Using MEA Technology to Measure Functional Responses from Human iPSC-Derived Sensory Neurons

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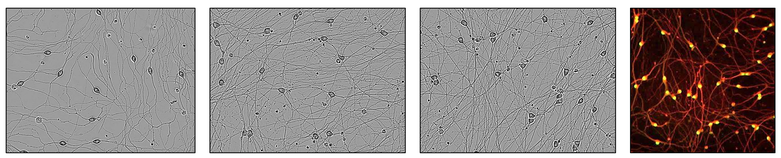
OVERVIEW

The purpose of this work was to develop reliable and diverse methods for measuring the electrical activity of human iPSC-derived sensory neurons using microelectrode array (MEA) technology.

INTRODUCTION

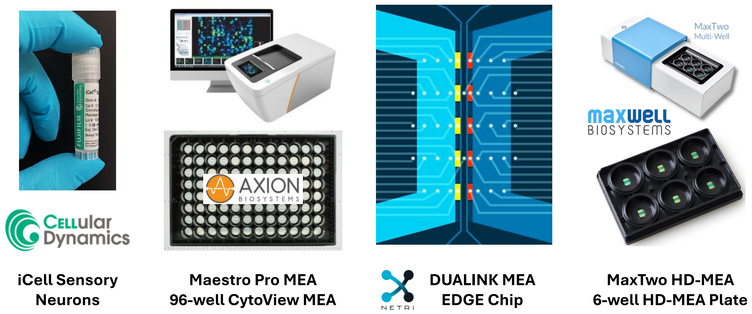
The availability of cryopreserved human iPSC-derived sensory neurons presents a valuable source of cells for applications in pain research and drug discovery. Microelectrode array (MEA) technology is a common method for measuring neuronal function, however, sensory neurons are different from cultures of excitatory neurons in that this cell type does not form synaptic connections with other sensory neurons and should not fire high numbers of action potential spontaneously if they are pure nociceptive cells.



Day 7 | Day 14 | Day 21 | BRN3A / Peripherin

METHODS

iCell® Sensory Neurons with media and supplements were provided by FUJIFILM Cellular Dynamics. MEA plates were coated with PDL solution from GIBCO and Recombinant Laminin-511 iMatrix from amsbio. The Maestro Pro™ MEA system and 48- or 96-well Cytoview MEA plates from Axion BioSystems were used to test the response of iCell Sensory Neurons to specific sensorineural agonists (e.g., capsaicin, menthol, ATP, etc.) and alternative culture conditions, including the "inflammatory cocktail" composed of materials (e.g., IL-6, IL-6/SR, IL-1β, NGF, Oncostatin M, Prostaglandin E2, and TNF-α) intended to stimulate baseline spike activity above quiescence. NeuroFluidics™ devices (e.g., DUALINK MEA EDGE) from NETRI were used to merge electrophysiology with microfluidics for next-level testing. Electrical stimulation to evoke action potentials from sensory neurons was performed on the MaxTwo multi-well high-density MEA (HD-MEA) platform with 6-well HD-MEA plates from MaxWell Biosystems.



iCell Sensory Neurons | Maestro Pro MEA 96-well CytoView MEA | DUALINK MEA EDGE Chip | MaxTwo HD-MEA 6-well HD-MEA Plate

RESULTS

iCell Sensory Neurons responded to depolarization with potassium chloride (KCl) at every time point tested (Days 7-35), but the most consistent and robust responses to agonists like capsaicin usually required ≥4 weeks in culture on standard CytoView MEA plates. Similar times in culture were required for the inflammatory cocktail to be effective. On the contrary, MEA recordings from cells in the microfluidic devices showed strong spike activity under homeostatic conditions as early as 2 weeks in culture. The response to agonists is still being tested in the lab. Electrical stimulation with multiple pulses and increasing amplitudes generated repeatable spike activity in every HD-MEA well tested in experiments on Day 55.

See each of the 3 panels to the right on this poster for data and more details.

CONCLUSIONS

We have developed application protocols on these various platforms for long-term culturing iCell Sensory Neurons on the MEA plates and for recording functional electrical responses. These data show that iCell Sensory Neurons have low spontaneous spike activity (as expected) that can be modulated in biologically relevant ways. Quiet but active!

