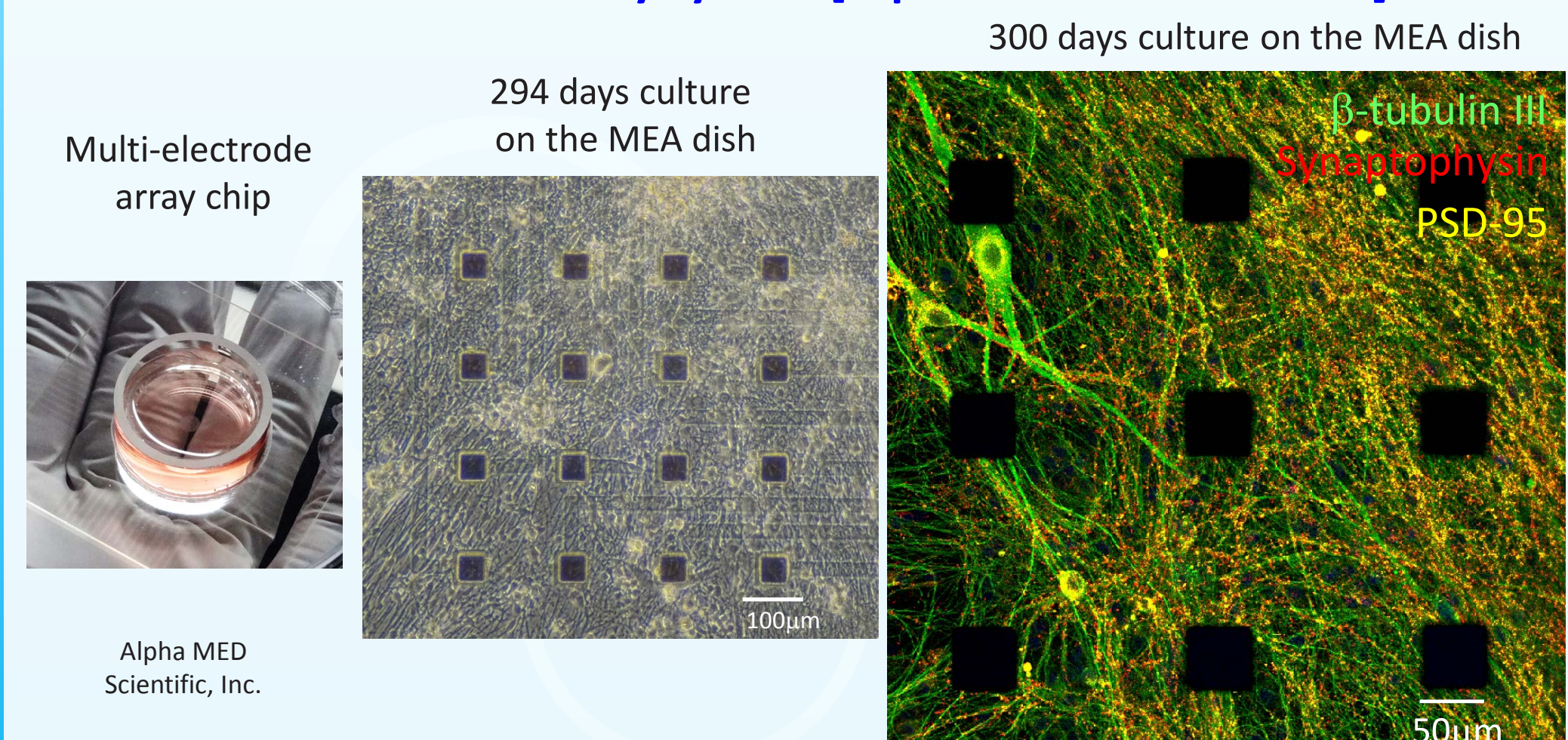


Fig. 1. Pyramidal neuron-like morphology and synaptogenesis at 300 DIV. (A) Scale bars = 50 μm

Multi-electrode array 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 MED Scientific, Japan). The MEA chips contain 64 electrodes (MED-PS15A) 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

