Pain responses in human iPSC-derived sensory neurons using MEA system

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Introduction

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 human pain research and drug screening is important issue. Here, we used human iPSC-derived sensory neurons and the multi-electrode array (MEA) system to detect the electrophysiological responses by chemical and thermal stimuli.

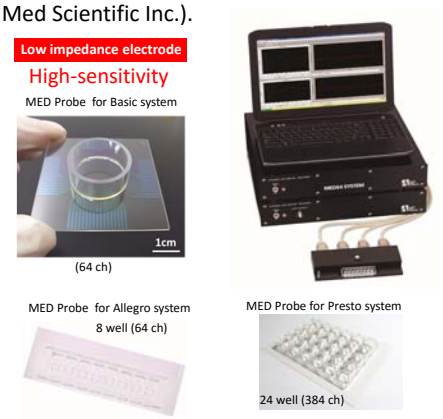
Methods

Culture of hiPSC-derived sensory neurons

Human iPSC-derived sensory neurons (Axol Bioscience Inc., UK) were cultured at 5.0 × 10^5 cells/cm^2 on 64-channel MEA chips (MED-P515A; Alpha Med Scientific) coated with Axol Sure Bond Coating Solution (Axol Bioscience) at 37° C in a 5% CO_2/95% air atmosphere.

MEA system

Spontaneous extracellular field potentials were acquired at 37° C under a 5% CO_2 atmosphere using a 64-channel MEA system(1) (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 sortings were performed using Mobius software (Alpha Med Scientific Inc.).



Conclusion

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

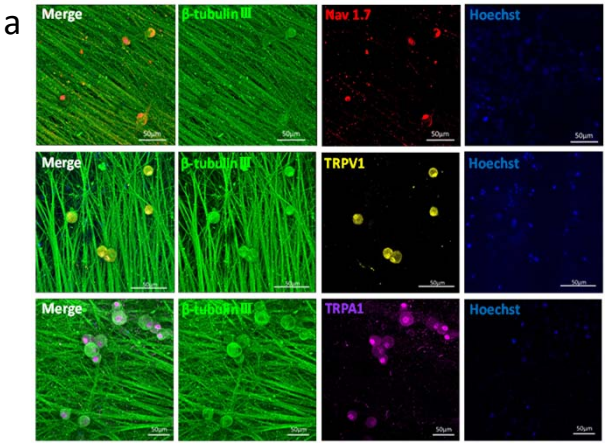
➢We detected the responses to temperature change, capsaicin, menthol, and wasabi by change of spike rate.

➢ Human iPSC-derived neurons were classified into 27 types depending on physiological responses against 3 compounds.

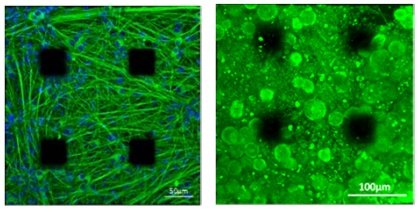
➢ Percentage of the neurons having positive function against capsaicin were high both hiPSC-derived neurons and rat DRG neurons.

➢Our studies show that electrophysiological measurement in cultured hiPSC derived sensory neurons using MEA system are suitable to toxicological assay and drug screening in peripheral nerves.

Result 1 Sensory neural marker expression



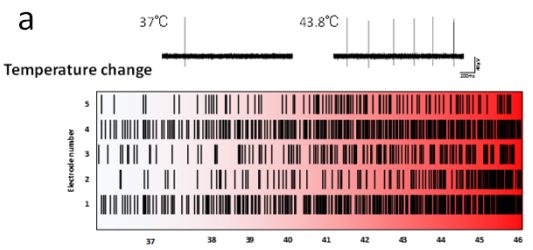
b Sensory neurons on the MEA

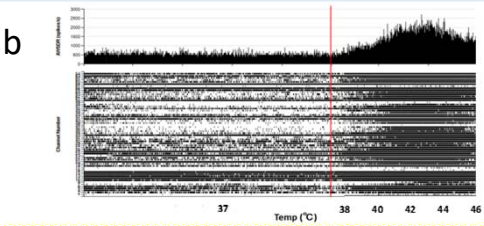


Immunostaining in cultured hiPSC-derived sensory neurons. (a) Nav 1.7, TRPV1, TRPA1 expression at 8 *weeks in vitro* (WIV). (b) Sensory neurons on the MEA chip. Right: 2 WIV. Left: 8 WIV. Green: β-tubulin Ⅲ.

