Induction of plasticity phenomena in human induced pluripotent stem cell-derived cortical neurons

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Background

Plasticity such as long-term potentiation (LTP) and long-term potentiation depression (LTD) in neuronal networks has been analyzed using in vitro and in vivo techniques in simple animals to understand learning, memory, and development in brain function. Human induced pluripotent stem cell (hiPSC)-derived neurons may be effectively used for understanding the plasticity mechanism in human neuronal networks, thereby elucidating disease mechanisms and drug discoveries. In this study, we attempted the induction of LTP and LTD phenomena in a cultured hiPSC-derived cerebral cortical neuronal network using multi-electrode array (MEA) systems.

Material & Methods

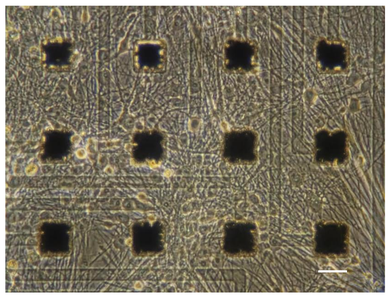


Figure 1. Long-term culture of hiPSC-derived cortical neurons on the MEA chip

hiPSC-derived cortical neurons were cultured on a multi-electrode array (MEA) chip. Phase-contrast images at 112 days in vitro (DIV). Scale bars= 50 µm.

・Cell Culture

hiPSC-derived cerebral cortical neurons (hyCCNs; Axol Bioscience Inc., UK) were cultured (density, 1.0 × 10^6 cells/cm^2) on MEA chips (MED-P515A; Alpha Med Scientific). The cultures were grown at 37℃ in a 5% CO_2/95% air atmosphere. Half of the media was exchanged from 5 to 7 days.

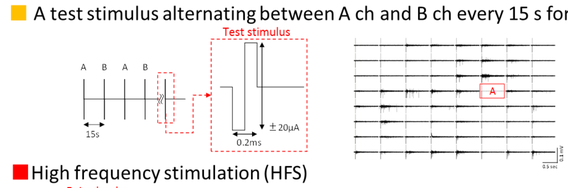
・Extracellular recording

The extracellular signals in evoked responses and spontaneous firings were obtained by the MEA system (MED64-Basic; Alpha Med Scientific) and stored on a personal computer. A sampling rate of 20 kHz/channel and low cut filter of 100 Hz was used. The cultures were maintained at 37℃ in a 5% CO^2 incubator during the recordings and stimulation. Firing analyses were performed using Mobius software (Alpha Med Scientific) and MATLAB.

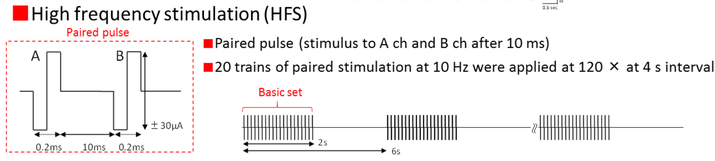
Induction of LTP・LTD ⇒ High frequency stimulation(HFS)

【Protocol】

- Selecting two electrodes (A and B ch)
- A test stimulus alternating between A ch and B ch every 15 s for 60 min (before)



- High frequency stimulation (HFS)



- Paired pulse (stimulus to A ch and B ch after 10 ms)
- 20 trains of paired stimulation at 10 Hz were applied at 120 × at 4 s intervals.
- A test stimulus alternating between A ch and B ch every 15 s for 60 min (after)

