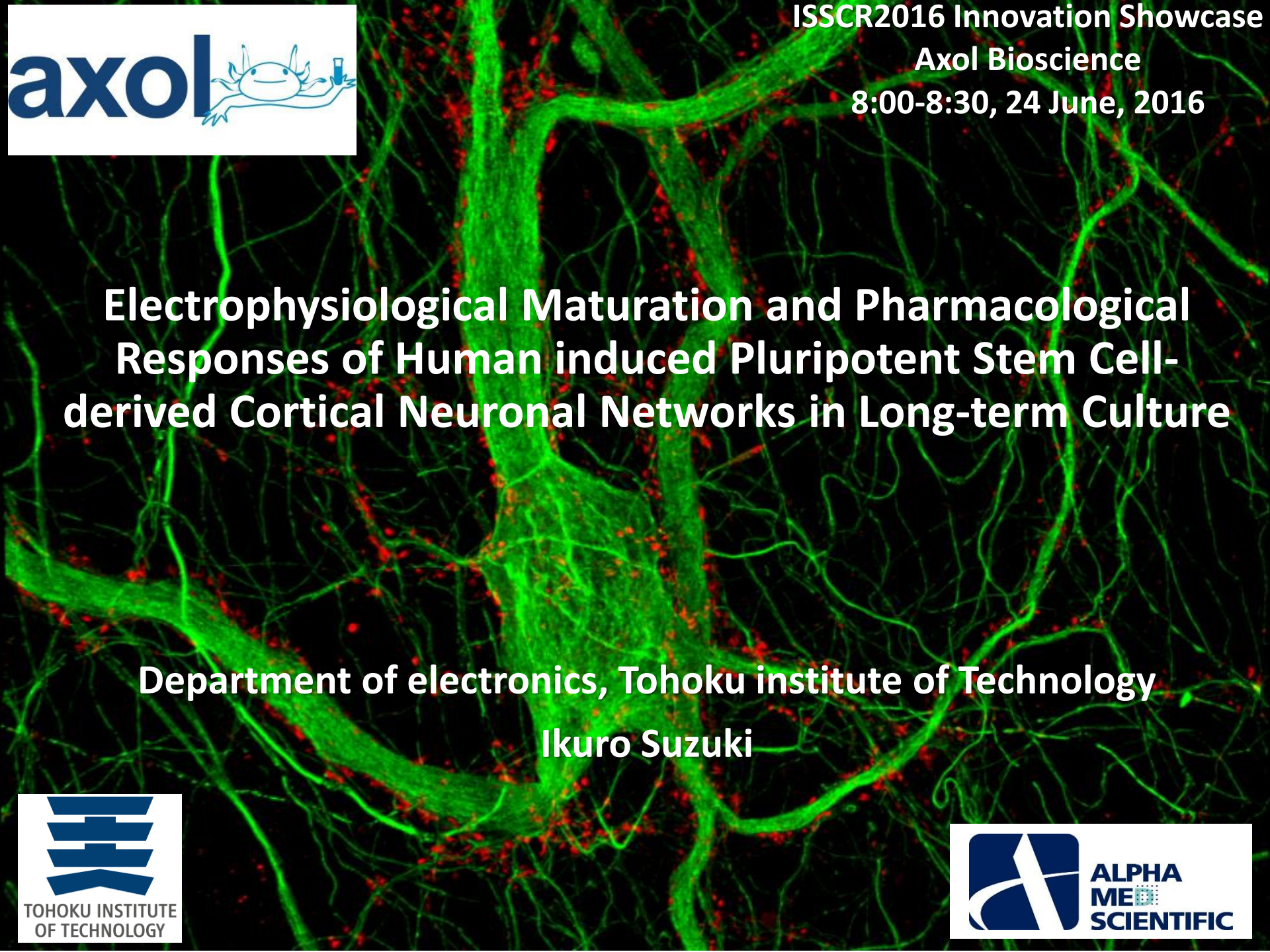


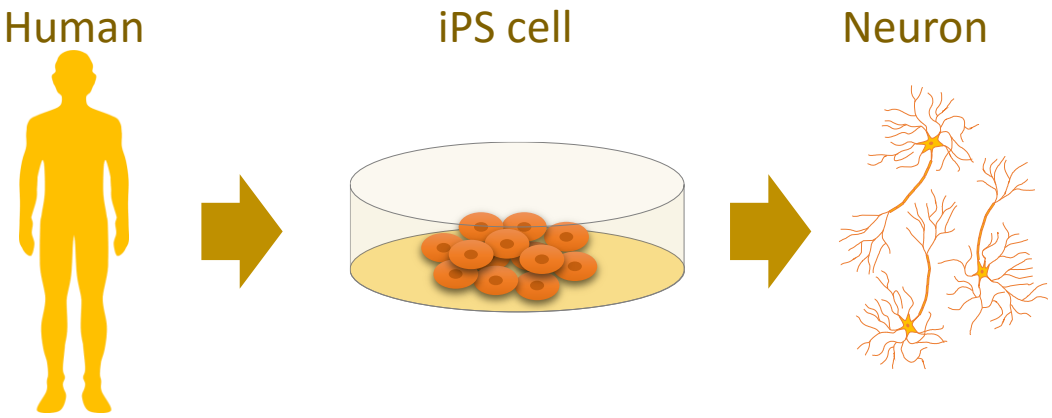
ISSCR2016 Innovation Showcase
Axol Bioscience
8:00-8:30, 24 June, 2016

Electrophysiological Maturation and Pharmacological Responses of Human induced Pluripotent Stem Cell-derived Cortical Neuronal Networks in Long-term Culture

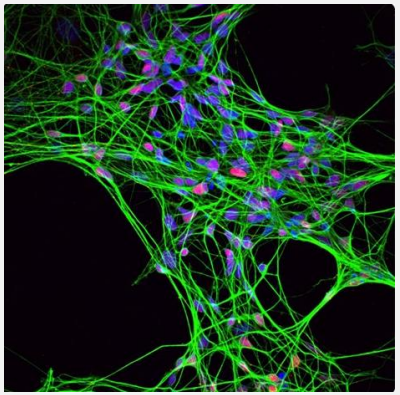
Department of electronics, Tohoku institute of Technology
Ikuro Suzuki

Background

Development of differentiation technology from Human iPS cells into neurons



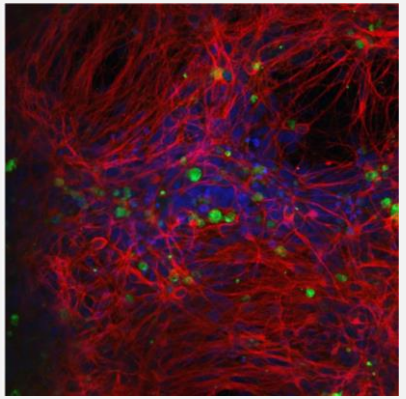
Normal neuron



Glutamate
GABA
Dopamine
Acetylcholine
⋮

Disease model

Alzheimer's
Parkinson's
Huntington's
Rett Syndrome
Epilepsy
⋮



Application to drug discovery and toxicological assay

Purpose

Important issues

The maturation of cultured human iPSC-derived neurons

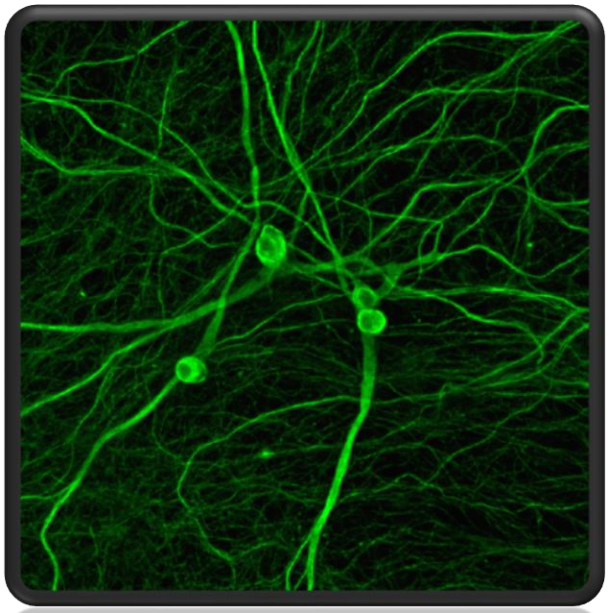
The establishment of methods for functional evaluation

Purpose

Investigation of Electrophysiological properties and pharmacological responses in long-term cultured hiPSC-deried neruons using MEA system

human iPSC-derived neurons

Human Cerebral Cortical Neurons (hCCNs)

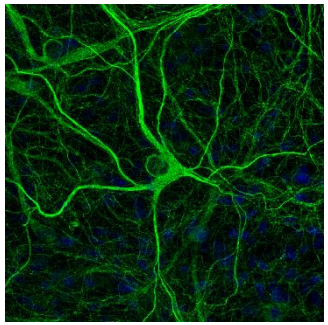


hCCNs express typical markers of cerebral cortical neurons, such as Tbr1, Ctip2, Brn2 and Cux1.

Shi Y, et.al, *Nat Neurosci* **15**, 477 (2012).
Shi Y, et.al, *Nat Protoc* **7**, 1836 (2012).

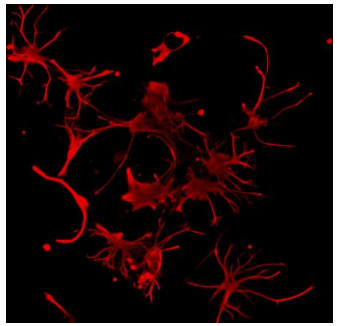
【Co-culture with astrocyte】

Neurons



\+

Astrocytes



Odawara A, et.al, *Sci Rep* **6**, 26181 (2016).
Odawara A, et.al, *BBRC* **496**, 856 (2016).

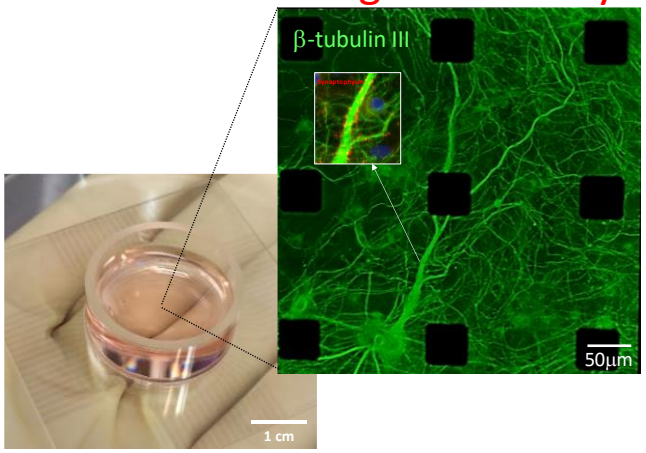
Electrophysiological methods

ALPHA MED SCIENTIFIC

Multi-electrode array system (MED64)

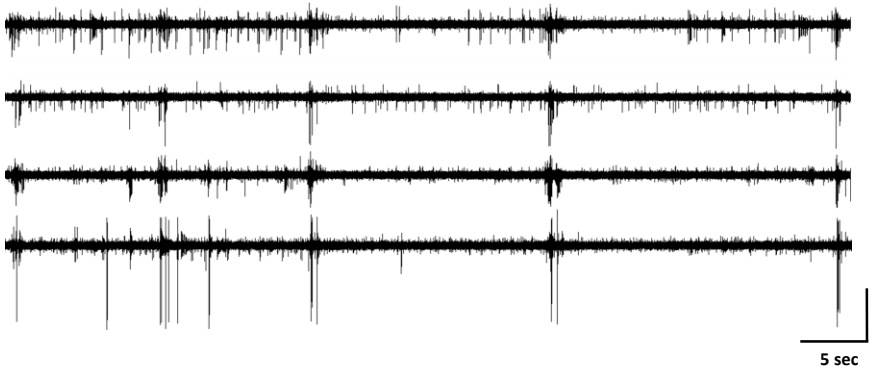
Low impedance electrode

High-sensitivity



(64 ch)

