Electrophysiological Nociceptive and Itch Assessment of Human iPSC-Derived Sensory Neurons Using Multi-Electrode Array (MEA) And Their Potential as an Alternative Model for Dorsal Root Ganglia

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Introduction

Sensory neurons transmit pain signals generated by trauma, temperature, or chemicals to the central nervous system. Primary afferent nerves, evoked by non-nociceptive and nociceptive stimuli, reside in the dorsal root ganglion (DRG) of the spinal cord. Primary DRG cells have been used as an *in vitro* model of nociceptive responses for decades. However, they are low-yielding, challenging to culture, and difficult to access. To negate accessibility issues and improve ease of culture, REPROCELL has developed an alternative sensory neuron model using human-derived induced pluripotent stem cells (iPSC). This model was analyzed to confirm its phenotypical relevance and functionality. The expression of sensory markers were detected by immunocytochemistry. Multi-Electrode Array (MEA) analysis was used to confirm whether these induced hiPSC-derived sensory nerves display the same functionality as primary DRG cells.

Stemgent® StemRNA™ Sensory Neuron

- Frozen as a suspension of individual cells
- Pack size: > 1.0 × 10^6 cells/vial
- Quality Control: Expression of Peripherin and Brn3a
- Standard Medium: Sensory Neuron Culture Medium