Response to Sensory Neuron Stimulation can be Captured using MEA Technology

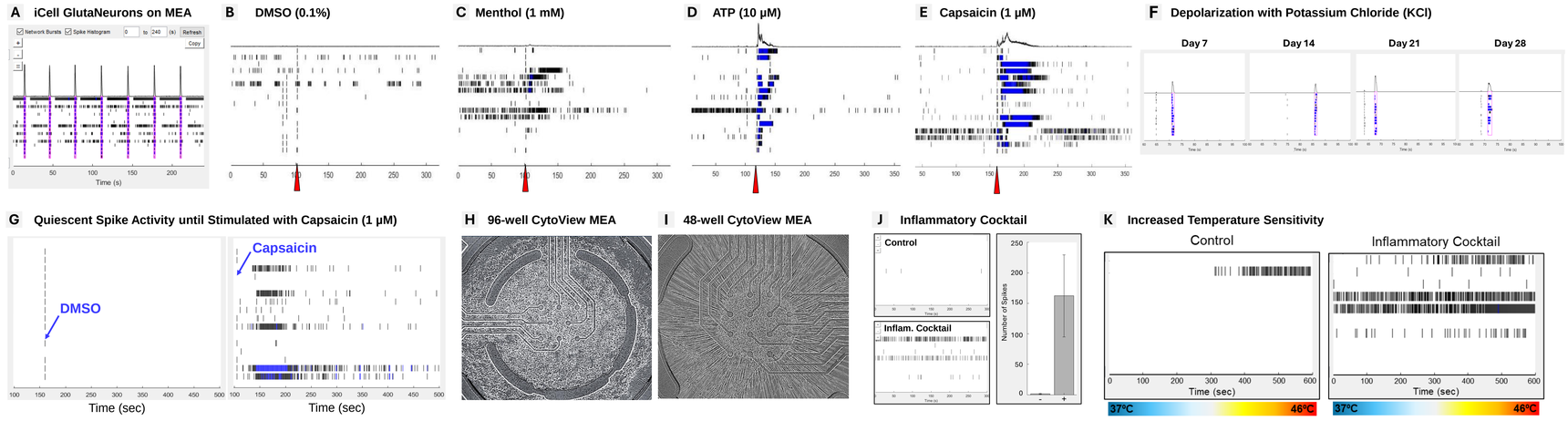


Figure 1. MEA is a sensitive assay to evaluate sensory neuron function and model hyperexcitability. (A) Human iPSC-derived neurons typically contain some excitatory cells that drive spontaneous firing of action potentials that synchronize over time to form network bursts. Protocol development at FUJIFILM CDI for the differentiation of iPSC into sensory neurons focused on the functional response to sensorineural agonists. Compared to the vehicle control (B) 0.1% DMSO, cells were shown to respond to (C) menthol, (D) ATP, and (E) capsaicin. Protocol refinements to improve sensory neuron purity resulted in quiescent baseline MEA recordings. (F) Treatment with potassium chloride at weekly intervals was performed to depolarize the cells and demonstrate an electrophysiological response. Raster plots show no detectable spike activity until cells are stimulated. (G) The recommended timing to test for sensory stimulation is ≥4 weeks in culture on the MEA. (H) and (I) Maintaining the cultures of sensory neurons to stay over the MEA electrodes can be challenging. We have observed better success in 96w format, but any clustering in 48w plates that might occur does not begin until after 3-4 weeks in culture and typically does not negatively impact MEA signal. Additional approaches to increase baseline spike activity of sensory neurons for assay include (J) exposing the cells to an "inflammatory cocktail" (IL-1β, IL-6, IL-6/SR, NGF, Oncostatin M, Prostaglandin E2, and TNFα) for several days, or (K) raising the temperature of the MEA system from 37ºC → 42ºC.

