Electrophysiological Pain Responses in Cultured Human iPSC-Derived Sensory Neurons Using High-Throughput Multi-Electrode Array System

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Purpose

Dorsal root ganglion (DRG) sensory neurons are pain-related neurons and have a variety of sensory receptors that are activated by chemical, thermal, and mechanical stimuli. Establishment of pharmacological assay in pain research and drug screening is important issue. In addition, human induced pluripotent stem cell (hiPSC)-derived sensory neurons may be effectively used for drug discovery and toxicity testing. The purpose of this study was to evaluate the physiological responses against typical pain-related molecules, temperature change and anti-cancer drug in cultured sensory neurons using high-throughpupt multi-electrode array (MEA) system.

Material & Methods

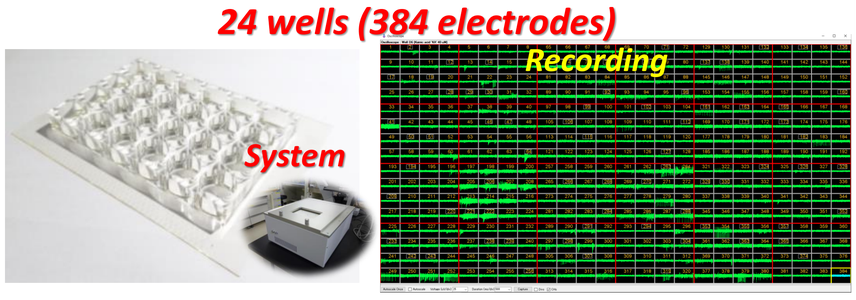
Human iPSC-derived sensory neurons [Axol Bioscience]

Human iPSC-derived sensory neurons (Axol Bioscience Inc., UK) were cultured at 8.0 × 10^5 cells/cm^2 on 384-channel 24 well MEA chip and 64-channel MEA chips (Alpha Med Scientific) coated with Axol Sure Bond Coating Solution (Axol Bioscience) at 37° C in a 5% CO_2/95% air atmosphere. Immunofluorescent images were obtained by confocal microscopy.

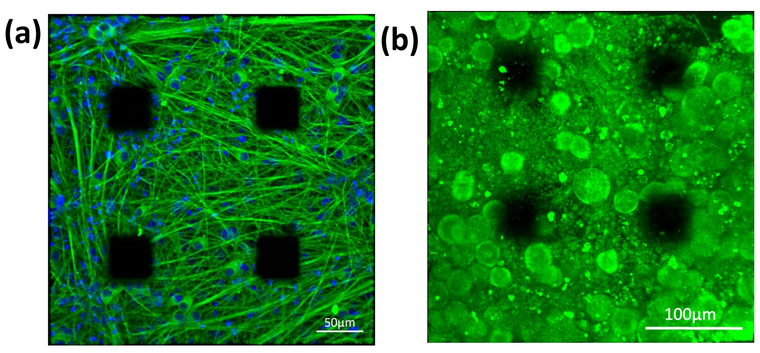
High-Throughput MEA system [Alpha med scientific]

Spontaneous extracellular field potentials were acquired at 37° C under a 5% CO_2 atmosphere using the high-throughput multielectrode array system, where we simultaneously record extracellular potentials for 16 channels per well across 24-well plates (Presto, Alpha Med Scientific) and a 64-channel MEA system (MED64-Basic; Alpha Med Scientific) at a sampling rate of 20 kHz/channel. Signals were low-pass filtered at 100 Hz and stored on a personal computer. Firing analyses and spike sorting were performed using Mobius software (Alpha Med Scientific Inc.).

