

## 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 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-throughput multi-electrode array (MEA) system.

## Material & Methods

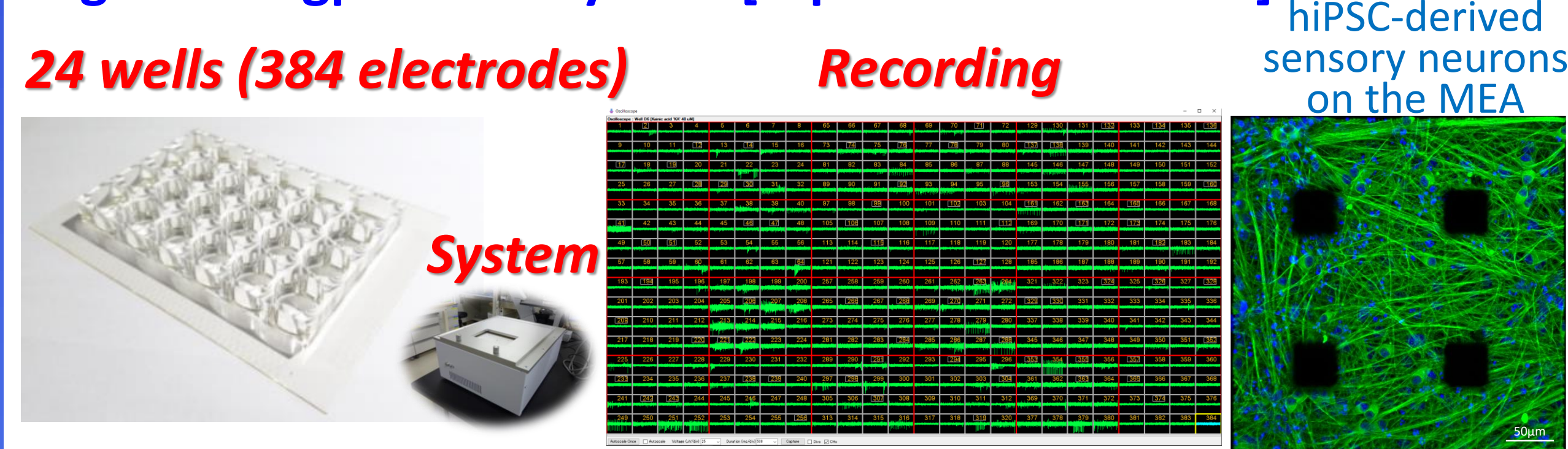
### Human iPSC-derived sensory neurons [Axol Bioscience]

Human iPSC-derived sensory neurons (Axol Bioscience Inc., UK) were cultured at  $5.0 \times 10^5$  cells/cm<sup>2</sup> 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<sub>2</sub>/95% air atmosphere. Immunofluorescent images were obtained by confocal microscopy.

### Rat DRG neurons

To compare with human iPSC-derived sensory neurons, Rat DRG neurons were used in Fig.2D. DRG neurons were obtained from 10 weeks male Rat by dissection. DRG neurons were cultured on 64-channel MEA chips (MED-P515A; Alpha Med Scientific) coated with Laminin-511.

