In vitro pain responses of dorsal root ganglion neurons using multi-electrode arrays

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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 pain research and drug screening is important issue. Here, we used the multi-electrode array (MEA) system to detect the electrophysiological responses by chemical and thermal stimuli in cultured DRG neurons. After 2 days of culture on the MEA, we observed spontaneous activities and chemical responses. Addition of the capsaicin, menthol and wasabi induced significant changes of the firing rate and concentration-dependent responses. Furthermore, temperature elevation increased the number of firings and it showed the largest increase at 43 degrees. We also detected the responses to temperature and capsaicin in hiPSC derived sensory neurons at 14 DIV. We confirmed that the typical response of DRG neurons can be easily obtained using MEA system. These results suggested that electrophysiological measurements in DRG neurons using a MEA system may be beneficial for clarifying the functions of DRG neurons and human iPSC derived sensory neurons in pain research and for drug screening applications.

Methods

Culture of Rat DRG neurons

DRG neuros were obtained from wister rat the age over 10 weeks. Rat DRG neurons were cultured at 1.0 × 10^4 cells/cm^2 on 64-channel MEA chips (MED-P515A; Alpha Med Scientific) coated with Laminin 511 at 37° C in a 5% CO_2/95% air

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 and evoked 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.).

Result 1

Pain responses in cultured rat DRG neurons

Rat DRG neurons

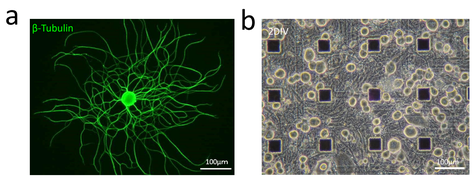

Figure.1 Cultured adult DRG neurons. (a) a single neuron at 2 *days in vitro* (DIV). (b) DRG neurons at 2 DIV on the MEA chip.

Temperature

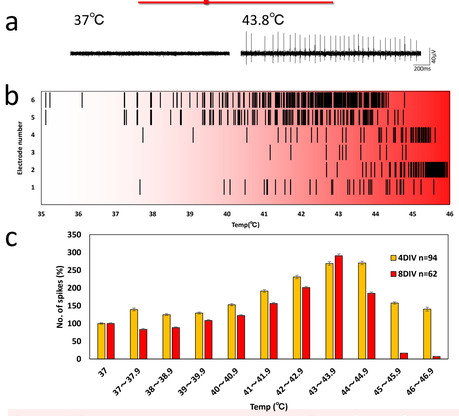

Figure.2 Responses to temperature. (a) Typical waveform at 37 and 43.8 ℃. (b) Raster plots to temperature. (c) Change of spike rate to temperature at 4 and 8 DIV.

Capsaicin

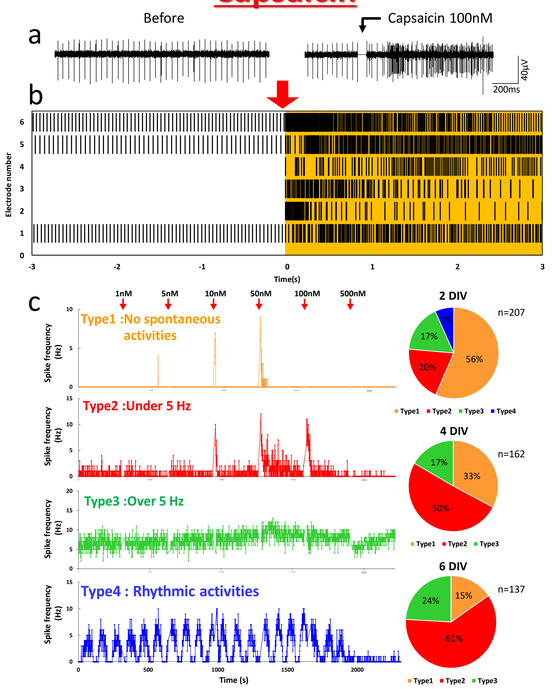

Figure.3 Responses to capsaicin. (a) Typical waveform before and 100 nM capsaicin. (b) Raster plots to before and after capsaicin administration. (c) Response patterns before administration and rate with culture day.

Menthol

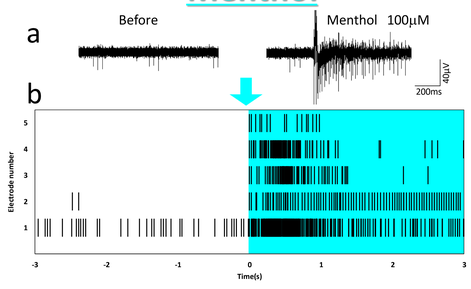

Figure.4 Responses to menthol. (a) Typical waveform before and 100 μM menthol. (b) Raster plots to before and after menthol administration.