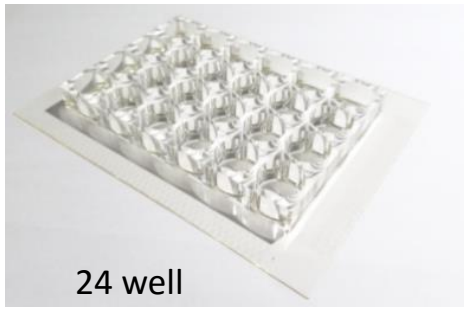
Extracellular recordings of action potentials



MED Probe
for Allegro system

8 well
(64 ch)

MED Probe
for Presto system



24 well
(384 ch)

Futures for MEA

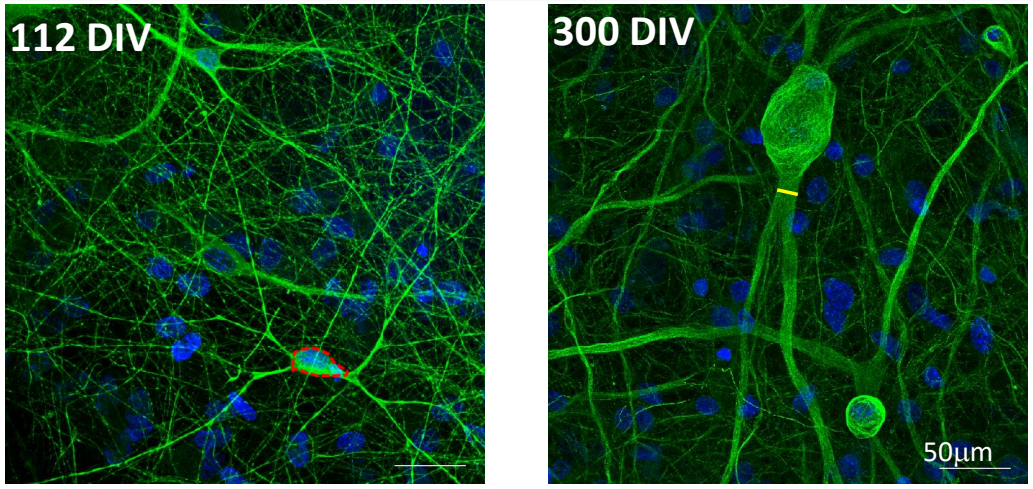
- Non invasive recording
- High time resolution (/50μs)
- Flexible electrical stimulation
- Network analysis

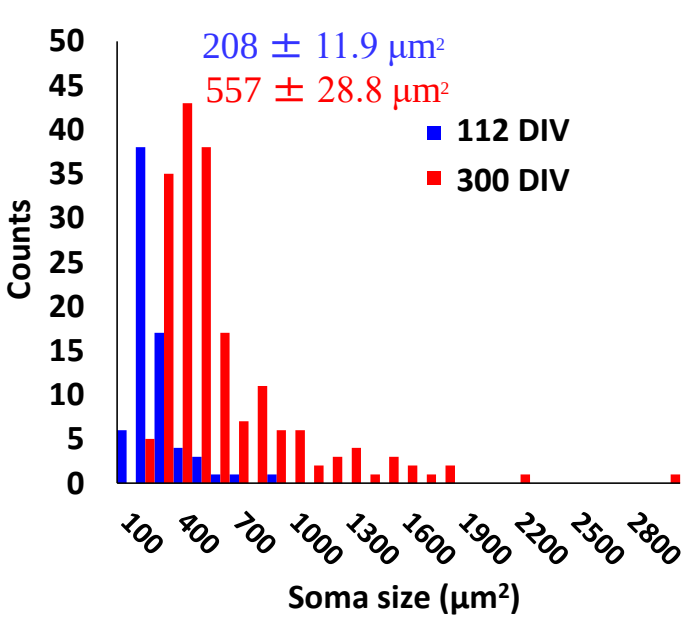
Today's Topics

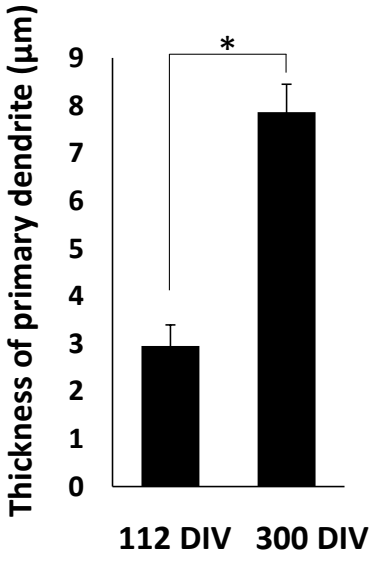
Electrophysiological properties and pharmacological responses in hiPSC-derived cerebral cortical neurons (hCCNs)

- Morphology in long-term culture
- Development of spontaneous firings
- Pharmacological properties
- Induction of Epileptiform activity and effects of AEDs
- Induction of Long term potentiation (LTP) and depression (LTD)

Results : Morphology (soma size and width of primary dendrite)



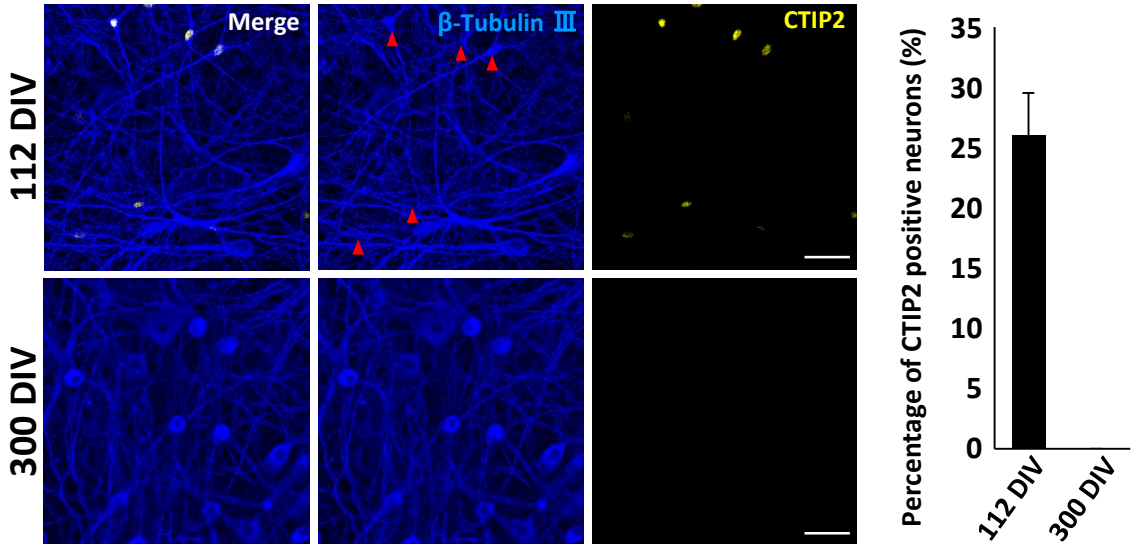




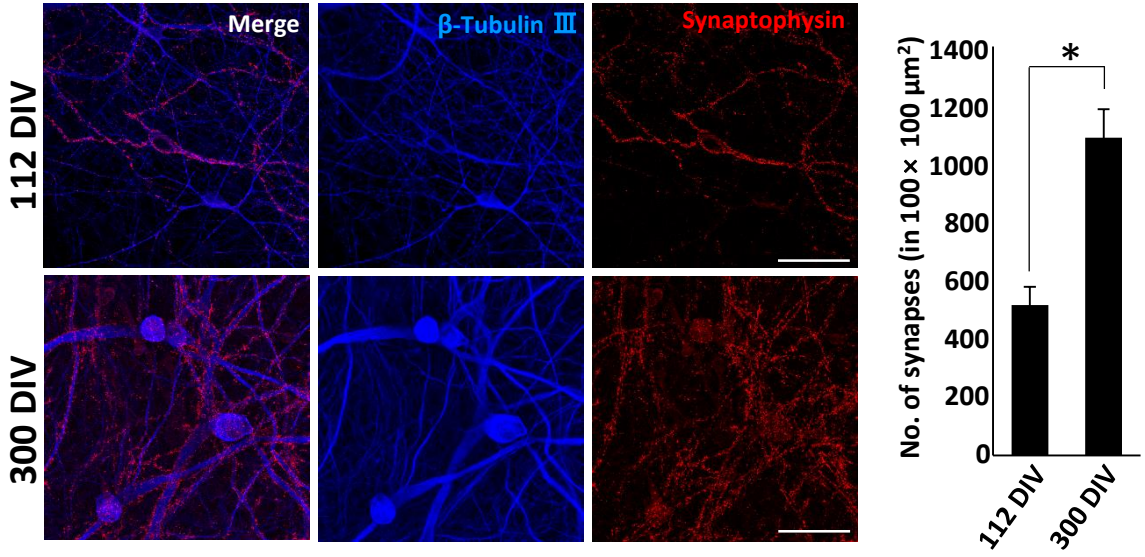
Cultured neurons at 300 DIV were morphologically mature, exhibiting thick dendrites and a large soma.

Odawara A, et.al, *Sci Rep* **6**, 26181 (2016).

Results: Comparison of immature neuron number and synaptic density between 112 and 300 DIV cultures



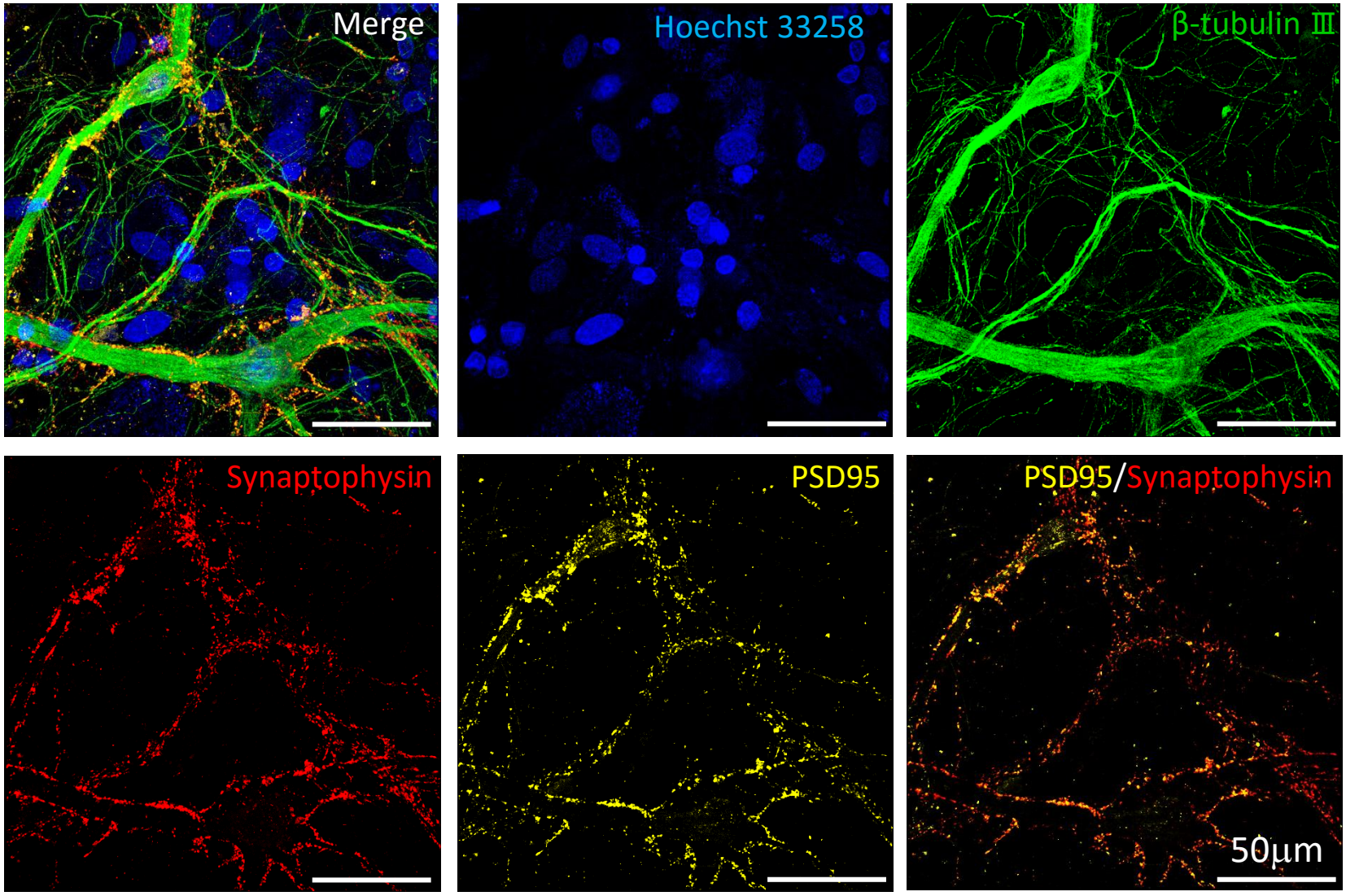
At 112 DIV, 26.0% of neurons were still CTIP2-positive.