Sensory Neuron Electrophysiology is Enhanced on Organ-on-a-Chip (OoC) Microfluidic Devices

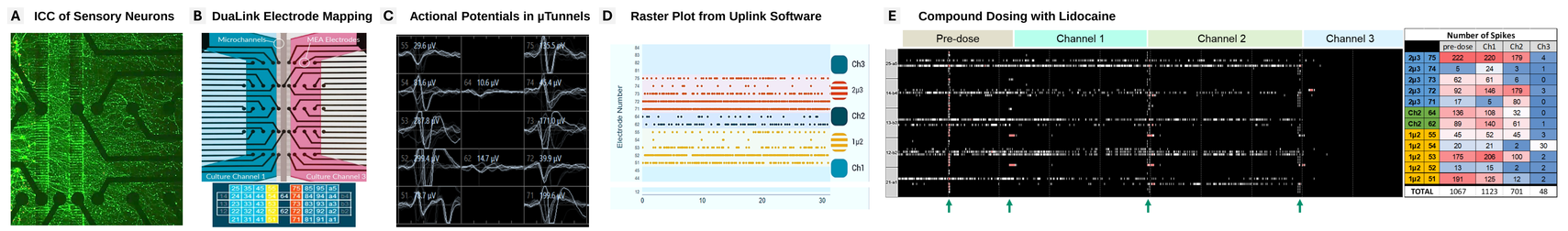


Figure 2. OoC accelerates maturation of sensory neuron function. (A) iCell Sensory Neurons were plated on a DuaLink MEA microfluidic device (NETRI) where cells readily project through the microchannels (µTunnels) over time. Pictured here are cells with a live cell Calcein AM stain on DIV 29 (image courtesy of NETRI). (B) Each DuaLink MEA chip has 48 electrodes embedded within it. Electrodes that are located within the microchannels are colored in yellow and red and numbered as 51-55 and 71-75. (C) While sensory neurons are presented across all surfaces, spontaneous spike activity is detected primarily by the MEA electrodes in the microchannels. Strong action potentials with spike amplitudes averaging ~50-100 µV were recorded as early as DIV 15 in culture. Robust recordings have been acquired out past DIV 50 in this system. (D) The Uplink software integrates with Axion system software for data analysis and can generate unique raster plots. (E) Importantly, compound dosing is possible with the DuaLink MEA chip. iCell Sensory Neurons were dosed with Lidocaine (1 mM) in each individual channel (Ch1 or 1µ2, Ch2, and Ch3 or 2µ3) at ~90 sec intervals. The number of spikes captured is totaled in the table. All spike activity was silenced when the drug was added to Ch3, which is where cells were seeded initially. Artifacts from touching the plate are indicated by the arrows.

Evoked Responses from Electrical Stimulation of iCell Sensory Neurons using HD-MEA Technology

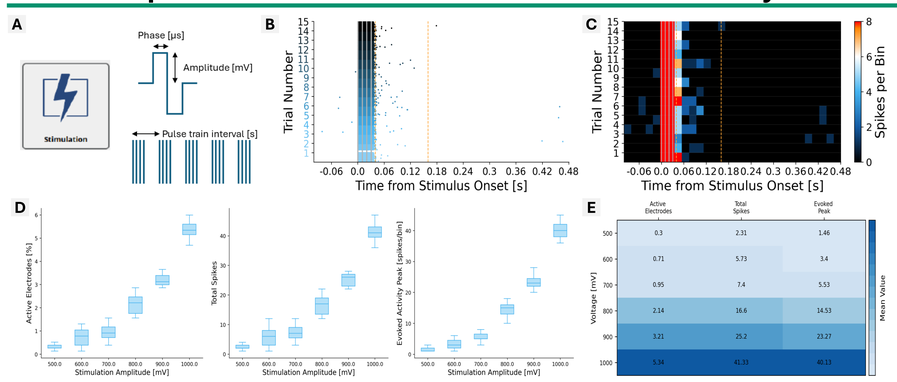


Figure 3. Electrical stimulation. (A) A sequence composed of 4 pulses per burst (inter-pulse interval of 10 ms) and 15 bursts per pulse train (inter-burst interval of 0.5 s) was used. Each individual pulse had a phase of 600 µs and amplitudes that ranged from 500-1000 mV during the pulse train. Spike activity recorded for each trial (every repetition of a stimulus) was then analyzed and stimulation-evoked responses are seen in the (B) Raster Plot and (C) Evoked Network Response Plot with spikes binned every 20 ms. (D) Box plots depicting Active Area, Total Spikes, and Evoked Activity Peak for each stimulation amplitude are presented. (E) The means summary table is the average of all individual trials for each metric across the range of amplitudes tested. The Stimulation Assay in MaxLab Live software enables the MaxTwo HD-MEA system to explore an additional approach for recording neuronal activity from iCell Sensory Neurons.

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