Capturing Functional Responses of iCell® Sensory Neurons on MEA

iCell Lab Note

Introduction.

Micro-electrode array (MEA) is a key technology to assess neuronal activity. Testing the function of human iPSC-derived sensory neurons (SNC) on MEA is challenging because the spontaneous activity for this cell type should be much lower compared to cultures of cortical excitatory neurons. In contrast recent publications showing high levels of activity for iPSC-derived SNCs on MEA, here we demonstrate that iCell® Sensory Neurons recapitulate the quiescent nature of human dorsal root ganglion neurons with an essentially silent activity profile under homeostatic conditions and only become excitable when treated with relevant sensory molecules or exposure to an inflammatory cocktail. This lab note provides technical guidance for culturing iCell Sensory Neurons on a CytoView MEA plate with representative data captured on the Axion BioSystems Maestro Pro™ MEA platform.

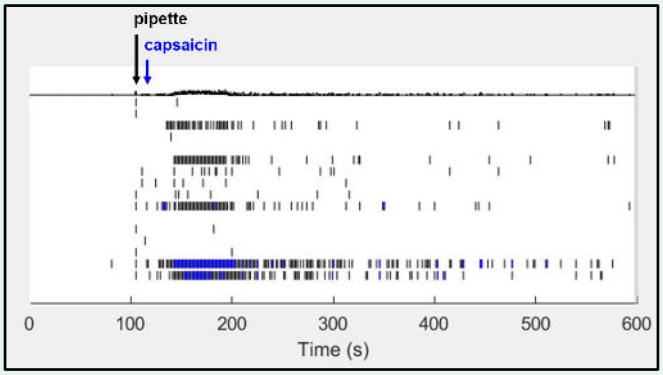

Figure 1. Capsaicin Response Captured on MEA. iCell Sensory Neurons were cultured on a 48-well MEA plate until Day 35. Typical baseline recordings have very little spike activity (see first 100 sec of raster plot). The MEA system initially responds when the pipette touches the well (**black →**) prior to dispensing the solution of TRPV1 agonist Capsaicin (1 µM; **blue →**), which then results in an immediate and extended MEA response from the cells. Control wells received 0.1% DMSO and no spikes were observed (data not shown).

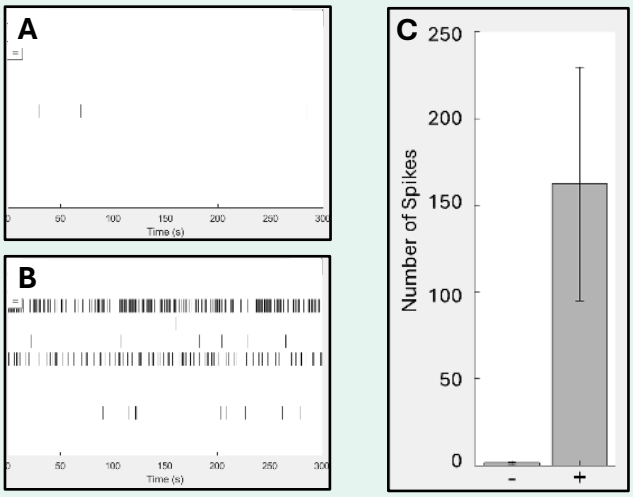

Figure 2. Inflammatory Cocktail Increases Baseline Activity. (A) Few spikes are recorded in the raster plot from iCell Sensory Neurons under normal conditions on a 96-well MEA plate. (B) Exposing the cells to an "inflammatory cocktail" (IL-1β, IL-6, IL-6/SR, NGF, Oncostatin M, Prostaglandin E2, and TNF-α), however, increases electrical activity of the neurons at baseline. (C) The increased number of action potentials / spikes detected is significant.

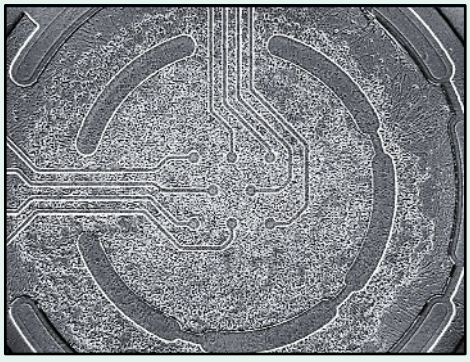

Figure 3. Representative Morphology. Whole well Incucyte® image of iCell Sensory Neurons from Day 28 that were dotted on a 96-well MEA plate treated with PDL and Recombinant Laminin-511. Cells typically remain in a monolayer across the surface area of the well. Slight retraction of neurons from the edges and clustering in the middle of the well can be managed with proper culture handling, seeding technique, feeding, and storage of materials.