Result 1

Induction of LTP and LTD by HFS

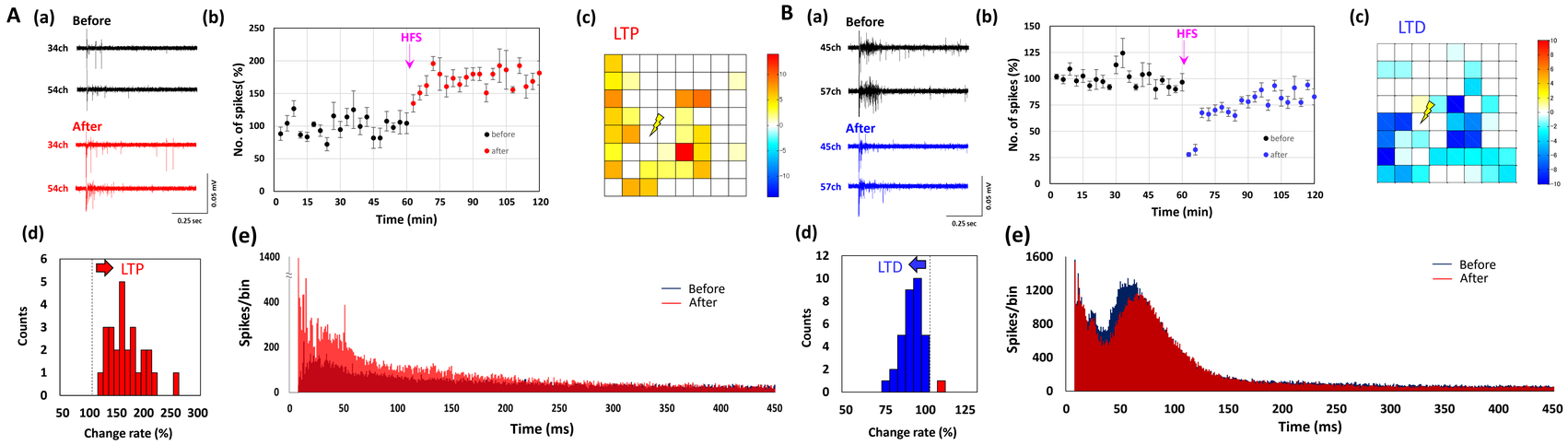


Figure 2. Induction of LTP and LTD by HFS

Induction of long-term potentiation (LTP) and long-term depression (LTD) by high-frequency stimulation (HFS). (A) Induction of LTP. (a) The waveforms represent the typical evoked responses before and after HFS. The number of spikes was increased after stimulation. (b) Time course of the number of spikes in evoked responses at 34 ch before and after HFS for 60 min, respectively. Test stimuli were applied to 35 ch every 30 s. The average before HFS for 60 min represents 100%. (c) Grids showing the 64 electrodes where colored electrodes changed the number of spikes per one stimulus. Electrodes that detected a higher increase of the number of spikes are shown in red (maximum: 14 spikes). (d) Histogram represents the number of electrodes in the change rate of the number of spikes. Bin size is 10%. (e) Poststimulus time histogram (PSTH) (n = 120 experiments, at 64 electrode) within 450 ms in evoked responses. Blue and red show before and after HFS, respectively. (B) Induction of LTD. (a) Typical evoked responses before and after HFS, (b) Time course of the number of spikes in evoked responses at 45 ch before and after HFS for 60 min, respectively. (c) Grids showing the 64 electrodes where colored electrodes changed the number of spikes. (d) The number of electrodes in the changed rate of the number of spikes. (e) PSTH before and after HFS.

We also detected LTP and LTD phenomena in a hiPSC-derived neuronal network as the change of spike pattern.

Result 2

Cross-correlation histogram (CCH)

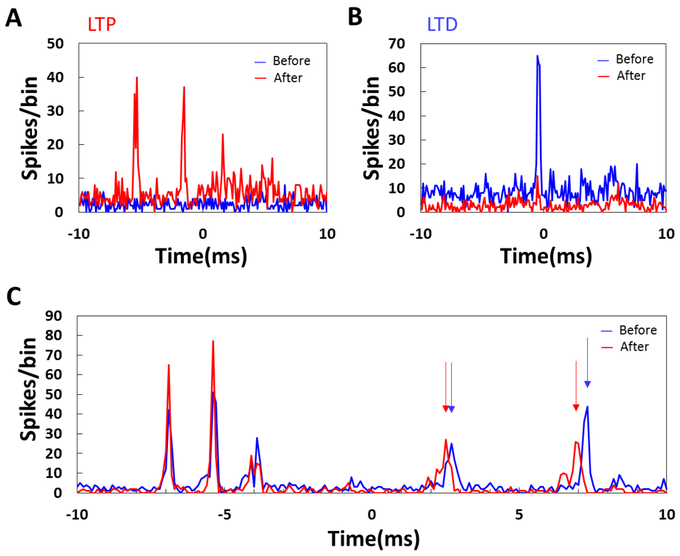


Figure 3. Cross-correlation histogram (CCH)