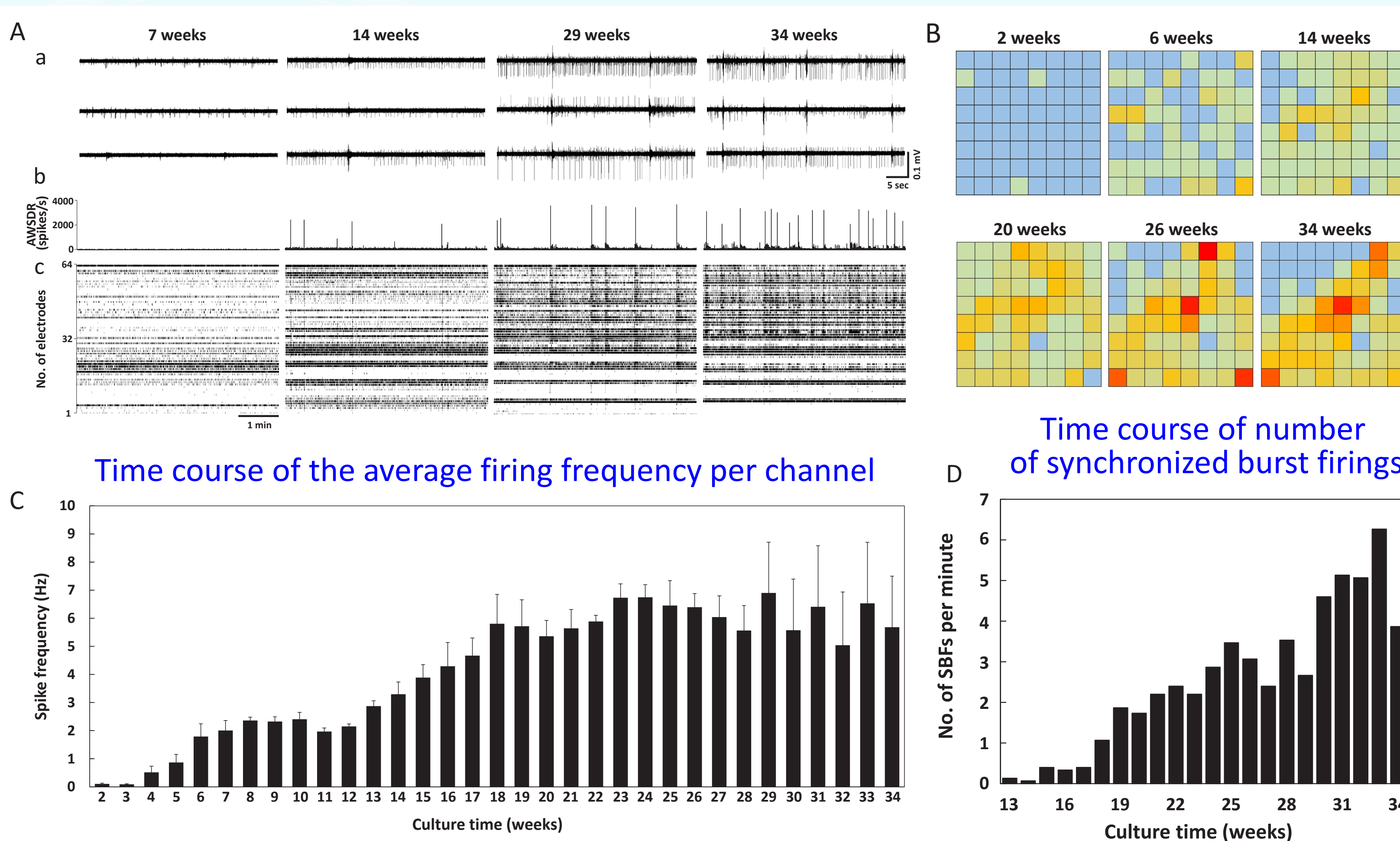


Fig. 2. (A) Changes in spontaneous firing pattern in the same long-term culture at 7, 14, 29, and 34 weeks *in vitro* (WIV). (a) Typical spontaneous firing patterns. (b) Rasters of the array-wide spike detection rate (AWSDR, spikes/s). Bin size is 1 ms. (c) Corresponding raster plots for all 64 electrodes 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 (\pm 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.