Synaptic density was about 50% lower than at 300 DIV.

Odawara A, et.al, *Sci Rep* **6**, 26181 (2016).

Results : Morphology (Colocalization of pre and post-synapses)

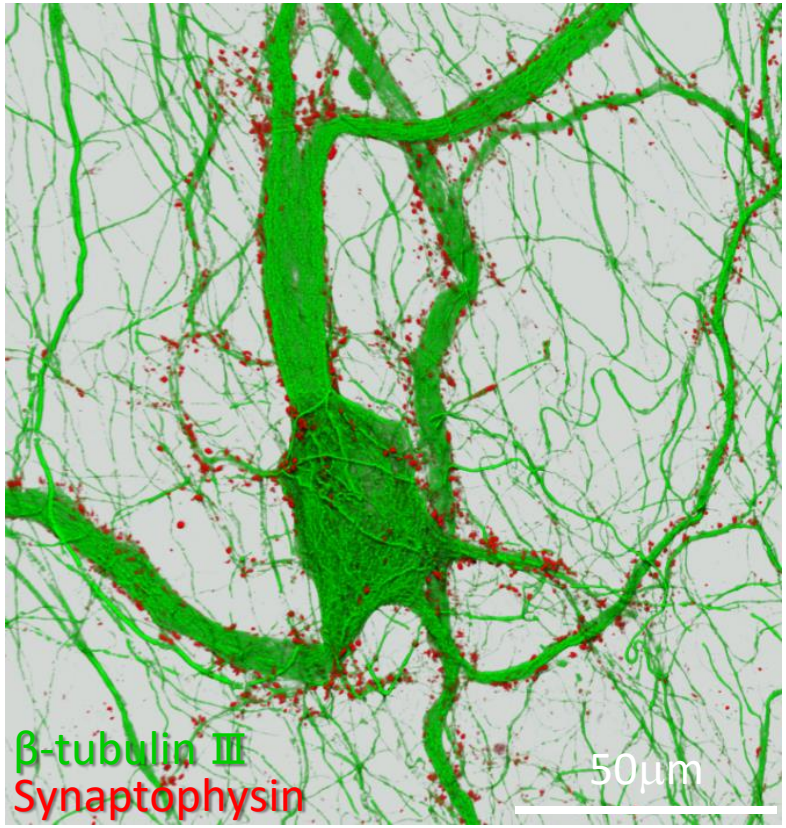


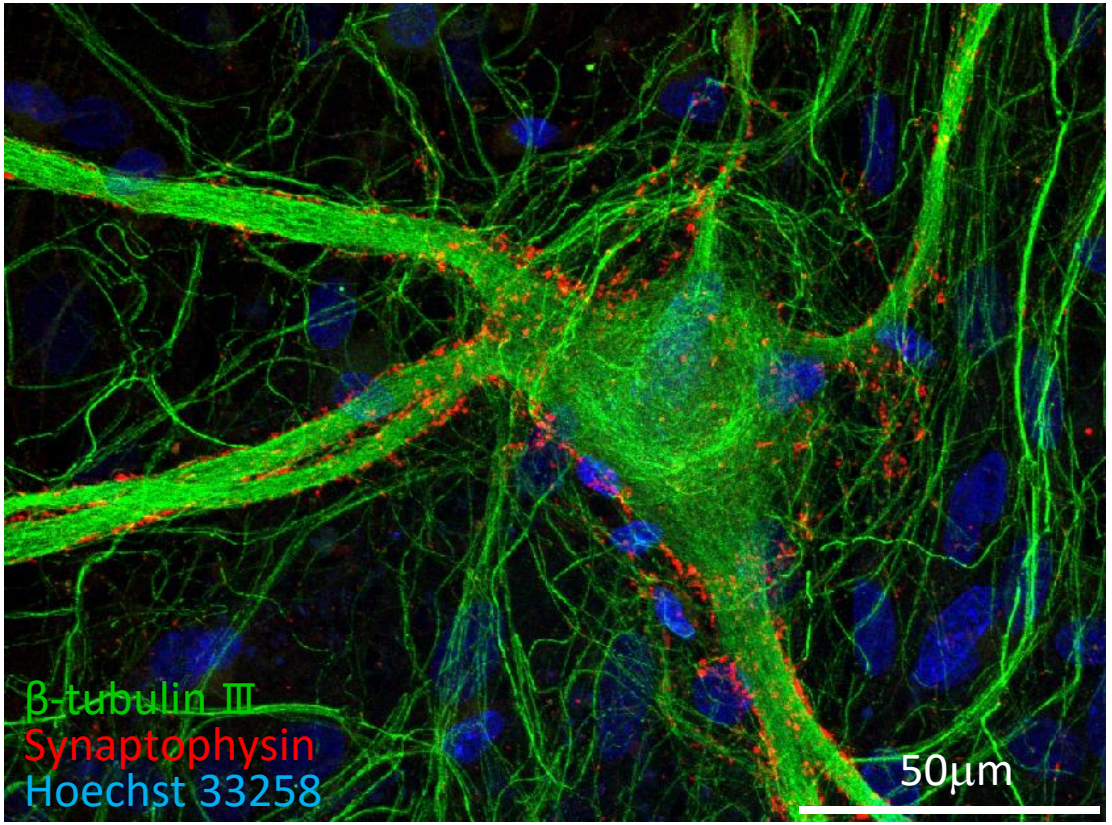
Colocalization of pre and post-synapses at 300 days culture

Odawara A, et.al, *Sci Rep* **6**, 26181 (2016).

Results : Morphology (Pyramidal-like morphology)

300 DIV





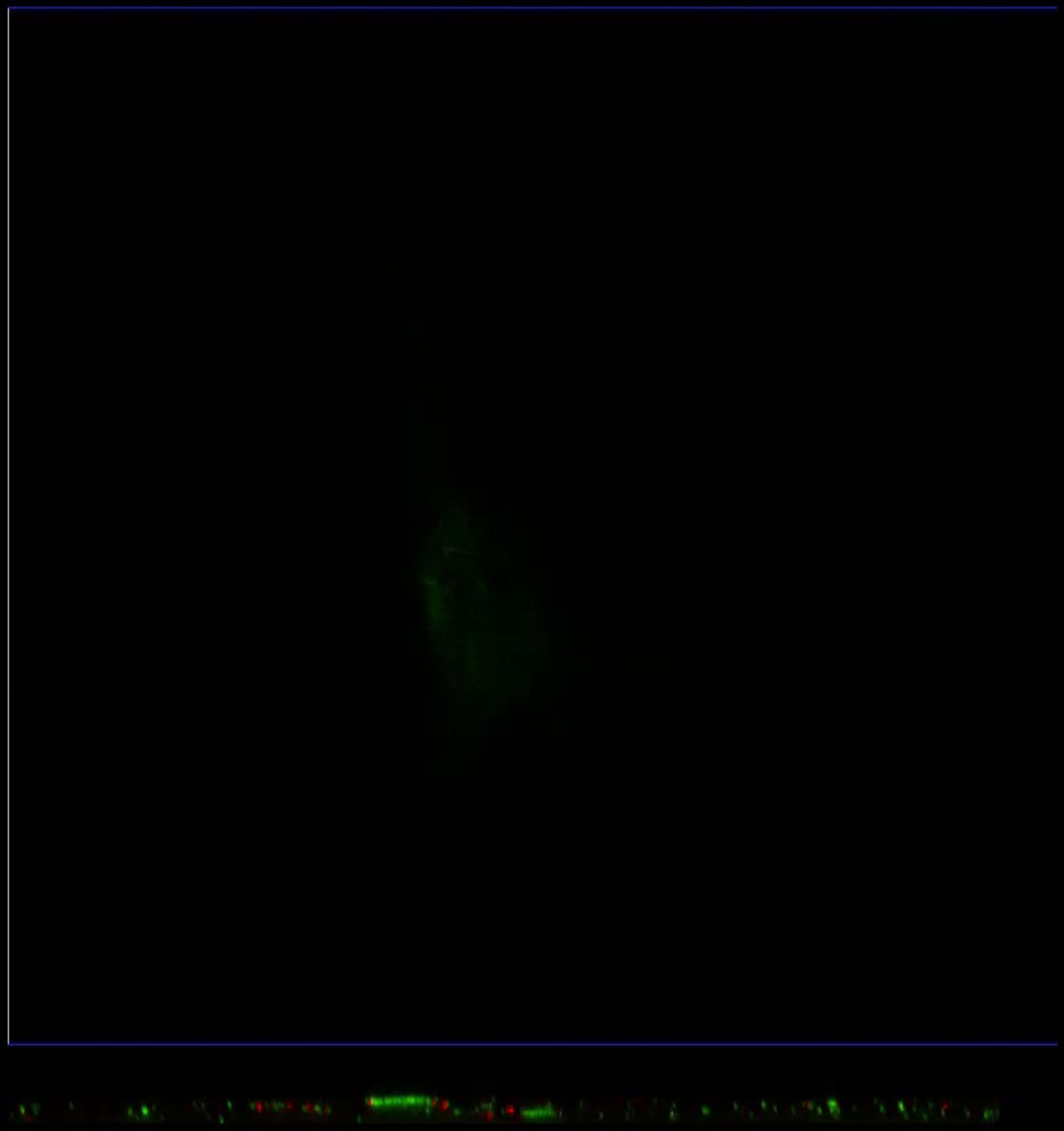
Pyramidal-like morphology with apical and basal dendrites.

Synapses were formed around thick dendrites and the soma.

Odawara A, et.al, *Sci Rep* **6**, 26181 (2016).