24 wells (384 electrodes)



hiPSC-derived sensory neurons on the MEA



(a) 2 weeks s in vitro. (2 WIV) (b) 8 WIV

Result 1 Sensory neural marker expression

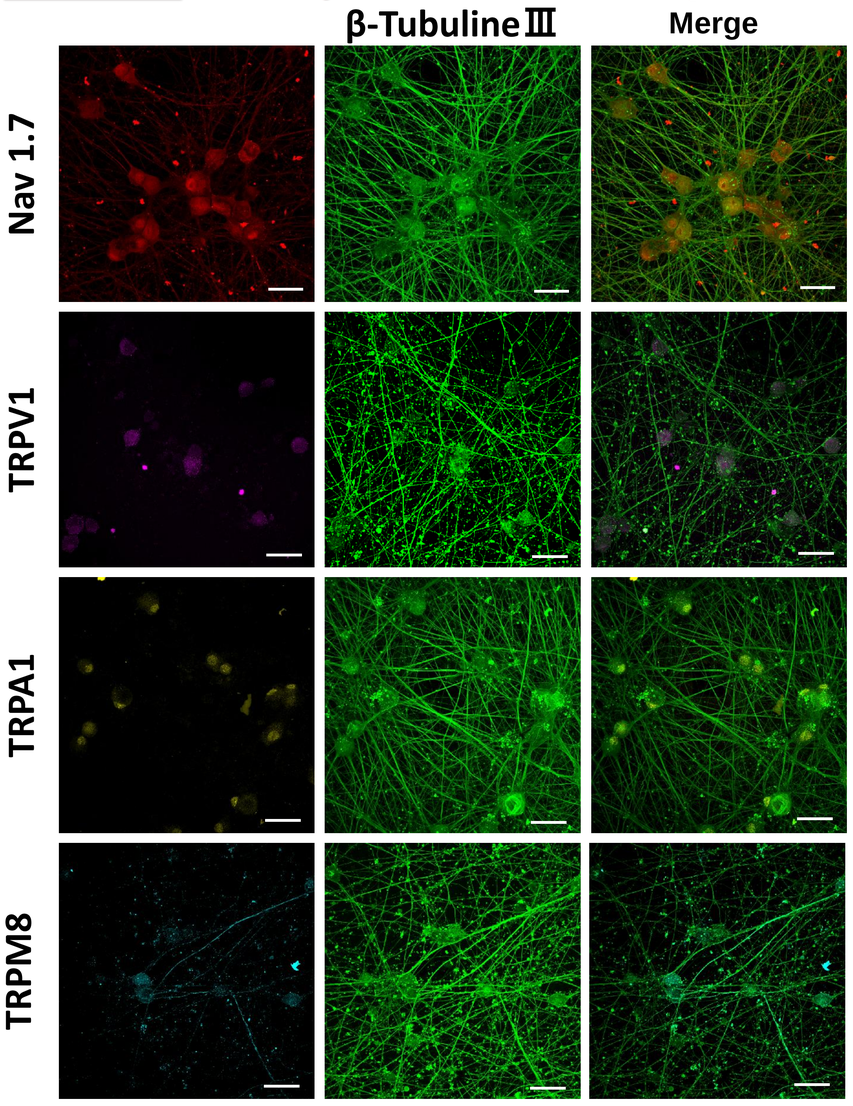


Fig.1 Immunostaining in cultured hiPSC-derived sensory neurons. Nav 1.7, TRPV1, TRPA1, and TRPM8 expression at 12 *weeks in vitro*.

➢Human iPSC-derived sensory neurons (Axol Bioscience) show the expression of typical sensory neural marker Nav1.7, TRPV1, TRPA1 and TRPM8.

Result 2 Electrophysiological responses by capsaicin, menthol, and AITC administration