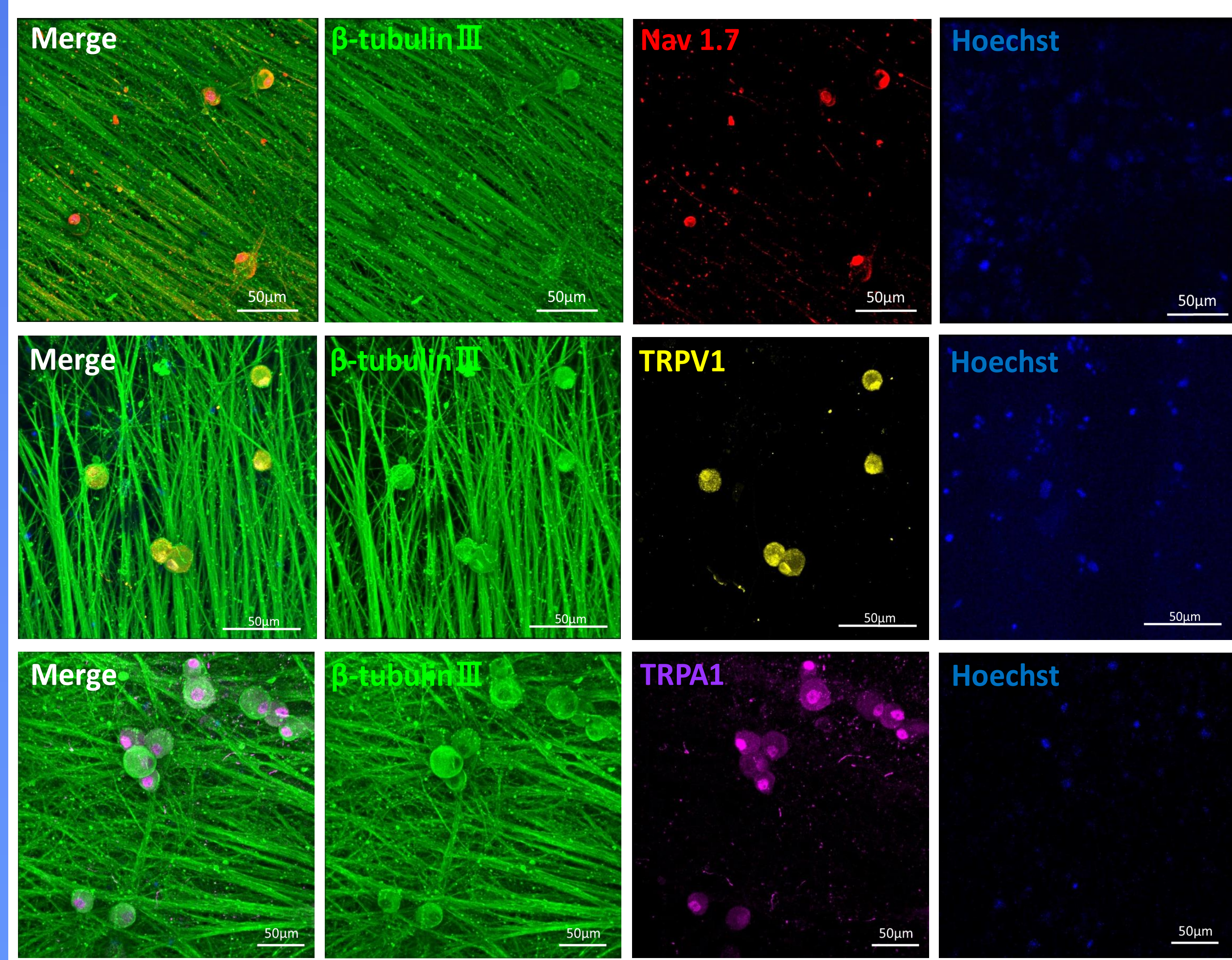
### High-Throughput MEA system [Alpha med scientific]



Low impedance and high sensitivity

Spontaneous extracellular field potentials were acquired at 37° C under a 5% CO<sub>2</sub> 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.).