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

Result 3: Pharmacological properties of evoked responses

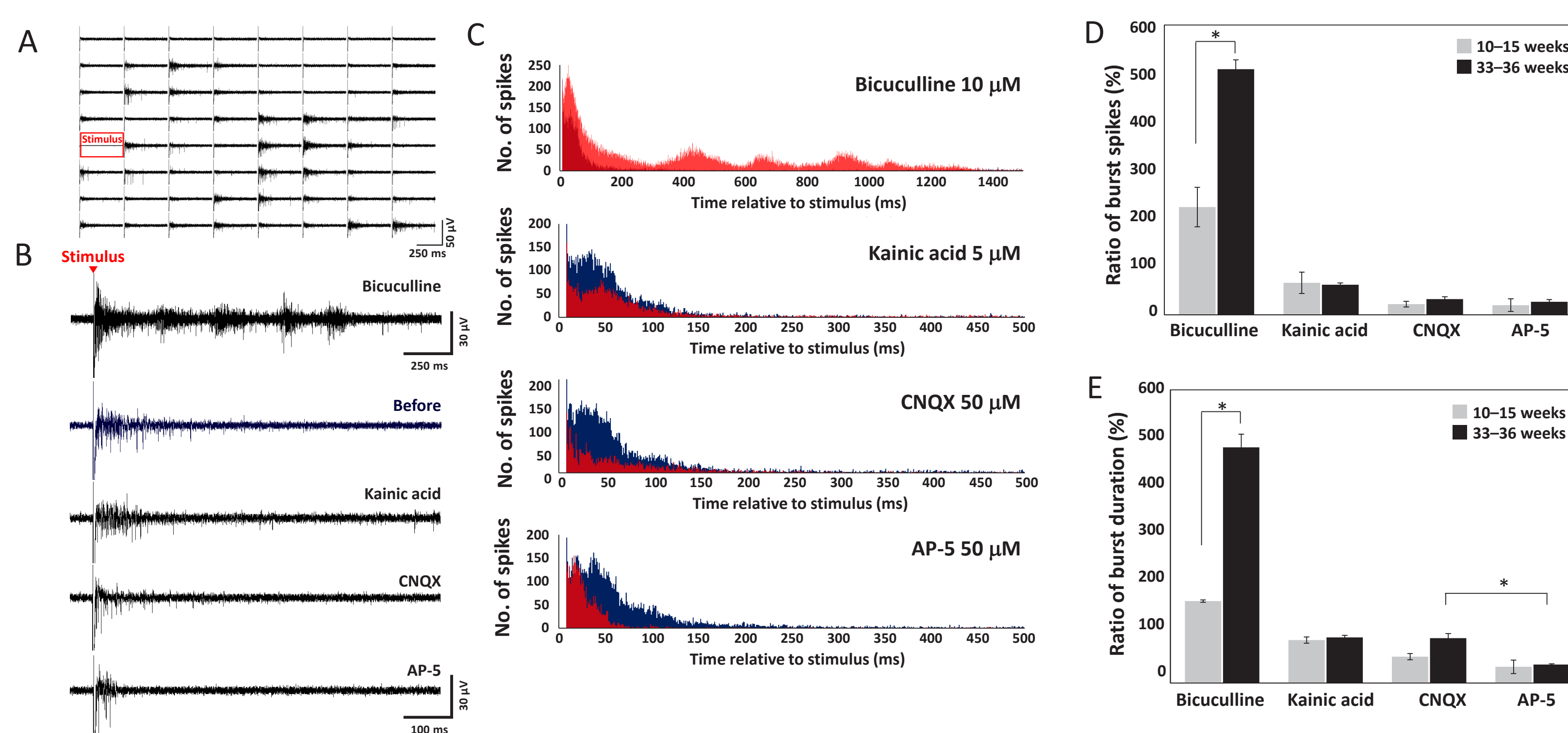


Fig. 4. Pharmacological properties of evoked responses. (A) Typical evoked responses from each of 64 electrodes following a single test stimulus at 33–36 WIV. Red square shows stimulus site (electrode 33). (B) Typical evoked responses before (top) and after the administration of the indicated neurotransmitter 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 2: Pharmacological properties of spontaneous activity