Results : Morphology (3D)

300 days culture



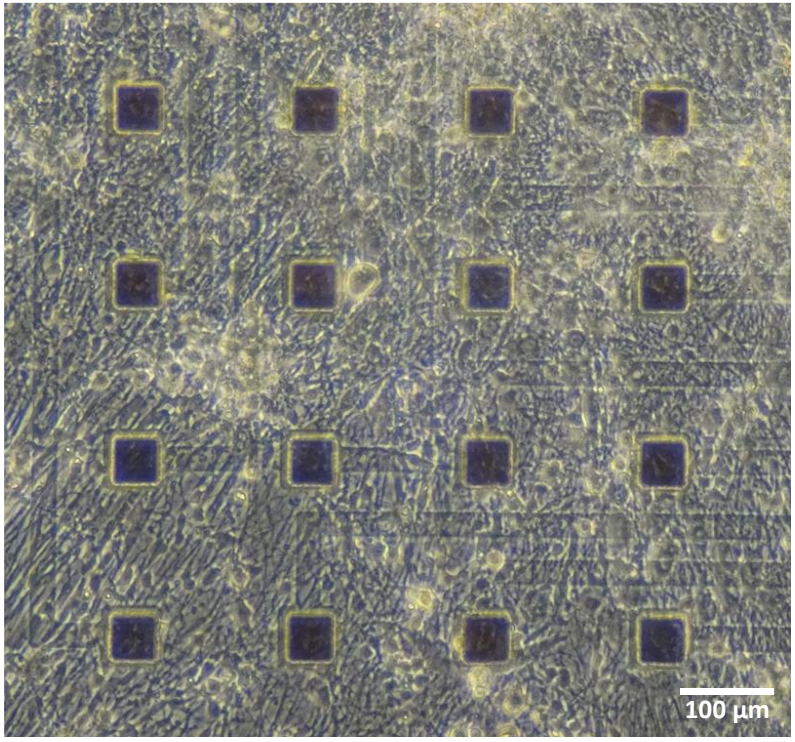
Today's Topics

Electrophysiological properties and pharmacological responses in hiPSC-derived cerebral cortical neurons (hCCNs)

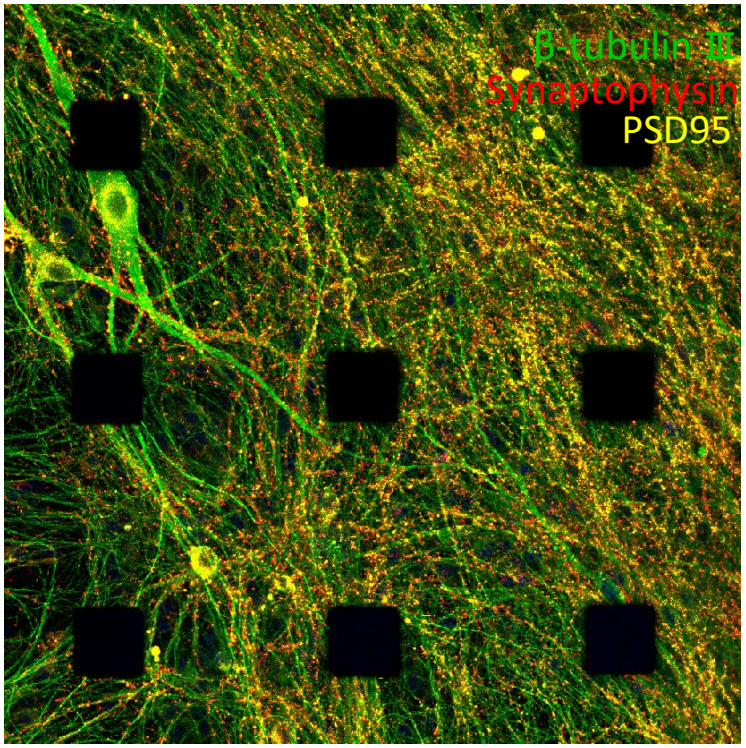
- Morphology in long-term culture
- Development of spontaneous firings
- Pharmacological properties
- Induction of Epileptiform activity and effects of AEDs
- Induction of Long term potentiation (LTP) and depression (LTD)

Results : Long-term culture on a MEA chip

294 DIV



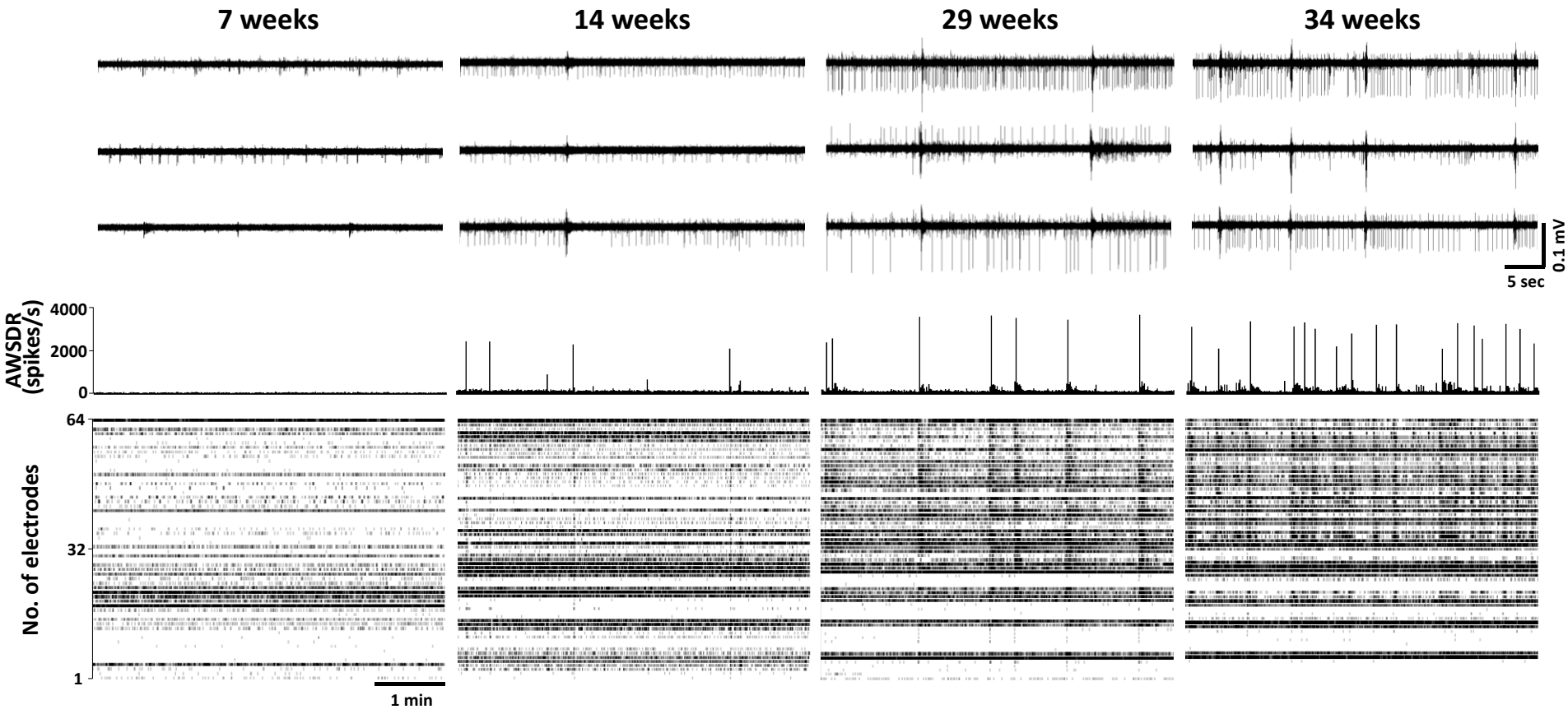
300 DIV



Human iPSC-derived cortical neurons grown on MEA chips survived over 1 year without cell aggregation, enabling the measurement of distributed network field activity.

Odawara A, et.al, *Sci Rep* **6**, 26181 (2016).

Results : Development of spontaneous firing during long-term culture



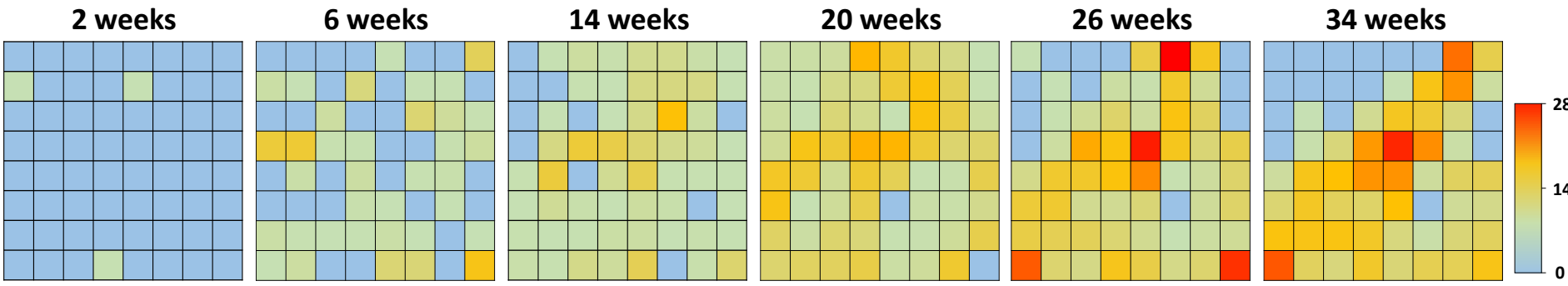
Synchronized burst firings by synaptic transmission were observed over 10 weeks.

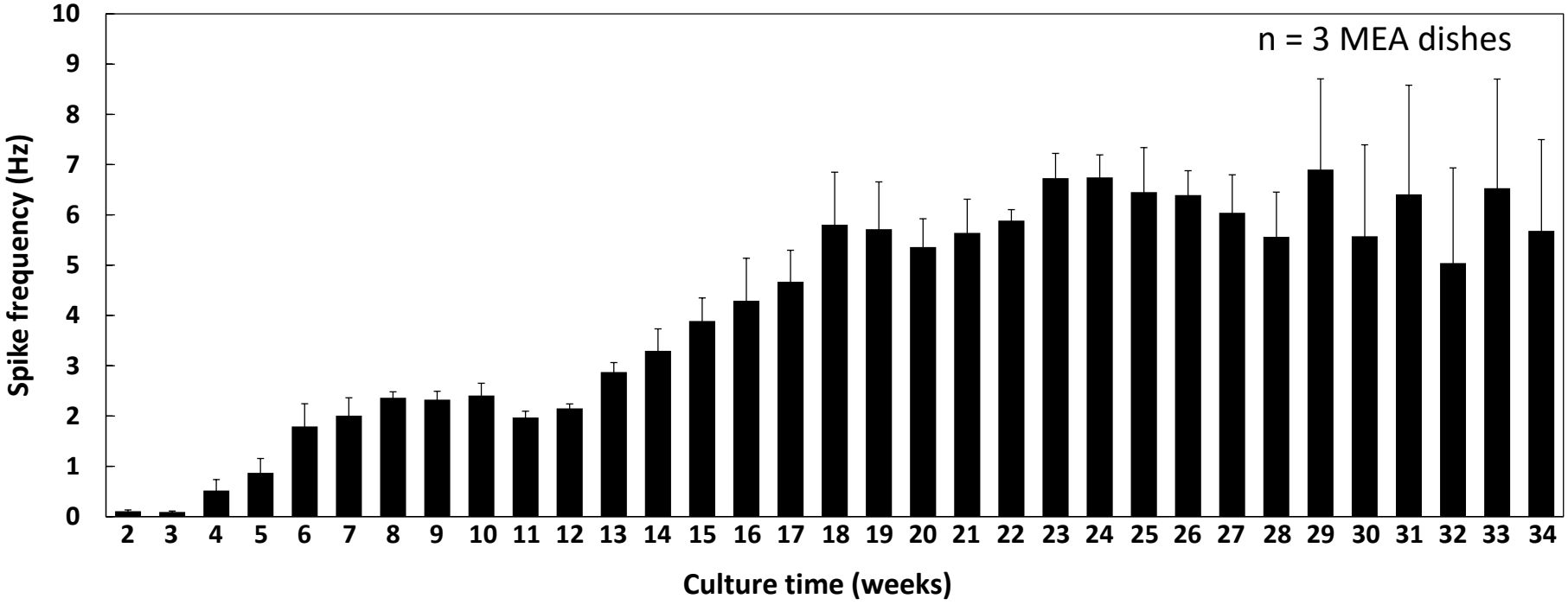
（Rat: over 1 weeks）

hiPSC-derived cortical neurons require much longer to achieve functional maturation.

Odawara A, et.al, *Sci Rep* **6**, 26181 (2016).

Results : Development of spontaneous firing during long-term culture





Firing frequency were increasing up to approx. 20 weeks culture.

Odawara A, et.al, *Sci Rep* **6**, 26181 (2016).