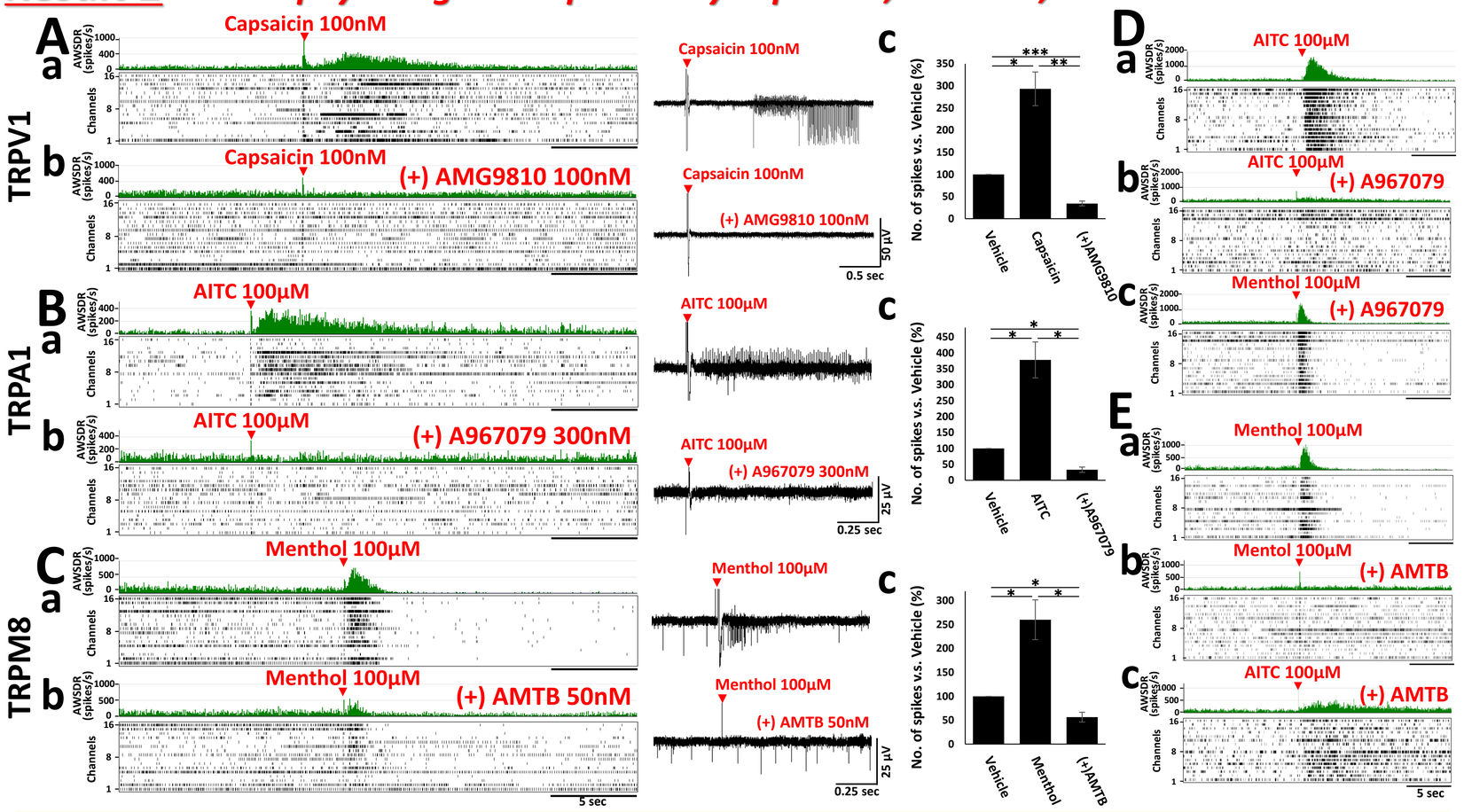


Fig.2 Responses to capsaicin, menthol, and AITC administration (A)Evoked responses to Capsaicin 100nM and block in the presence of TRPV1 antagonist AMG9810 100 nM. (B) Evoked responses to AITC 100μM and block in the presence of TRPA1 antagonist (+) A967079 300 nM. (C) Evoked responses to Menthol 100μM and block in the presence of TRPM8 antagonist AMTB 50 nM. (b) Responses against capsaicin, menthol and AITC were measured 1h after each antagonist administration. (A)(c) n=3, *p < 0.01, **p < 0.005, ***p < 0.001 (B)(c) n=8, *p < 0.001, (C)(c) n=5, *p < 0.005 (D)Conformation of inhibitor effects. (D)(a) Response to AITC 100 μM. (b)Response to AITC 100 μM after 1 hour A967079 300nM administration. (c) Response to Menthol 100 μM. (E) (a) Menthol 100 μM. (b)Response to Menthol 100 μM after 1 hour AMTB 50nM administration. (c) Response to AITC 100 μM.

➢We detected the evoked responses to capsaicin, menthol, and wasabi in cultured hiPSC-derived sensory neurons.
➢ Evoked responses to capsaicin, menthol, and AITC were TPRV1, TRPM8, and TRPA1 channel dependent responses.