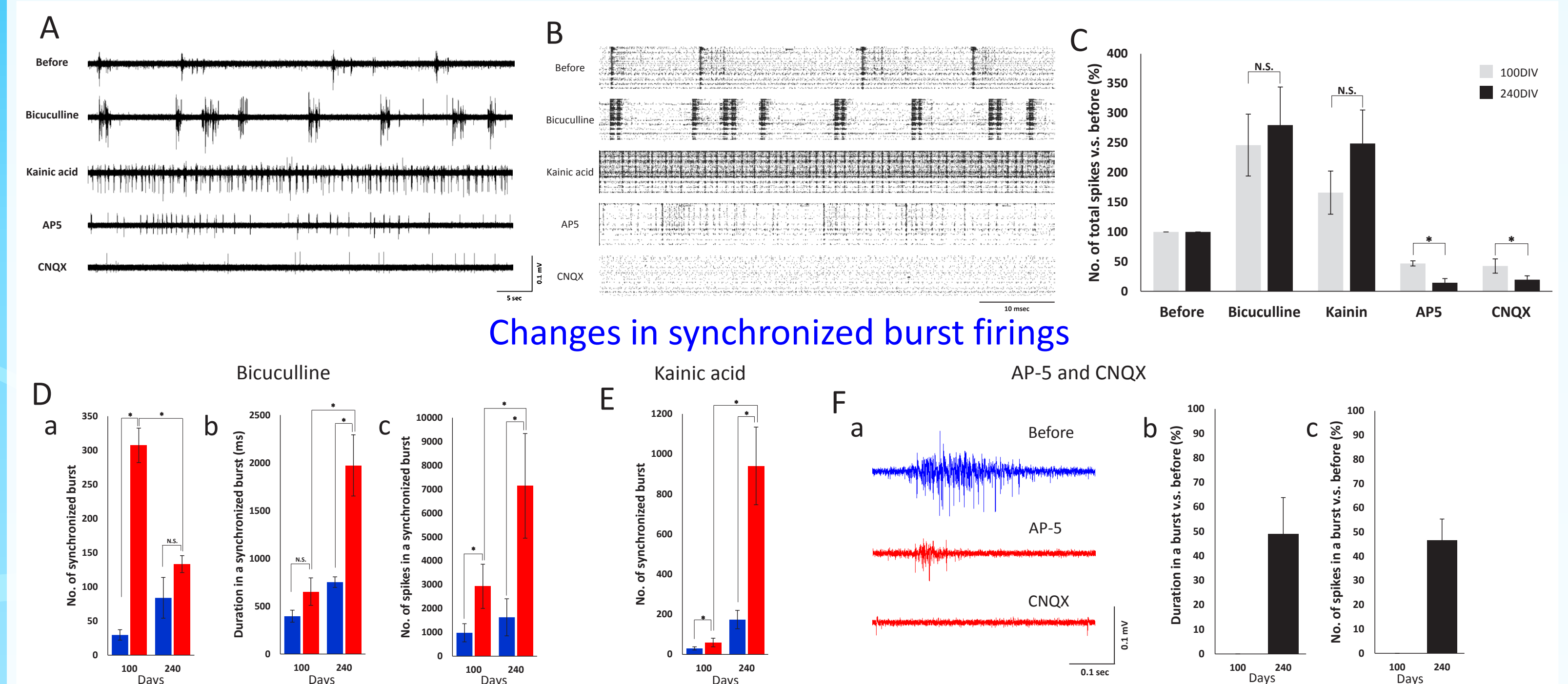


Fig. 3. Pharmacological properties of spontaneous firing activity. (A) Typical spontaneous firing at the same electrode in 33–36 WIV cultures before (top) and after the administration of 10 μM bicuculline, 5 μM kainic acid, 50 μM AP-5, 50 μM CNQX. (B) Raster plots of spontaneous firing for 1 min from 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 administration at 10–15 and 33–36 WIV. (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 by AP-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, $*p < 0.05$).