(A) Typical CCH in long-term potentiation (LTP) induction at 62 days in vitro (DIV). This represents CCH for ±10 ms at 17 ch versus trigger spikes at 16 ch. Red and blue show spike counts before and after HFS, respectively. Bin size is 100 ms. (B) Typical CCH in long-term depression (LTD) induction at 117 DIV. This represents CCH for ±10 ms at 50 ch versus trigger spikes at 45 ch. (C) A CCH for ±10 ms at 23 ch versus trigger spikes at 48 ch at 117 DIV. CCH represents the change in spike timing before and after HFS. Red and blue arrows indicate that the peaks of spike timing were shifted. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The cross-correlation of responses revealed that spike patterns with specific timing were generated during LTP induction and disappeared during LTD induction and that the hiPSC-derived cortical neuronal network has the potential to repeatedly express the spike pattern with a precise timing change within 0.5 ms.

Result 3

Induction of late-phase long-term potentiation (L-LTP)-like plasticity

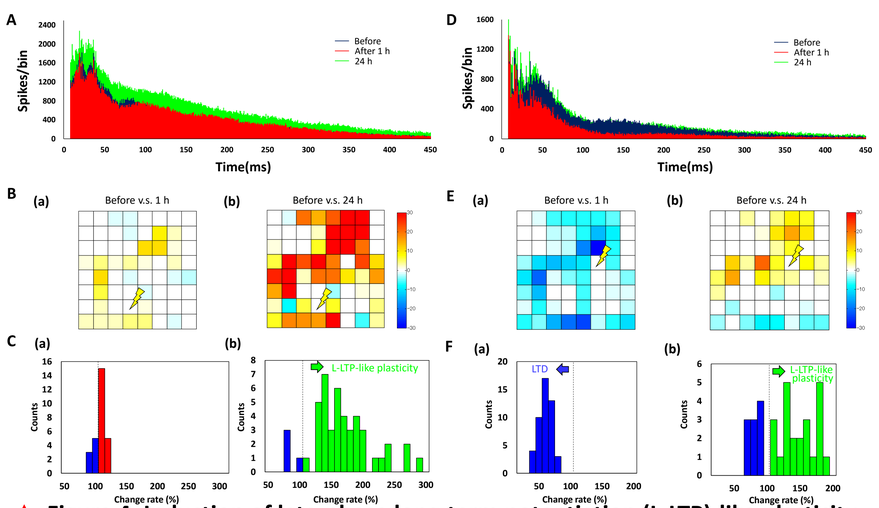


Figure 4. Induction of late-phase long-term potentiation (L-LTP)-like plasticity.

(A) Poststimulus time histogram (PSTH) (n = 120 experiments, at 64 electrodes) within 450 ms in evoked responses before (blue), after 1 h (red), and 24 h (green). Test stimuli were applied to 52 ch at 115 days in vitro. It was a different sample from that shown in Fig. 3 (B, E) Grids showing the 64 electrodes where colored electrodes changed number of spikes per one stimulus before and after 1 h (a) and before and after 24 h (B-b). After 24 h, 43 electrodes were increased and 4 electrodes were decreased. (E-b)After 1 h, 47 electrodes decreased. On the other hand, 23 electrodes increased and ten electrodes maintained a spike decrease. (C, F) Histogram of electrodes represent rate of changed number of spikes in an electrode. Bin size is 10%. (a) Change rate before and after 1 h. (b) Change rate before and after 24 h (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.) (D) PSTH at test stimulus site 30 ch.

We also detected the phenomenon for late-phase LTP (L-LTP) like plasticity .

Conclusion

- HFS induced LTP and LTD phenomena in hiPSC-derived cortical neurons.
- Spike patterns were generated or disappeared in induction of plasticity.
- hiPSC-derived neurons express the spike pattern with a precise timing change.
- HFS induced L-LTP-like plasticity and the change of synchronized burst firing.
- MEA system is beneficial for clarifying the function of hiPSC-derived neurons.

Reference

A.Odawara, H.Katoh, N.Matsuda, I.Suzuki. “Induction of long-term potentiation and depression phenomena in human induced pluripotent stem cell-derived cortical neurons.” BBRC, 469 (2016), 856-62.

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