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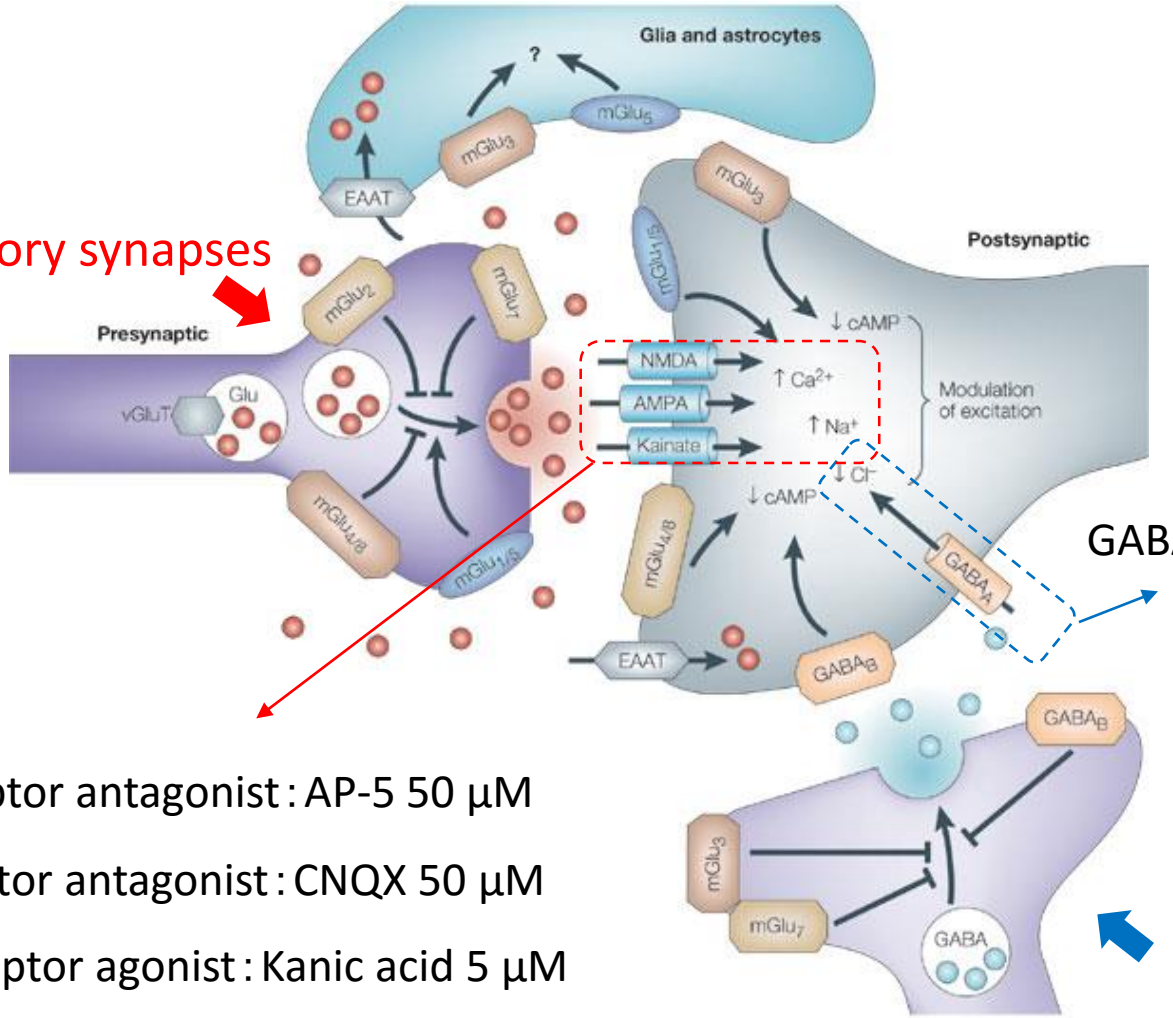
Pharmacological test

Synapse-related typical drugs

Excitatory synapses



GABA$_{-A}$ receptor antagonist
: Bicuculline 10 μM

NMDA receptor antagonist : AP-5 50 μM

AMPA receptor antagonist : CNQX 50 μM

Kainate receptor agonist : Kanic acid 5 μM

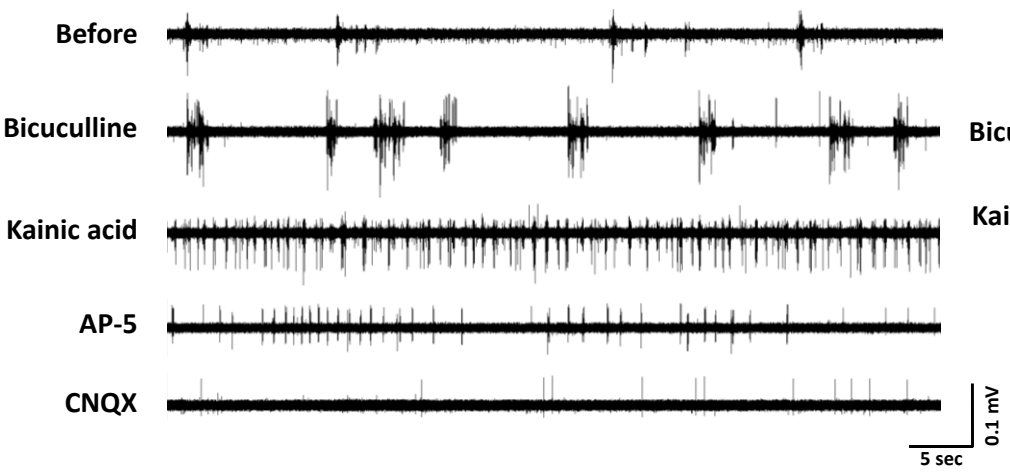
Inhibitory synapses

Nature Reviews | Drug Discovery

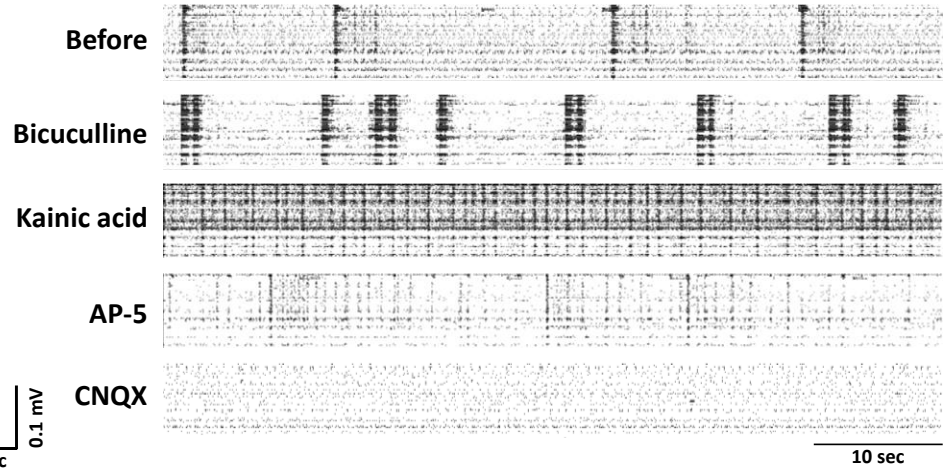
Results : Pharmacological properties of spontaneous firings

33-36 weeks

Typical spontaneous firings
at same electrode

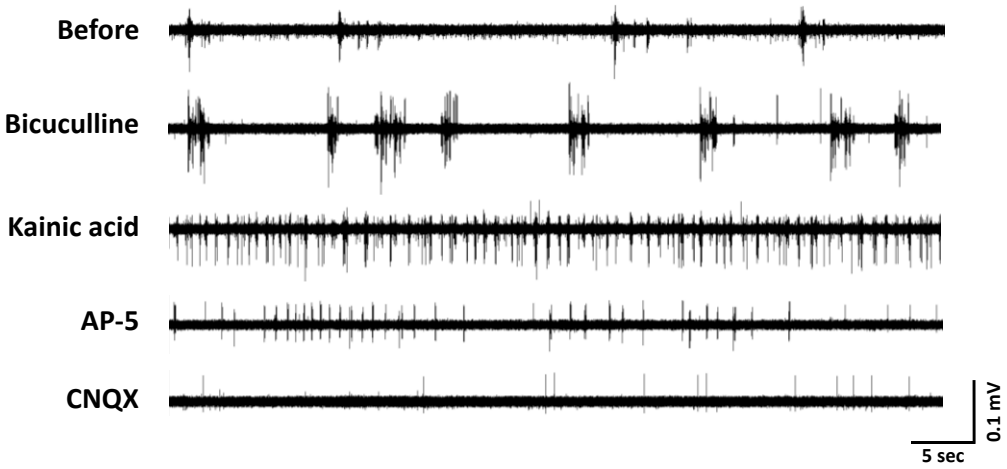


Raster plots
for 1 min all 64 electrodes

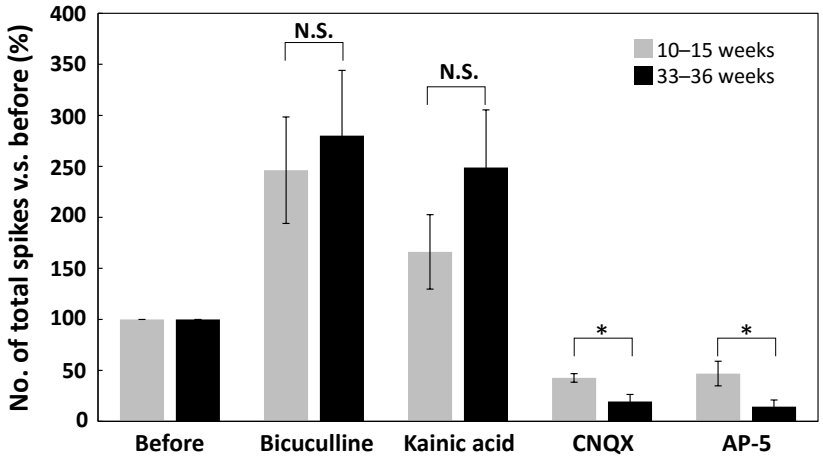


Odawara A, et.al, *Sci Rep* **6**, 26181 (2016).

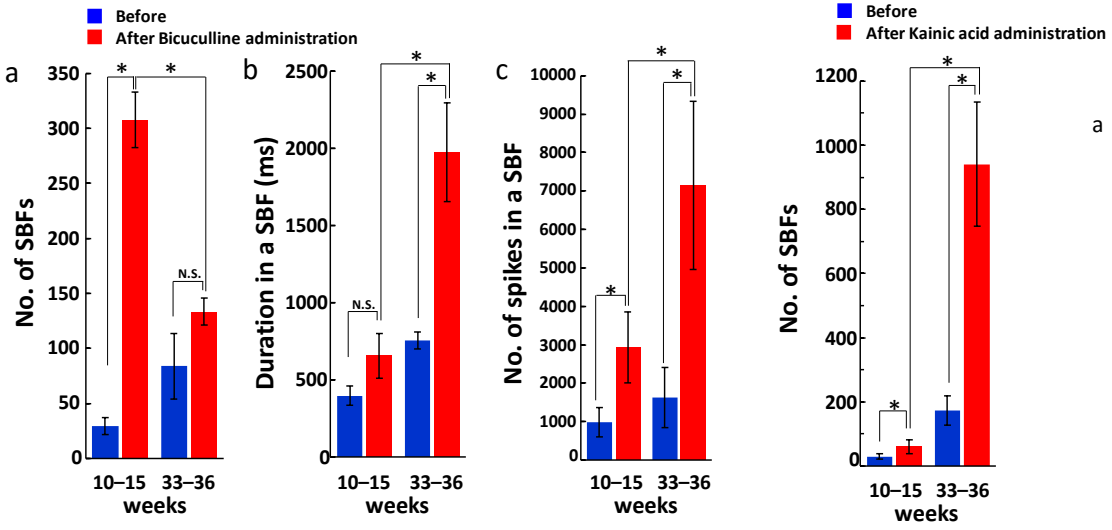
Results : Comparison between 10-15 and 33-36 weeks cultures

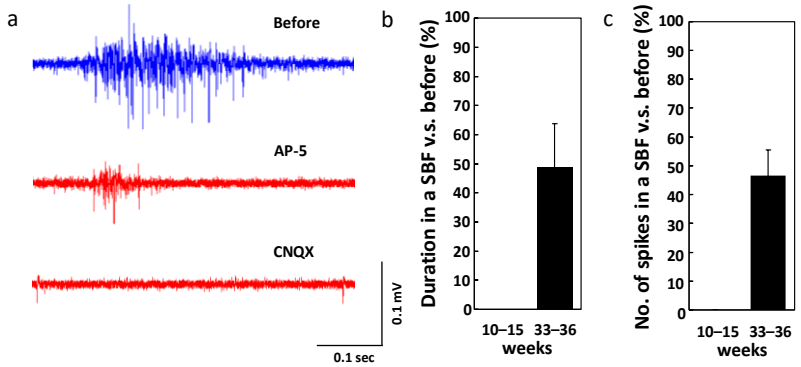


Total number of spikes(100%, before administration)