Result 3 Responses by temperature change

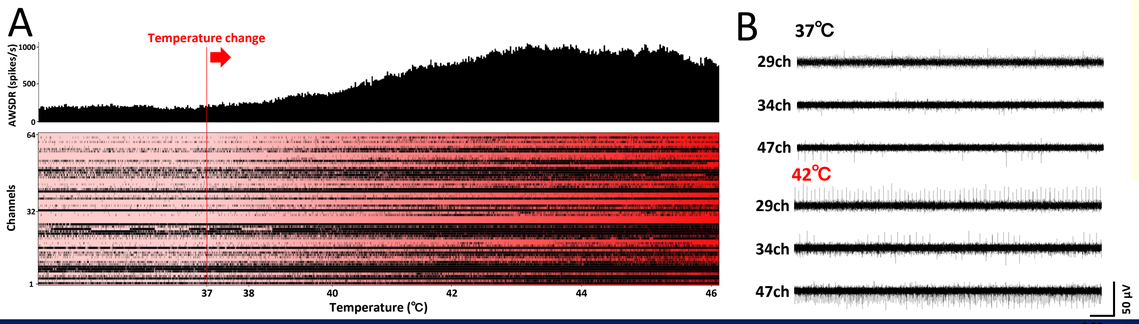


Fig.3 Responses in hiPSC-derived sensory neurons by temperature change. (A) Raster plots at 64 electrodes from 37 to 46 ℃. Red line: starting time.(B) Typical waveform at 37 and 42 ℃.

➢ The firing peak was 43℃. It was consistent with the activation temperature of TRPV1 channels.

Result 4 Anti-cancer drug Vincristine and Oxaliplatin administration

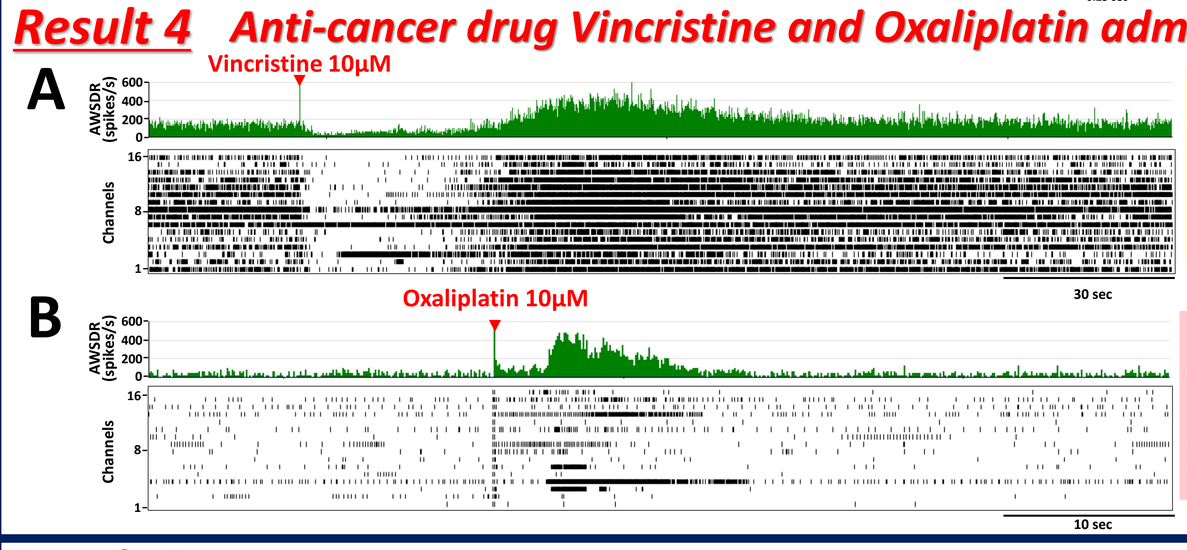


Fig.4 Responses by anti-cancer drugs vincristine and oxaliplatin
Raster plots and AWSDR before and after Vincristine 10μM (A) and Oxaliplatin 10μM (B) administration.

➢ Acute response to Vincristine and Oxaliplatin was shown.
➢ Time to response after administration is slow compared to capsaicin, menthol and AITC.