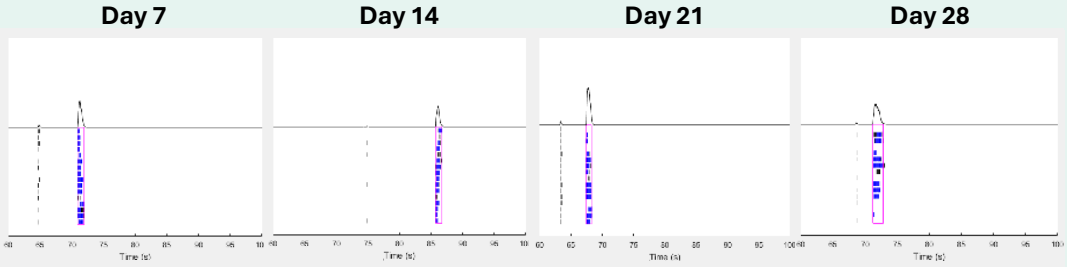

Figure 4. Quiescent Nature. iCell Sensory Neurons, 21527 were treated with KCl over time to check for spike activity in response to depolarization. The raster plots show that no spike activity is detected until the cells are stimulated.

Methods.

Prepare Complete Medium and thaw iCell Sensory Neurons according to the User's Guide.

- Day 0: Prepare MEA plate by coating with 80 µl of PDL (dilute to 50 µg/ml with water and incubate for 1-3 h at 37ºC), wash 3X with sterile water, and dry in a biological safety cabinet for 1 h.
- Prepare Dotting Medium by diluting Laminin-511 to 20 µg/ml in Complete Medium.
- Centrifuge cells and resuspend to a density of 6.25 x 10^6 cells /ml in Dotting Medium. Use lot-specific CoA info to determine viable number of neurons.
- Dot 8 µl of cell suspension onto MEA plate to yield 50,000 cells/well.
- Allow cells to adhere in incubator for 45-60 min and then gently fill wells with 200 µl of Complete Medium.
- Culture the cells in a 37ºC / 5% CO_2 incubator.
- Day 1: Perform a complete 100% media change.
- Days 2-beyond: Change 50% media 2X per week.
- The recommended timing to test for sensory stimulation or to add inflammatory cocktail is ≥4 weeks in culture.

Table 1. Materials Needed

Product	Vendor	Cat. #
iCell Sensory Neurons Kit, 01279 [1]	FCDI	R1250
▪ iCell Sensory Neurons Base Medium	incl. in kit	M1052
▪ iCell Sensory Neurons Supplement (100X)	incl. in kit	M1053
Poly-D-Lysine (PDL) Solution [2]	GIBCO	A3890401
Recombinant Laminin iMatrix-511 Silk	AMSBIO	AMS.892021
CytoView MEA 96-well plate [3]	Axion	M768-tMEA-96
Capsaicin [4]	Sigma	PHR1450
Inflammatory Cocktail [5]	Various	N/A

[1] iCell Sensory Neurons from Donor 21527 (Cat # R1252) are also available.
[2] Other polymer solutions, including PEI and PLO, have been tested but do not result in improved baseline activity.
[3] Different size CytoView MEA plates, such as 24- or 48-well, may be used.
[4] Cells have been shown to respond to increased temperature or other sensory agonists (i.e., menthol, ATP, or PIEZO compounds) in calcium signaling assays or on MEA (data not shown).
[5] Please inquire for more detailed information.

Summary.

This lab note provides a brief overview and optimized instructions for culturing iCell Sensory Neurons on a CytoView MEA plate and recording neuronal activity with the Axion Maestro Pro MEA system. Using this assay, we have demonstrated that these iPSC-derived sensory neurons have low baseline activity and can be stimulated with specific agonists (e.g., Capsaicin) to activate an acute response, or cultured in the presence of an inflammatory cocktail of reagents that results in increased spike activity (hyperexcitability) above baseline quiescence.

Highlights.

iCell Sensory Neurons have low spontaneous spike activity (as expected) that can be modulated in biologically relevant ways.

An optimized protocol enables long-term culture of sensory neurons resulting in robust functional responses on MEA.

iPSC-based model demonstrates efficacy for evaluating novel analgesics and modelling sensory neuron hyperexcitability.

Scan here to download the iCell Sensory Neurons User's Guide.

Contact **Technical Support** for more protocol details and supportive data.
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