## Result 1 Sensory neural marker expression



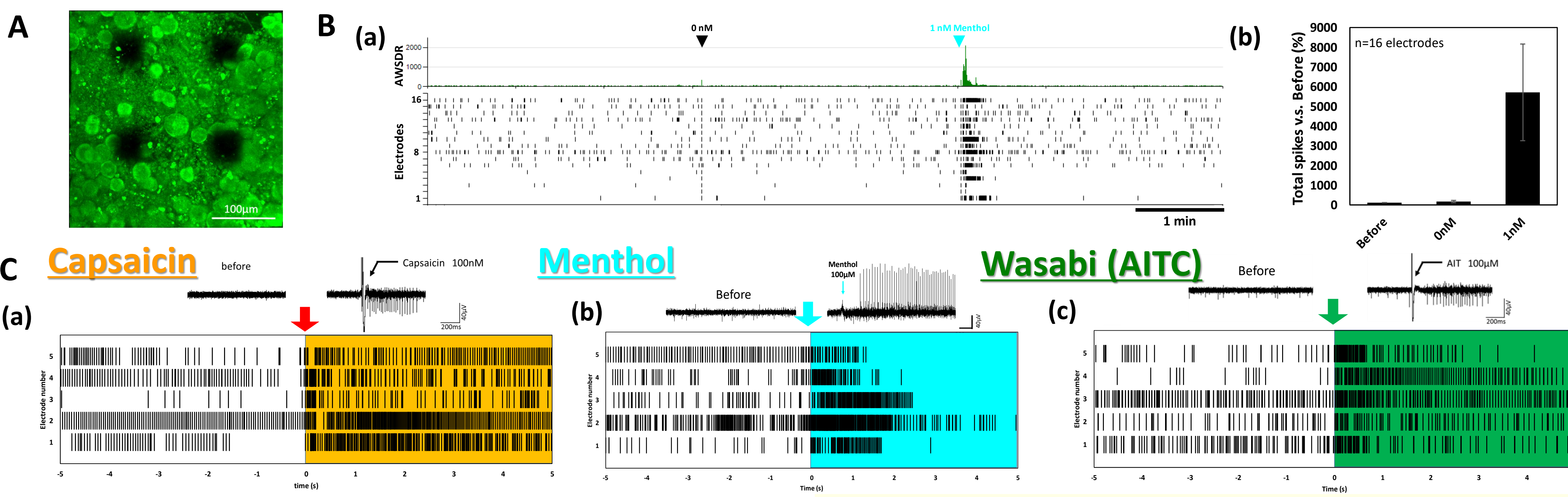
**Fig.1 Immunostaining in cultured hiPSC-derived sensory neurons.** (a) Nav 1.7, TRPV1, TRPA1 expression at 8 weeks *in vitro* (WIV). (b) Sensory neurons on the MEA chip. Right: 2 WIV. Left: 8 WIV. Green:  $\beta$ -tubulin III.

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

## Conclusion

In conclusion, we detected physiological responses against typical pain-related molecules (capsaicin, menthol, and AITC), temperature change and anti-cancer drug in cultured hiPSC-derived sensory neurons and confirmed the expression of typical sensory neural marker. Our studies show that electrophysiological measurement in cultured hiPSC derived sensory neurons using high-throughput MEA system are suitable to toxicological assay and drug screening in peripheral nerves.

## Result 1 Physiological responses by capsaicin, menthol, and AITC administration



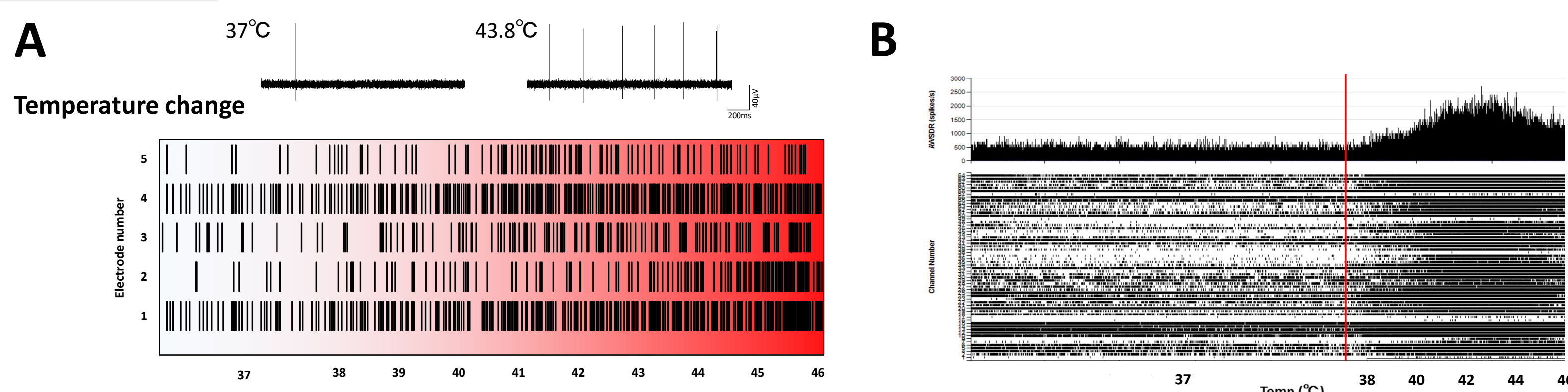
**Fig.2 Responses to capsaicin, menthol, and AITC administration at 8 WIV.**

(A) Immunohistochemical staining of hiPSC-derived sensory neurons on the MEA. Green:  $\beta$ -tubulin III. 8 WIV. (B) Raster plots by menthol administration. (C) Typical responses of capsaicin (100nM), menthol (100  $\mu$ M), and 100  $\mu$ M Allyl isothiocyanate (AITC). (D) 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),  $\pm$ : no change.