Analysis of synchronized burst firings (SBFs)



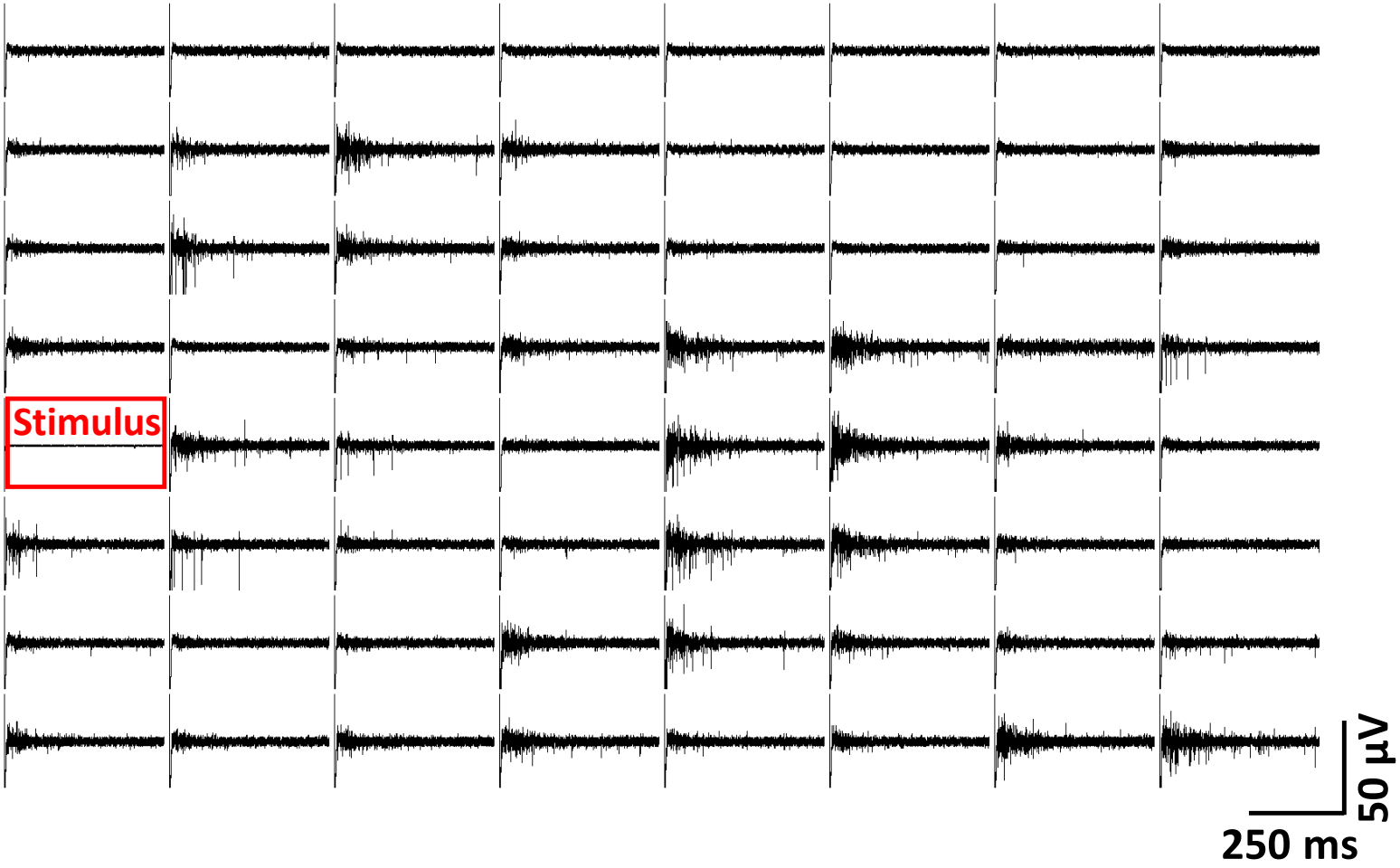


Spontaneous activity in 33–36 WIV cultures was more sensitive to GABA and glutamate receptor modulators.

Odawara A, et.al, *Sci Rep* **6**, 26181 (2016).

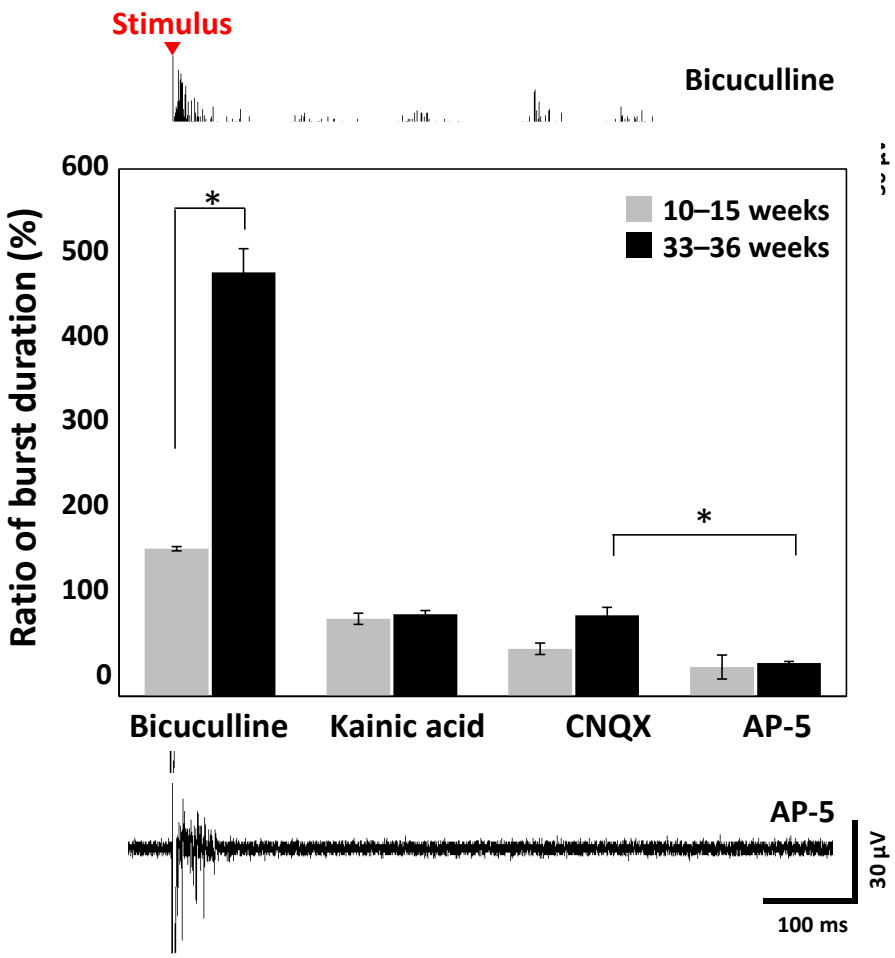
Pharmacological properties of evoked responses

Evoked responses at 64 electrode by test stimulus

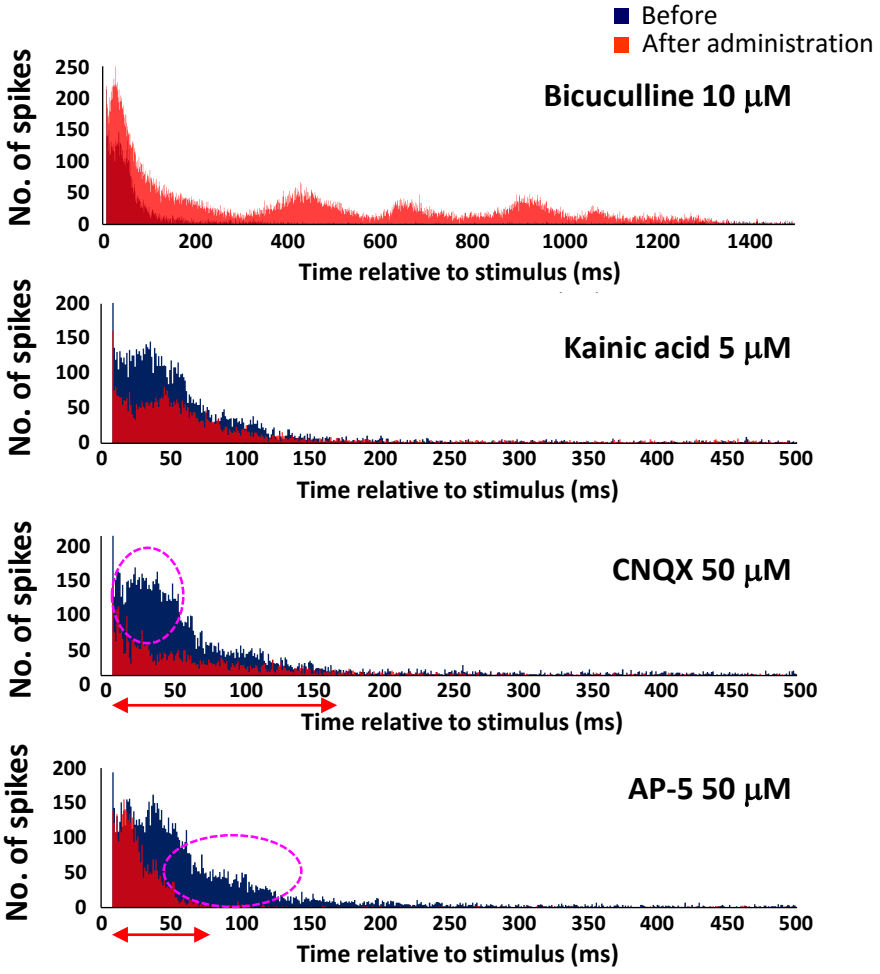


Results : Pharmacological properties of evoked responses

33-36 weeks



PSTH : Post stimulus time histogram



We detected each response of AMPA and NMDA receptors in 33–36 WIV cultures.

Odawara A, et.al, *Sci Rep* **6**, 26181 (2016).