Wasabi (Allyl isothiocyanate)

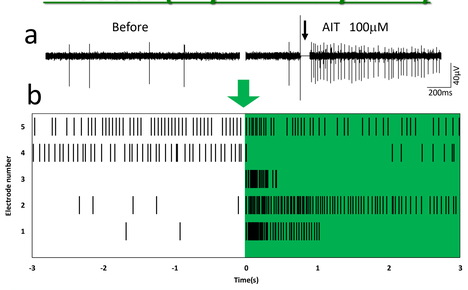

Figure.5 Responses to wasabi. (a) Typical waveform before and 100 μM wasabi. (b) Raster plots to before and after wasabi administration.

Percentage of rat DRG neurons that responded to three compounds

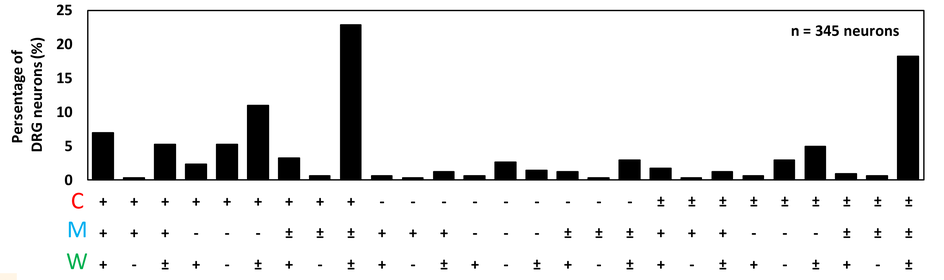

Figure 6. Percentage of rat DRG neurons that responded to three compounds (C: Capsacin 50 nM, M: Menthol 100 μM, W: Wasabi 100 μM). n =345 neurons.

Result 2

Pain responses in cultured human iPSC derived sensory neurons

Human iPSC derived sensory neurons

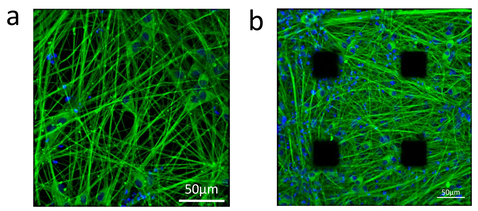

Figure.7 Cultured hiPSC derived sensory neurons. (a) 14 *days in vitro* (DIV). (b) Sensory neurons at 14 DIV on the MEA chip.

Temperature

Figure.8 Responses to temperature. (a) Typical waveform at 37 and 42.6 ℃. (b) Raster plots to temperature. (c) Change of spike rate to temperature at 14 and 21 DIV.

Capsaicin

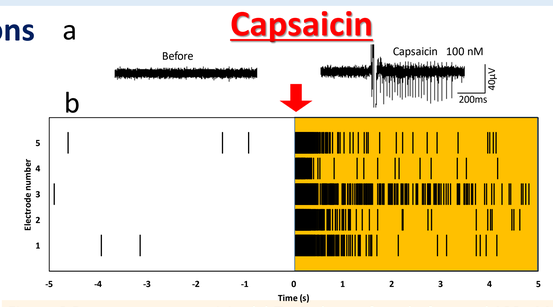

Figure.9 Responses to capsaicin. (a) Typical waveform before and 100 nM capsaicin. (b) Raster plots to before and after capsaicin administration.

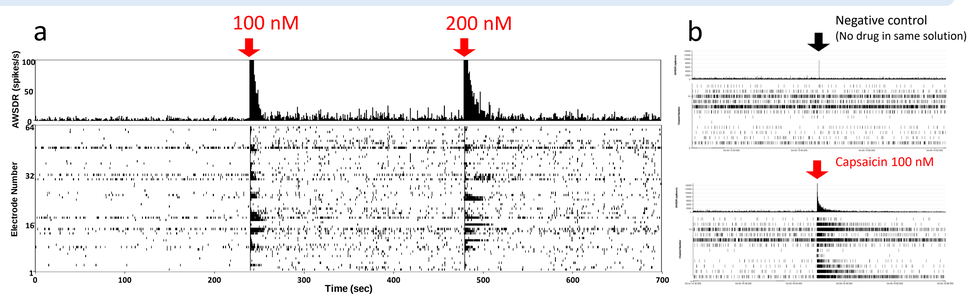

Figure.10 Raster plots of 64 electrodes and array wide spike detection rate (AWSDR) at 100 and 200 nM capsaicin administration. 14 DIV. (b) Raster plots and AWSDR at negative control and capsaicin 100 nM.

Conclusion

- Spontaneous firing and drug responses in cultured rat DRG neurons were detected at 2 DIV.
- We detected the responses to temperature change, capsaicin, menthol, and wasabi by change of spike rate.
- Responses can be separated to four patterns based on spontaneous firing patterns before administration.
- We classed functional neuronal type based on electrical responses to three compounds.
- MEA technology are suitable to functional profiling by characterizing subtypes in DRG neurons.
- We also detected the responses to temperature and capsaicin in hiPSC derived sensory neurons at 14 DIV.
- 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.

Reference (1) Odawara A, et.al., *Scientific Reports*, 6, 26181, 2016

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