> We detected the responses to capsaicin, menthol, and wasabi by change of spike rate in cultured hiPSC-derived sensory neurons.

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

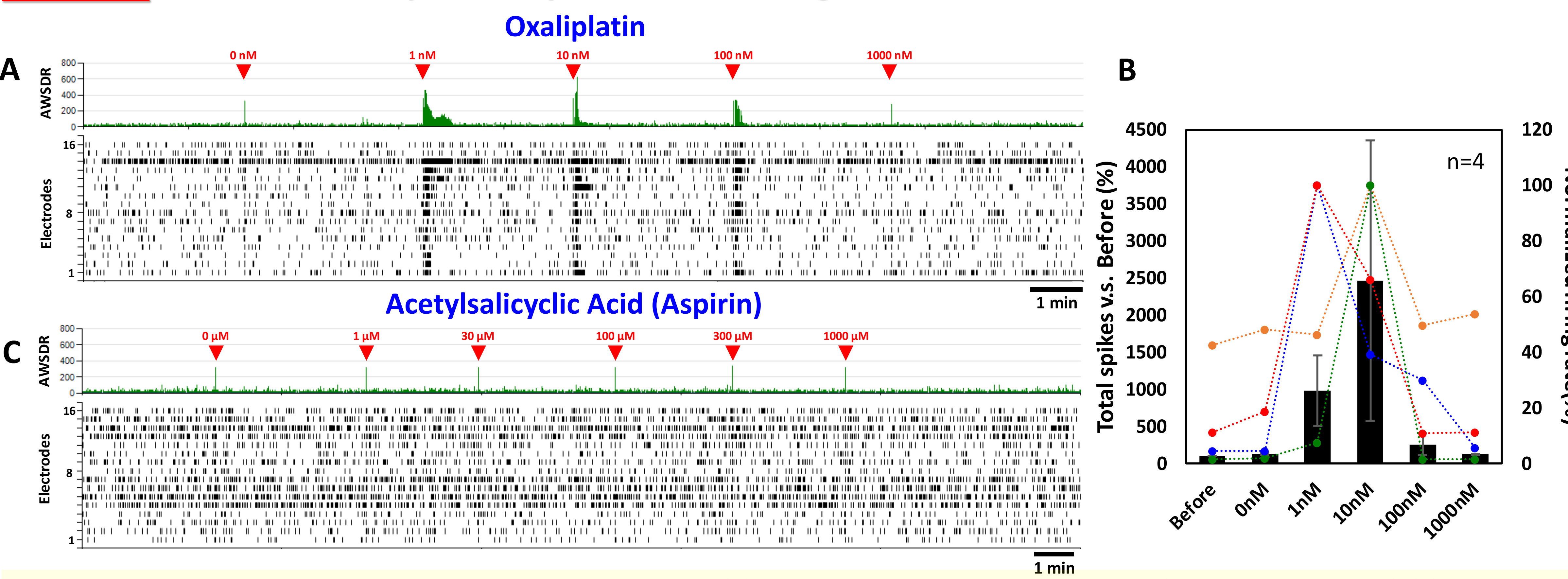
## Result 2 Electrophysiological responses by temperature change



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

> The firing peak was 43 °C. It was consistent with the activation temperature of TRPV1 channels.

## Result 3 Pain responses by anti-cancer drug administration



**Fig.4 Responses by anti-cancer drugs oxaliplatin and pain relief aspirin.** (A) Raster plots in oxaliplatin administration (0, 1, 10, 100, 1000 nM). (B) Firing change in oxaliplatin administration. N = 4 wells. (C) Raster plots in acetylsalicylic acid administration (0, 1, 10, 100, 1000  $\mu$ M).

> We detected the responses to anti-cancer drugs oxaliplatin in cultured hiPSC-derived sensory neurons.  
 > We also confirmed the no responses to pain relief aspirin in cultured hiPSC-derived sensory neurons.