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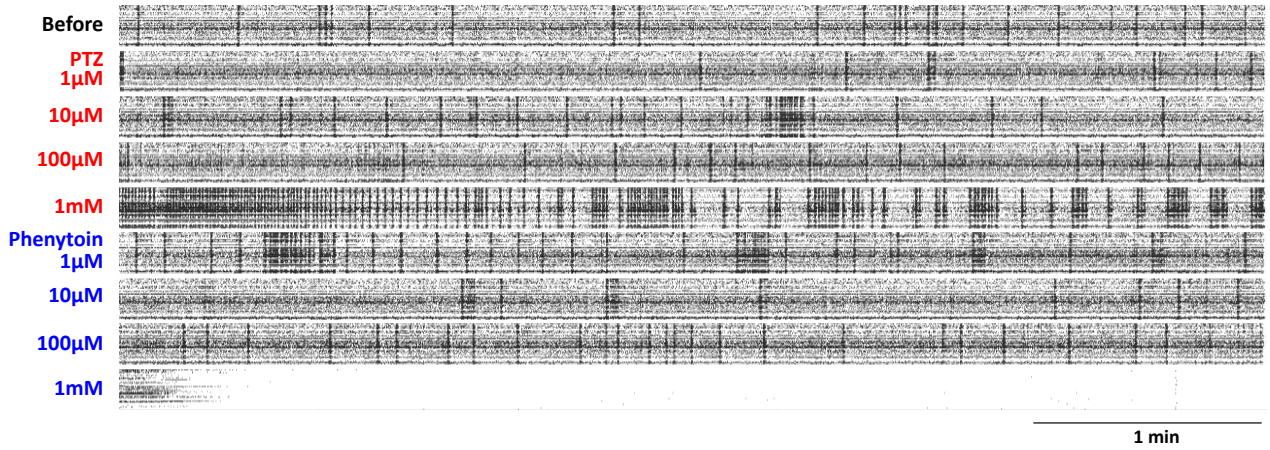
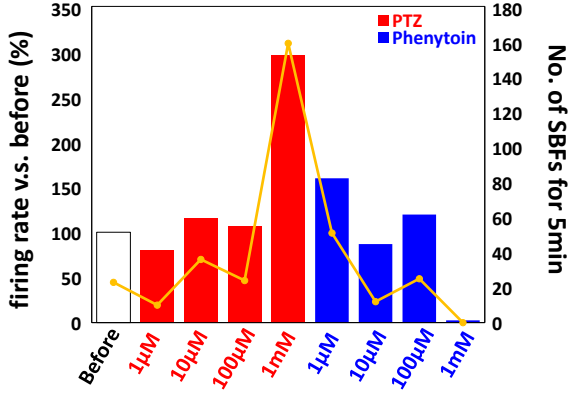
- Induction of Long term potentiation (LTP) and depression (LTD)

Results: Induction of epileptiform activity and effects of anti-epilepsy drugs

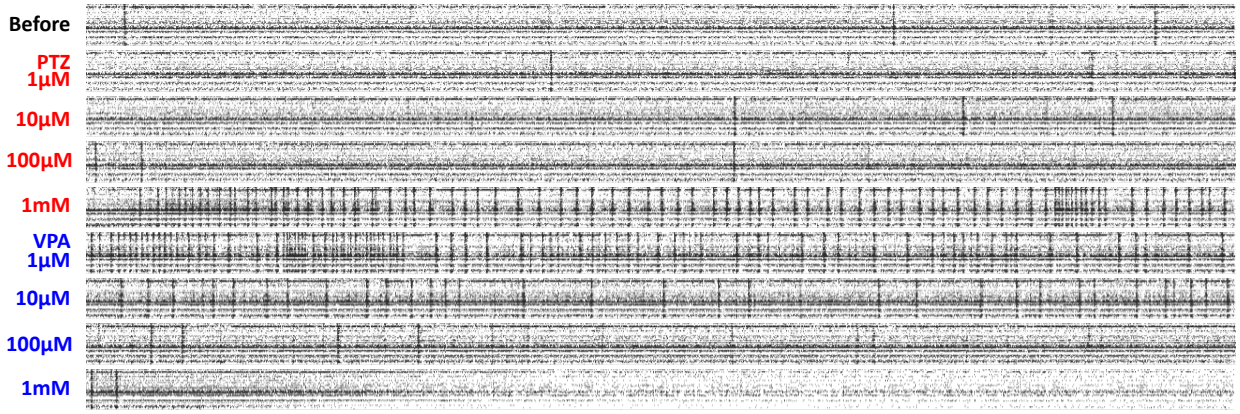
Epilepsy inducing drug : PTZ（PentyleneTetraZol）　　**Antiepilepsy drug :** Phenytoin, Sodium valproate (VPA)

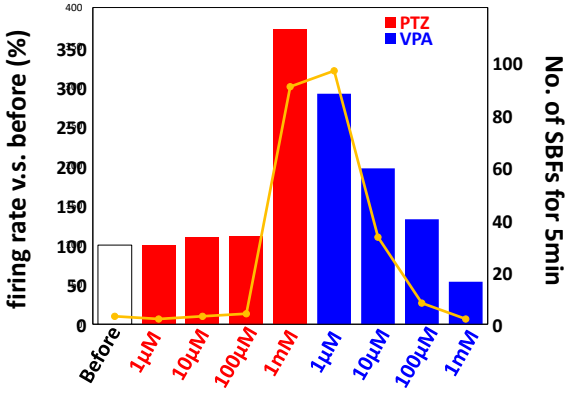
Phenytoin : Block of voltage-gated Na^+ channels





VPA : Enhancement of GABA inhibition





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

Odawara A, et.al, *Sci Rep* **6**, 26181 (2016).

Results: Induction of epileptiform activity and effects of anti-epilepsy drugs

Before

Today's Topics

Electrophysiological properties and pharmacological responses in hiPSC-derived cerebral cortical neurons (hCCNs)

- Morphology in long-term culture
- Development of spontaneous firings
- Pharmacological properties
- Induction of Epileptiform activity and effects of AEDs
- Induction of Long term potentiation (LTP) and depression (LTD)

Induction of Long term potentiation (LTP) and depression (LTD)

LTP・LTD

- Learning, Memory and Development in brain function
- Disease mechanisms and Drug discoveries

We attempted the induction of LTP and LTD phenomena as the change of spike pattern

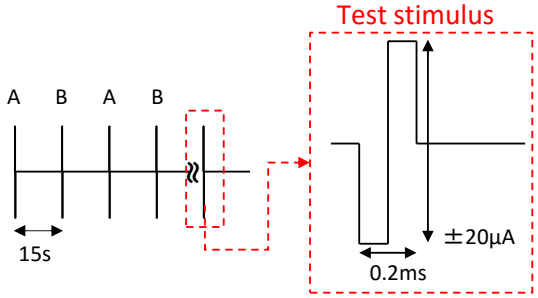
Induction of Long term potentiation (LTP) and depression (LTD)

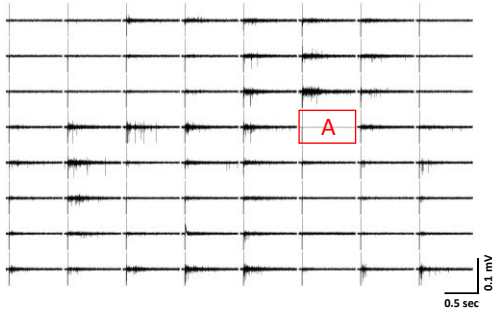
Methods

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

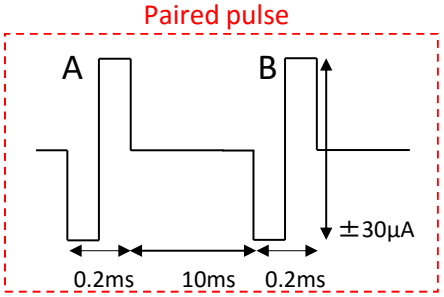
【Protocol】

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

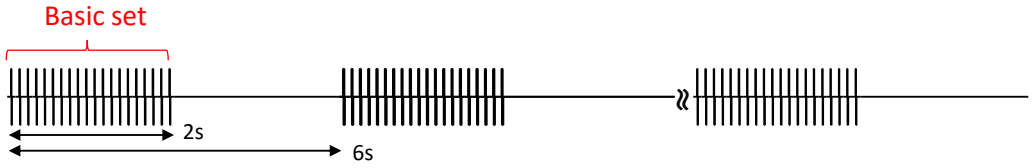




- High frequency stimulation (HFS)

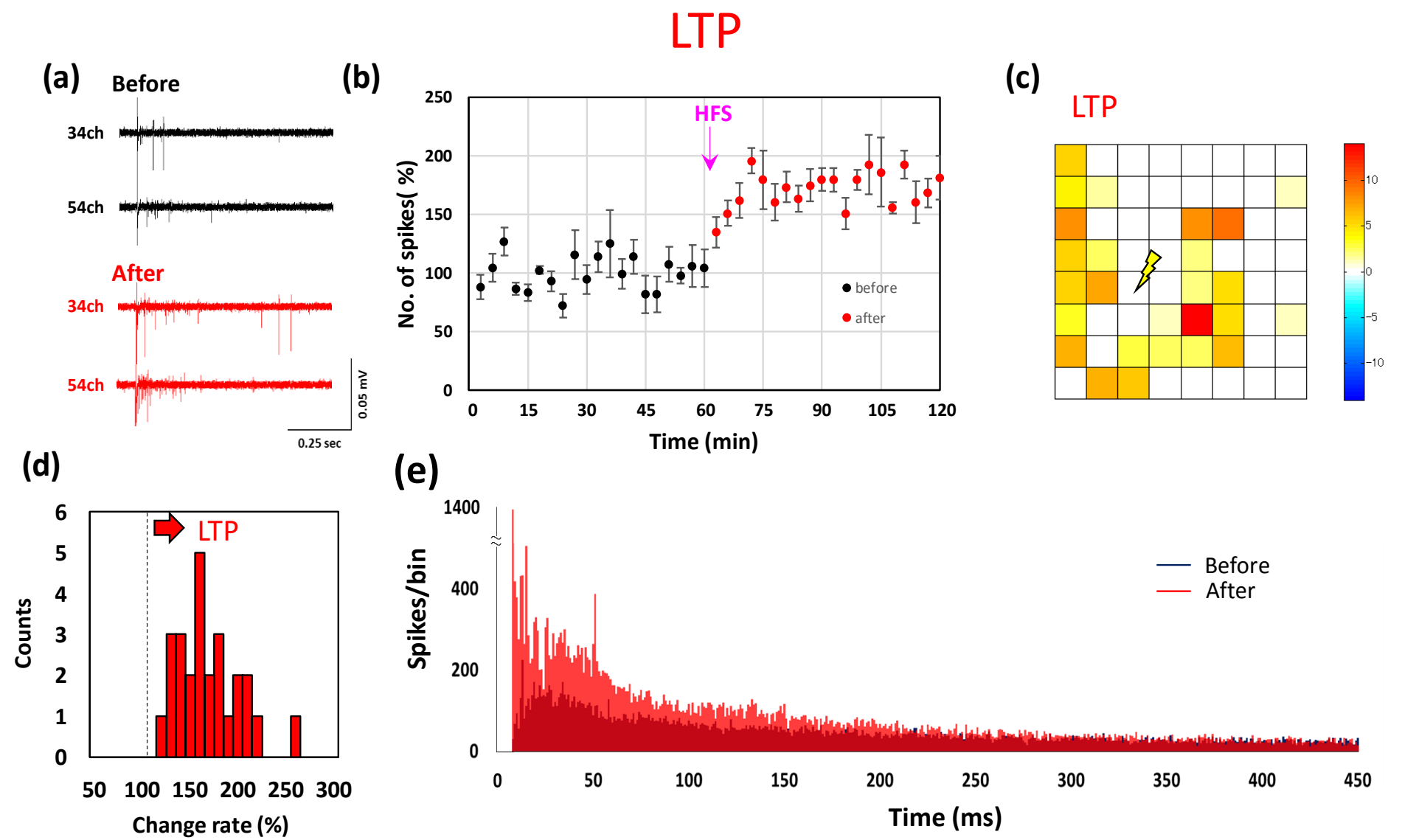


- Paired pulse (stimulus to A ch and B ch after 10 ms)
- 20 trains of paired stimulation at 10 Hz were applied at 120 × at 4 s intervals.