Result 2 Pain responses in hiPSC-derived sensory neurons

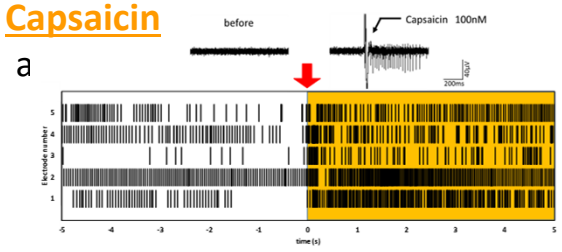
Temperature

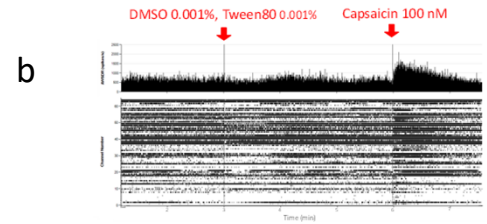




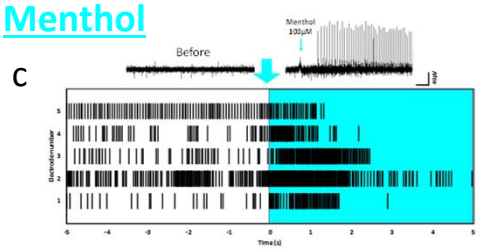
Physiological responses against temperature change (a) Typical waveform and Raster plots from 37 to 46 ℃. (b) Raster plots at 64 electrodes. Red line: starting time.

Capsaicin

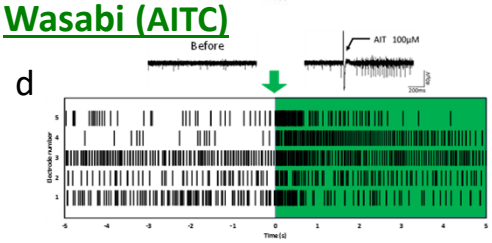




Menthol

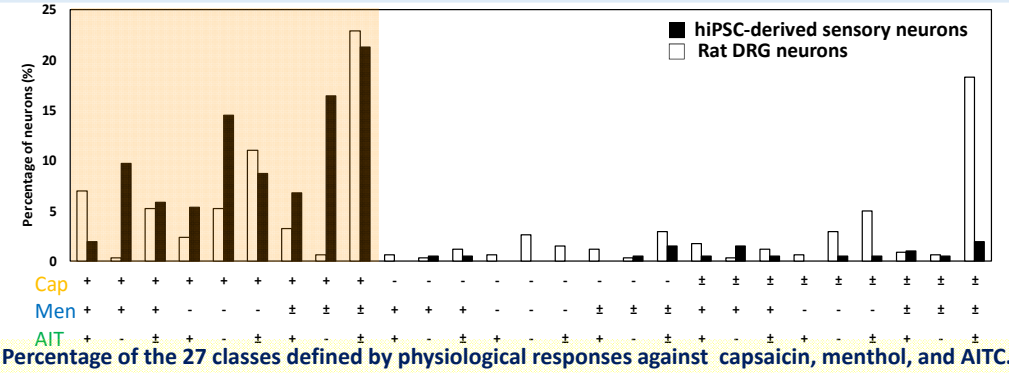


Wasabi (AITC)



Responses to capsaicin, menthol, and AITC administration at 8 WIV. (a) Responses to 100 nM capsaicin administration. (b) Raster plots with control at 64 ch. (c) 100 μM menthol. (d) 100 μM Allyl isothiocyanate (AITC).

Result 3 27 classes of hiPSC-derived sensory neurons and rat DRG neurons defined by physiological responses against 3 compounds



Percentage of the 27 classes defined by physiological responses against capsaicin, menthol, and AITC. Black bar: hiPSC-derived sensory neurons (n = 790 neurons). White bar: Rat DRG neurons (n = 345 neurons). +: positive function (increase of firings), -:negative function (decrease of firings), ±:no change.