Electrophysiological analysis by MEA

Fig.2. Electrophysiology experimental design

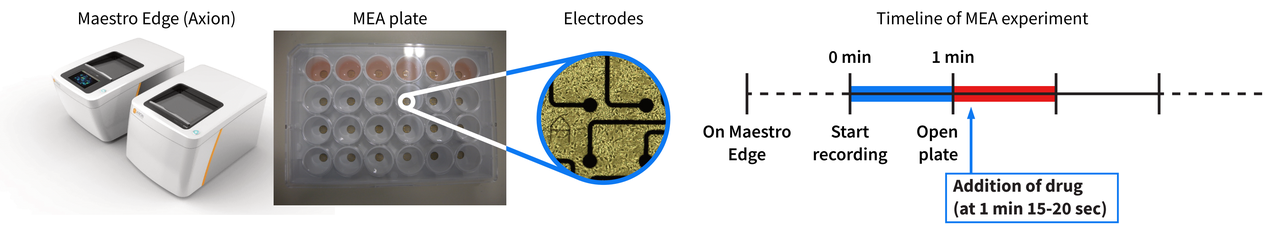

Fig.3. Electrophysiological response of sensory neurons to capsaicin

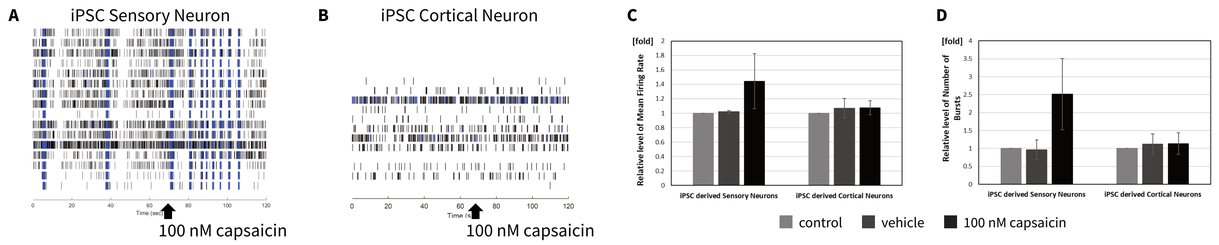

Fig.4. Electrophysiological response of sensory neurons to menthol

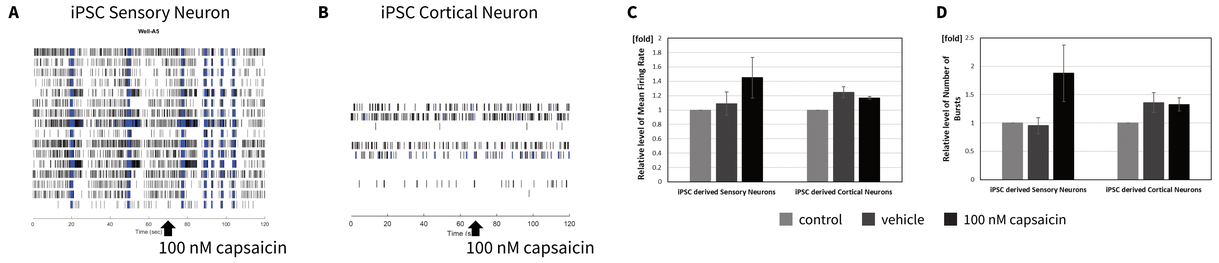

Fig.5. Electrophysiological response of sensory neurons to temperature

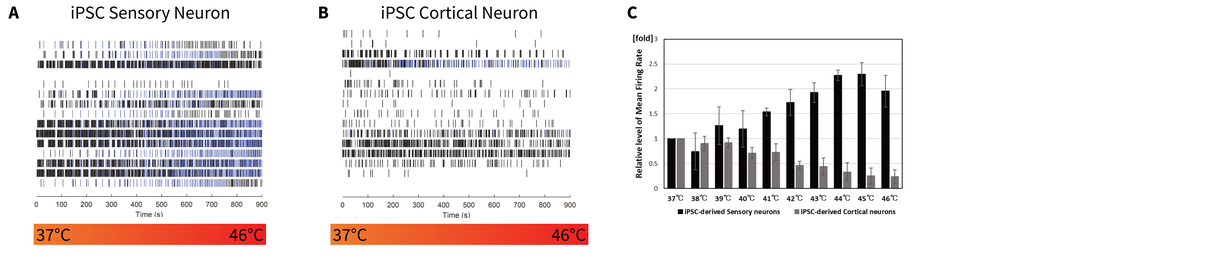

Fig.3-5. Electrophysiological analysis by MEA. Electrophysiological response of iPSC derived sensory neurons (n = 3), and iPSC derived Cortical neurons (ReproNeuro, RCDN001N) (n = 3) to (**Fig. 3**) 100 nM capsaicin 27 days after seeding, (**Fig. 4**) 100 nM menthol 32 days after seeding, (**Fig. 5**) temperature 28 days after seeding. (**A, B**) Raster Plot, (**C**) Mean Firing Rate (Hz), (**D**) Number of Bursts. For **Fig. 5**, temperature was increased from 37°C to 46°C. Cells seeded in CytoView MEA 24-White at 1.5 × 10^5 cells/well/1.1 mm^2. Maestro Edge (Axion) was used for MEA analysis.