Result 5 Cold hypersensitivity reaction in the presence of Oxaliplatin

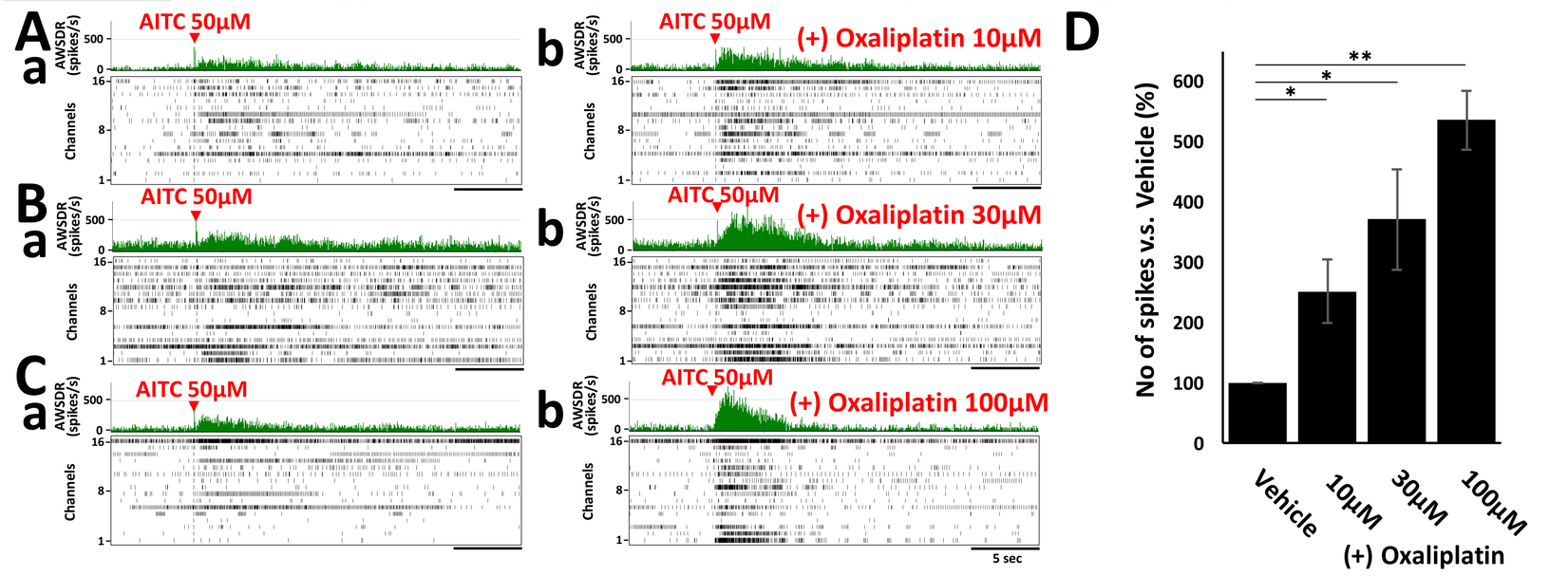


Fig.5 Cold hypersensitivity reaction in the presence of Oxaliplatin
(A)(a)Response to AITC 50μM(control). (b) Response to AITC 50μM in presence of oxaliplation 10μM. (B)(a)AITC 50μM(control). (b) Response to AITC 50μM in presence of oxaliplation 30μM. (C)(a)AITC 50μM(control). (b) Response to AITC 100μM in presence of oxaliplation 30μM. Responses were measured after 2 hours oxaliplatin 10, 30, 100μM administration. (D)Oxaliplatin dose-dependent firing rate. (n=6, *p < 0.05, **p < 0.0005)

➢Cold hypersensitivity response through TRPA1 channel in the presence of Oxaliplatin was detected using human iPS cell -derived sensory neurons and it was confirmed that the response to AITC increased dose -dependent in Oxaliplatin.

Conclusion

- TRP channel-dependent pain responses were detected in cultured human iPSC-derived sensory neurons.
- Acute pain response was detected by administration of anticancer drugs vincristine and oxaliplatin
- Cold hypersensitivity responses were detected in concentration dependent manner of oxaliplatin.

The MEA evaluation system using human iPSC-derived sensory neurons is effective as a pain assessment and detection method for human peripheral neuropathy.