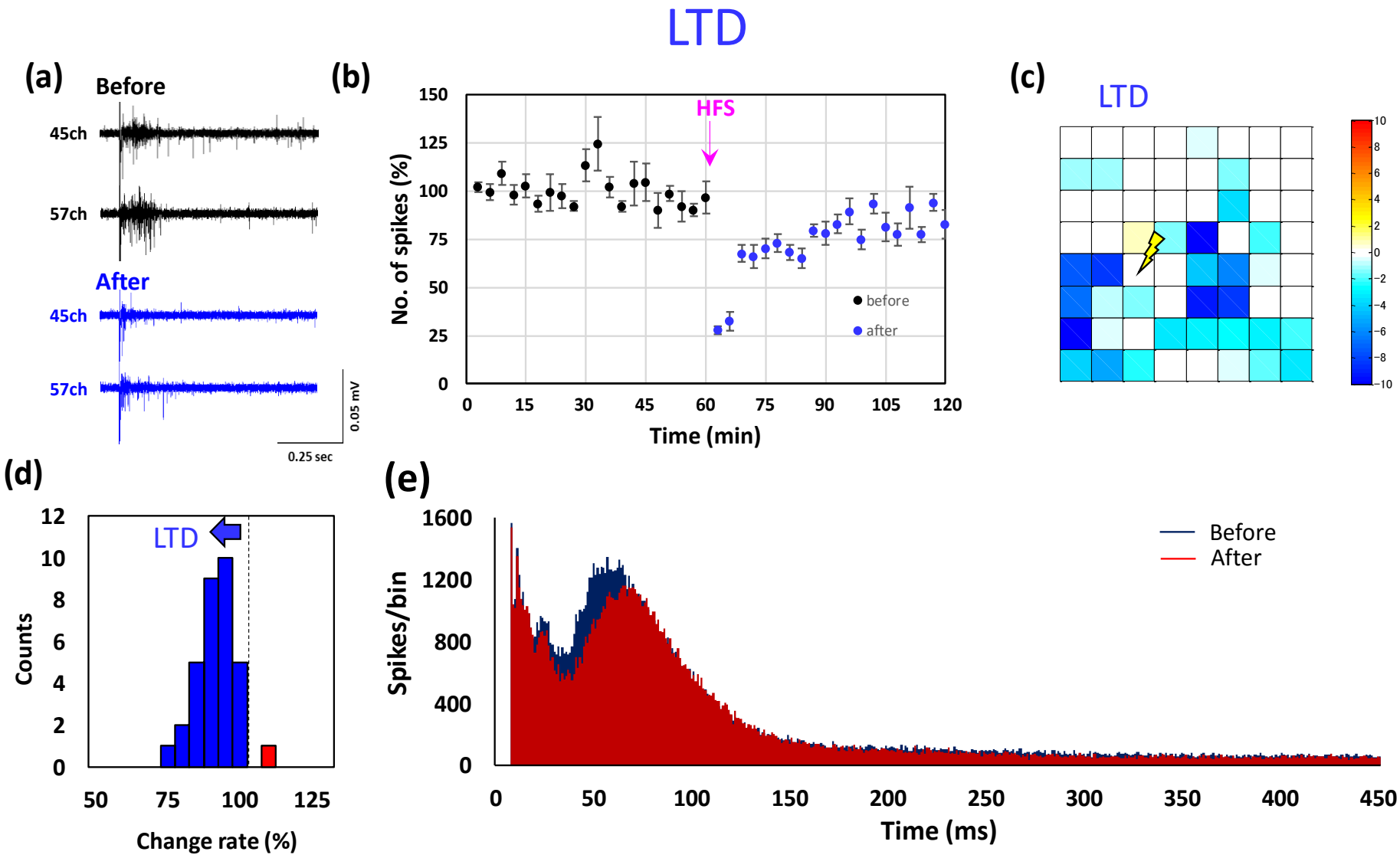
- A test stimulus alternating between A ch and B ch every 15 s for 60 min (after)

Results: Induction of Long term potentiation (LTP)



Odawara A, et.al, *BBRC* **496**, 856 (2016).

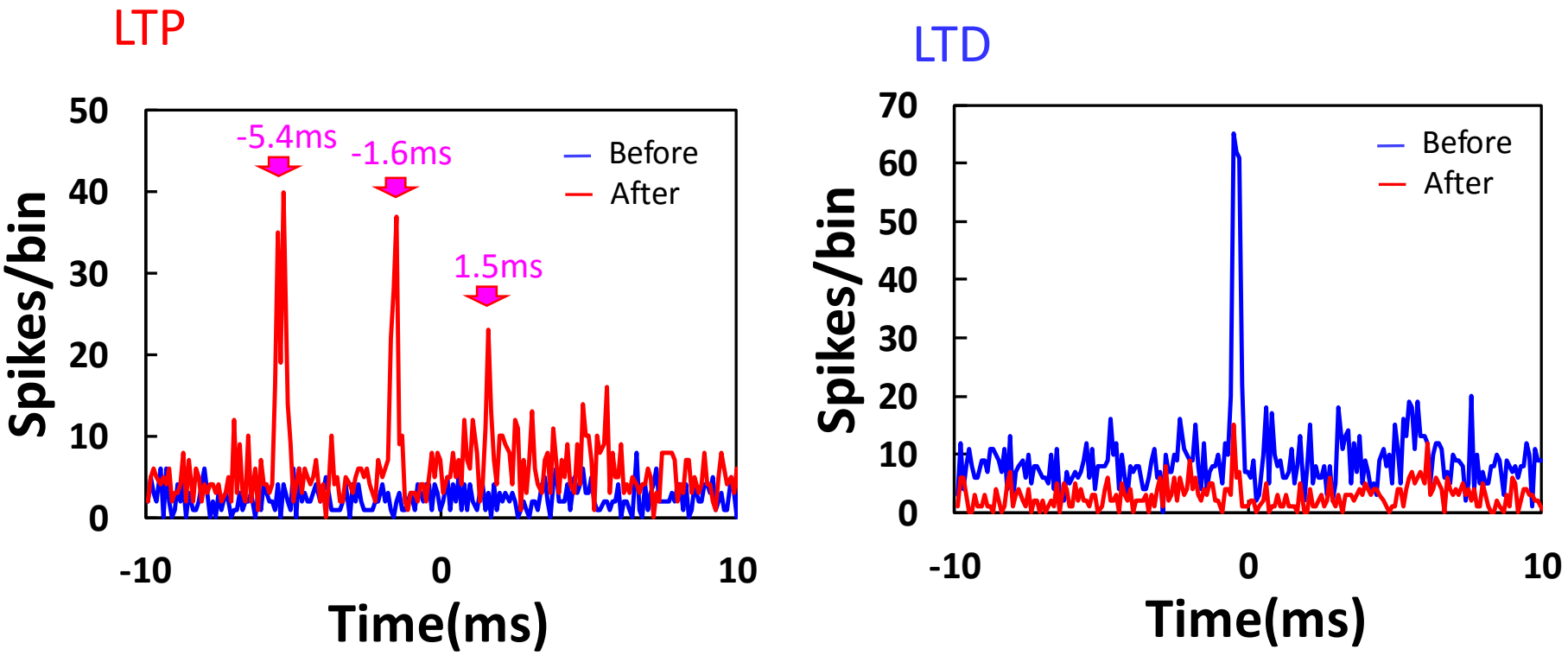
Results: Induction of Long term depression (LTD)



Odawara A, et.al, *BBRC* **496**, 856 (2016).

Results: Induction of LTP and LTD (Cross correlation Histogram ①)

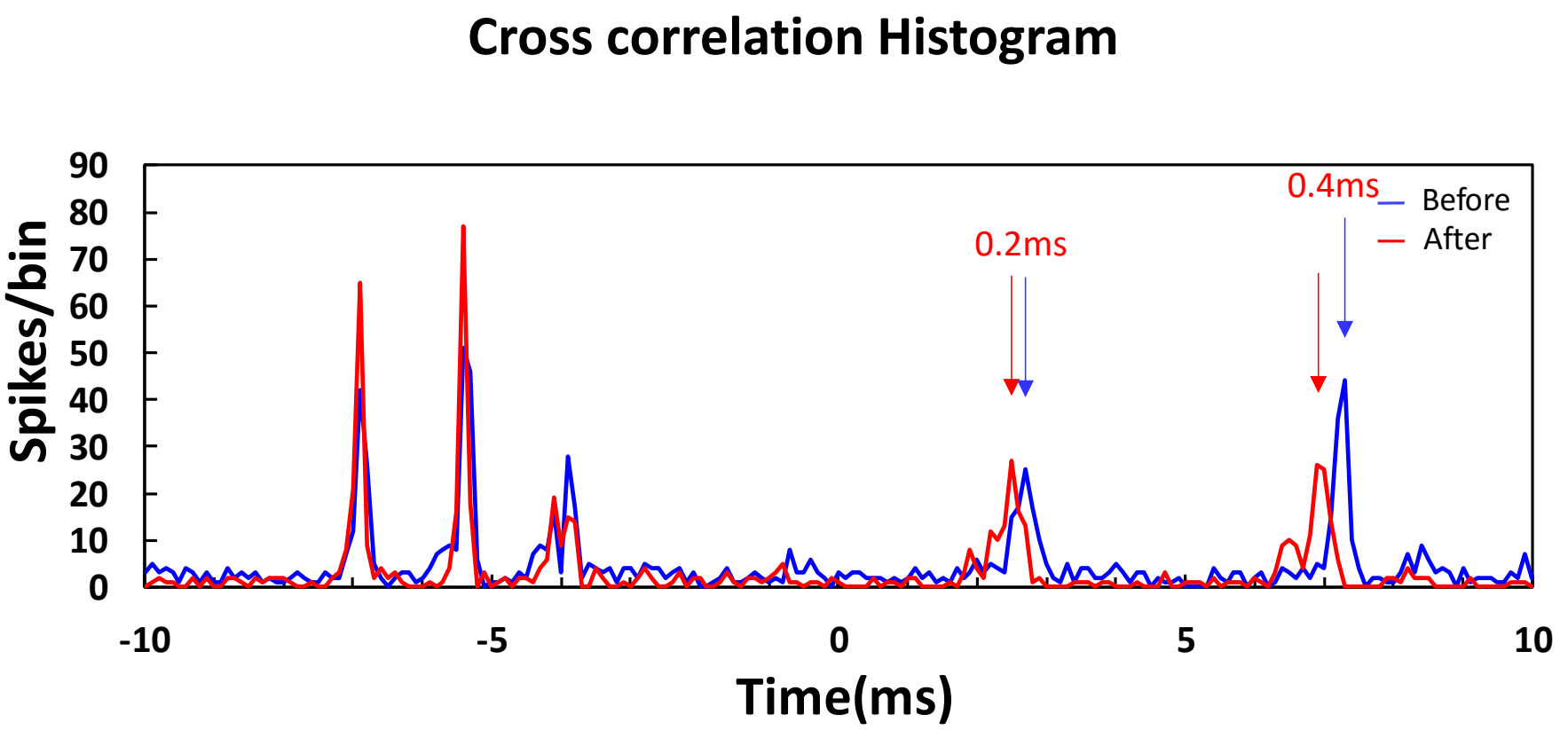
Cross correlation Histogram



Spike patterns with specific timing were generated during LTP induction and disappeared during LTD induction rather than the change of random firing pattern.

Odawara A, et.al, *BBRC* **496**, 856 (2016).

Results: Induction of LTP and LTD (Cross correlation Histogram ②)



The hiPSC-derived cortical neuronal network has the potential to repeatedly express the spike pattern with a precise timing change within 0.5 ms.

Odawara A, et.al, *BBRC* **496**, 856 (2016).

Summary

- We detected the development of spontaneous electrophysiological activity and pharmacological responses for over 1 year in cultured human iPSC-derived cerebral cortical neurons (Axol, hCCNs)
- The complete maturation of spontaneous firing, evoked responses, and modulation of activity by glutamatergic and GABAergic receptor antagonists/agonists required 20–30 weeks.
- Neural networks also demonstrated epileptiform synchronized burst firing (SBF) in response to pro-convulsants and SBF suppression using clinical anti-epilepsy drugs.
- We also detected LTP and LTD phenomena in a hiPSC-derived neuronal network as the change of spike pattern.

Long-term culture of hiPSC-derived neuronal neurons on MEAs proved useful for neuropharmacological, neurotoxicological assays and investigating the function of human neuronal circuits.

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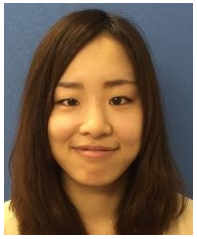
  A. Odawara, H. Katoh, N. Matsuda



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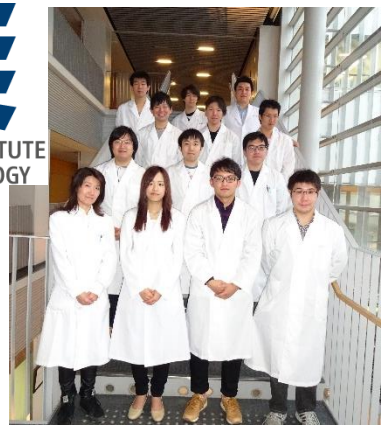

- Axol Bioscience (UK).
  N. W. Carpenter, Y. Shi,


axol

- Alpha Med Scientific (Japan).
  R. Yamazaki, H. Jiko


ALPHA MED SCIENTIFIC