Immunocytochemistry

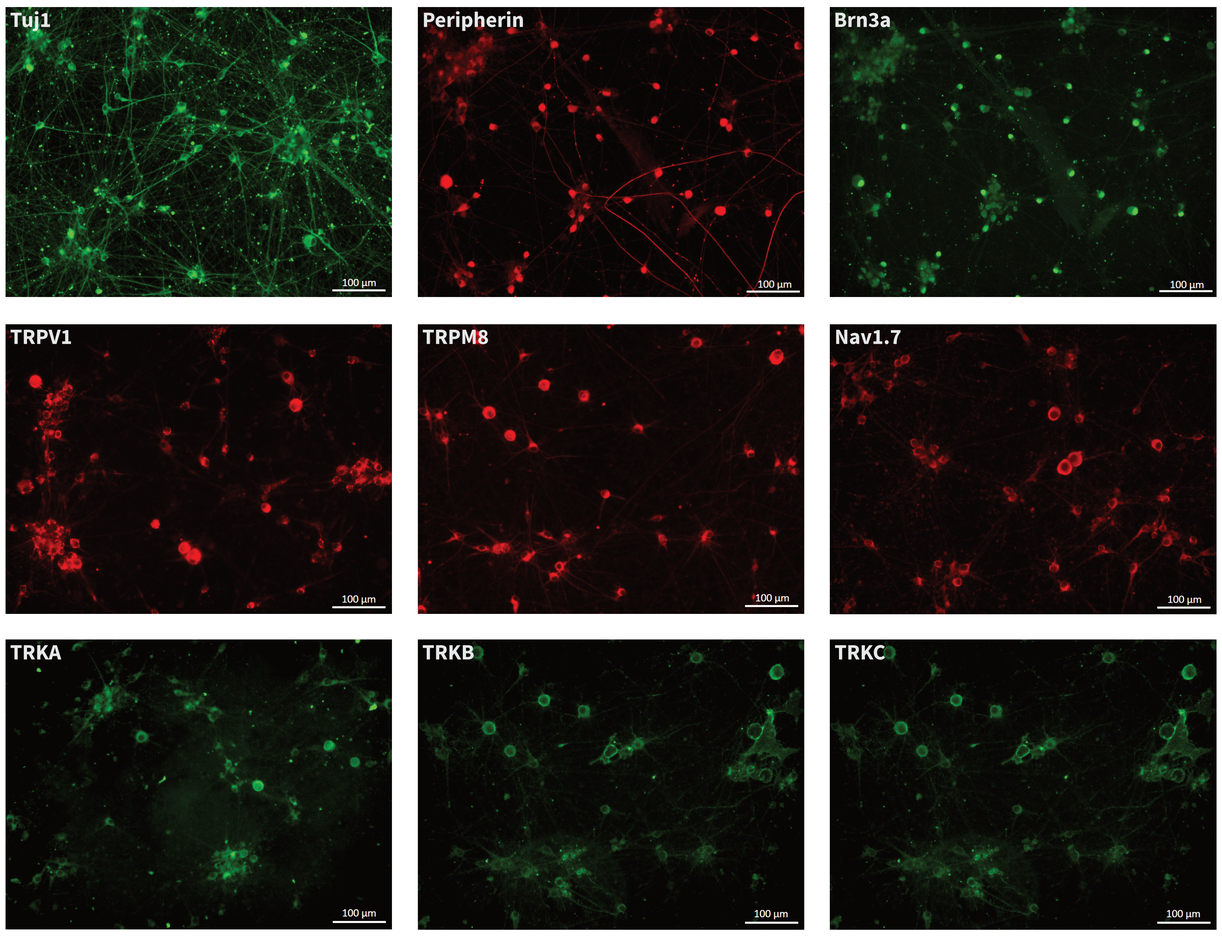

Fig 1 (right). Immunofluorescence staining day 28 after thawing and seeding. Neurons were seeded at 1.5 × 10^5 cells/well (24 well plates) in Sensory Neuron Culture Medium. They were stained for neural markers, peripheral nerve markers, and various sensory nerve-related proteins including: Tuj1 (mature nerve marker), Peripherin (peripheral nerve marker), Brn3a (sensory nerve marker), TRPV1 (capsaicin receptor), TRPM8 (menthol receptor), Nav 1.7 (Na channel), TRKA (NGF receptor), TRKB (BDNF receptor) , and TRKC (neurorophin-3 receptor).

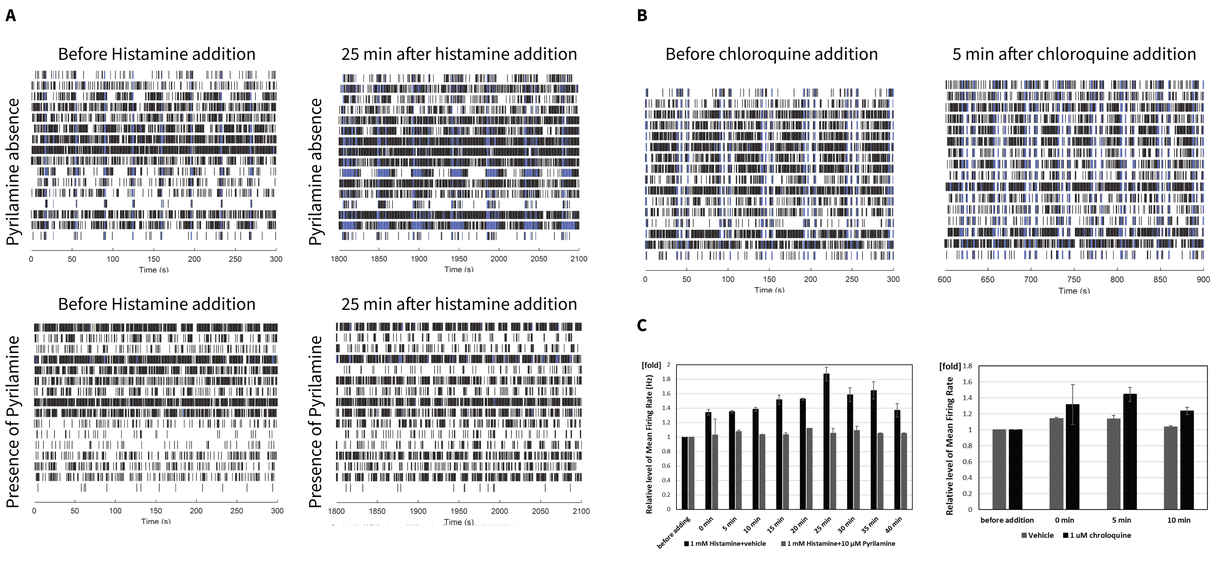

Fig. 6. Electrophysiological analysis by MEA. Electrophysiological response of iPSC derived sensory neurons to (6) 1 mM Histamine (n = 2), 10 µM Pyrilamine (n = 2) 61 days after seeding, 1 µM Chloroquine (n = 3) 45 days after seeding. (**A-C**) Raster Plot, (**D, E**) Mean Firing Rate (Hz).

Conclusion

The electrophysiological activity of Stemgent StemRNA Sensory Neurons was characterized in this study. These neurons express neuron-related proteins and respond to capsaicin, menthol, and temperature. Histamine activated the neurons and pyrilamine inhibited the activation. Chloroquine, a histamine-independent itch inducer, increased the mean firing rate of the sensory neurons. We conclude that these iPSC-derived sensory neurons could be a good tool for assessing drug responses, as a alternative to DRGs.