Glutamatergic receptors are more functional at 33–36 WIV compared to 10–15 WIV.

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

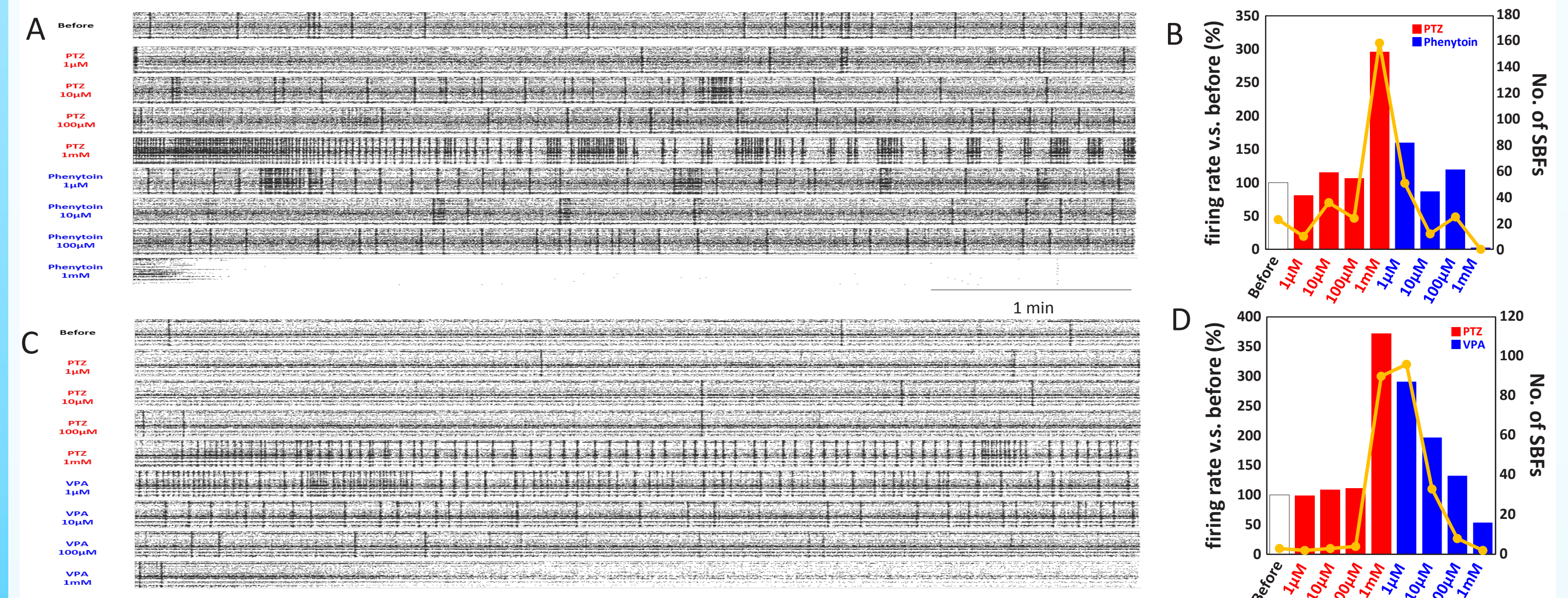


Fig. 5. Induction of epileptiform activity and anticonvulsant effects of anti-epilepsy drugs (AEDs). (A) Induction of epileptiform activity using pentylenetetrazole (PTZ) and the suppressive effect of phenytoin. PTZ was added at increasing concentrations (1 μM, 10 μM, 100 μM, and 1 mM). Phenytoin was 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) (1 μM, 10 μM, 100 μM, 1 mM, and 2 mM). The raster plots at 15 WIV (99 DIV). (D) Changes in firing rate and number of SBFs

We observe the induction of epileptiform activity by PTZ and suppressive effects by clinical AEDs.

Conclusions

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

Reference

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