BioResearch

In vitro Electrophysiological Screening with Dorsal Root Ganglion Neurons

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Introduction

The sensation of pain is transmitted from sensory nerve endings to the central nervous system by axons of peripheral neurons whose cell bodies reside in the dorsal root ganglion (DRG).[1] To date, pain research has been predominantly based on animal models, in part due to a lack of predictive *in vitro* screening methods.[2] A high-throughput *in vitro* assay for pain will significantly contribute to the discovery of therapies for pain, while also reducing the need for research animals. Here, we describe an *in vitro* model of pain designed for high-throughput experimentation using the Axion BioSystems Maestro™ platform and Lonza rat embryonic DRG neurons.

The Maestro microelectrode array (MEA) system provides a platform for label-free multi-well screening of electrically active cells, *in vitro*.[3] Cryopreserved dissociated DRG neurons from embryonic rats display excellent post-thaw viability and healthy neuronal morphology with extensive axonal outgrowth. DRG neurons can be cultured in multi-well MEA plates, enabling simultaneous recording of electrical activity from numerous electrodes in each culture well. These DRG cultures exhibit chemical sensitivities representative of those observed *in vivo*.

Materials and Methods

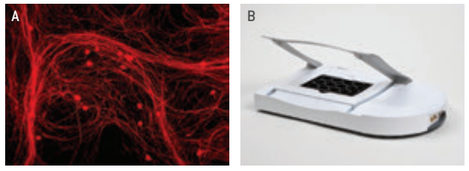


Figure 1: A) Cryopreserved Clonetics™ Rat Dorsal Root Ganglion Neurons are suspensions of high quality sensory neurons prepared by standardized methods, and are ready for immediate culture. **B) The Axion Biosystems Maestro MEA system** with 768 stimulating and recording electrodes for 12, 48 and 96 wells represents the next generation in multi-electrode array technology, providing unprecedented access to native cellular electrical signaling and connectivity.

Process

- **Cell Source:** Rat Embryonic Dorsal Root Ganglion Neurons (Lonza, R-eDRG-515)
- **MEA:** 48-well MEA (Axion BioSystems, if not indicated otherwise)
- **Cell Density:** 1×10^4 to 5×10^4 cells per well
- **MEA Surface Coating:** Polyethyleneimine (Sigma–Aldrich, Laminin (Sigma–Aldrich)
- **Cell Culture Medium:** PNGM™ BulletKit™ (Lonza , CC-4461) supplemented with 100 ng/mL NGF-β, 7.5 µg/mL 5'-fluoro-2'-deoxyuridine and 17.5 µg/mL uridine (all Sigma–Aldrich)

Acquisition

- **Inclusion Criteria after Stimulation:** Active Channel (> 5 spikes/min)[3]; Active Well (> 1 Active Channel)
- **Settings:** Signals acquired from 200–3000Hz, Spike detected at 6 × Std. Dev. of noise
- **Analysis:** MatLAB, Plexon Offline Sorter

Application

- **Compound Sensitivity:** Capsaicin (100nM, 1µM), DHEA (10 µM)
- **Electrical Stimulation:** ±750mV or ±50mV biphasic stimulus, 250µs duration, 5 × repeat

Figure 2: A) Timeline depicting cell seeding and maintenance for MEA experiments. Spontaneous action potentials in plated DRG neurons were clearly detectable as early as 3 days post-plating persisted though day 10. **B) Dosing workflow for TRPV1 agonist Capsaicin and additional compounds (optional).**

Results

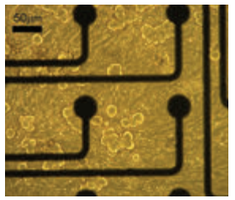
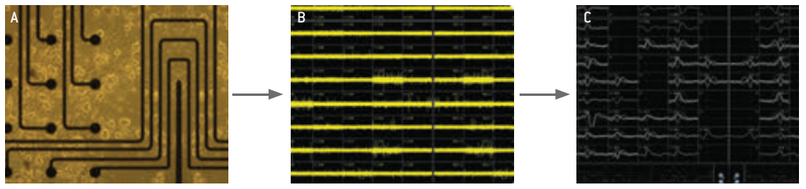


Figure 3 (Left): DRG neurons displayed excellent adhesion to 12-well MEA Plates. Clear neurite outgrowth was evident, and healthy cultures were maintained and recorded for at least 10 days through the use of antimitotic agents (7.5 µg/mL 5'-fluoro-2'-deoxyuridine and 17.5 µg/mL uridine; both Sigma–Aldrich) to prevent Schwann cell overgrowth. DRG neurons were seeded with 1×10^4 cells per well and cultured for 6 days. Excellent attachment and neurite growth can be seen for small, medium, and large diameter DRG neurons

Figure 4 (Below): Characterization of DRG neurons in multi-well MEA Plates. A) Healthy cultures were maintained for at least 10 days. **B)** Spontaneous action potentials in plated DRG neurons were clearly detectable as early as 3 days post-plating. **C)** Spike and raster plot data taken from AxIS software displays the number of active channels in one well following treatment with capsaicin.



Sensitivity of DRG neurons to noxious stimuli is largely caused by the TRP (Transient Receptor Potential) family of ionotropic receptors and cation channels, which display differing sensitivity profiles for stimuli including chemical agents[4–11]. Of particular relevance is the TRPV1 channel, also known as the capsaicin receptor and the vanilloid receptor 1.

Capsaicin, an active compound in chili peppers, is a high-affinity TRPV1 agonist. Capsaicin binding leads to a conformational change in the channel that lowers its activation energy, and sensitizes the channel to activation by heat and acidification. Capsaicin, therefore, provides a tool for induction of an *in vitro* correlate of pain under experimentally-tractable (37°C, neutral pH) conditions, providing a tool for screening compounds that inhibit the excitability of DRG neurons.

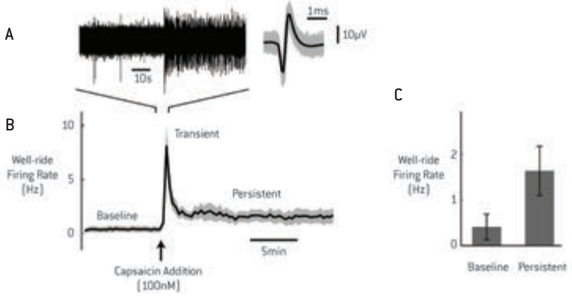


Figure 5: Capsaicin induces transient and persistent increases in firing rate of DRG neurons. A) Raw voltage trace from a single electrode in response to capsaicin addition, including the accompanying spike waveforms (mean – black, individual spikes – gray). **B)** Averaged capsaicin evoked activity across wells (N = 6, mean – black, gray – standard error of the mean), illustrating a transient increase in firing rate, followed by a persistent elevation in firing rate above the pre-dose baseline. **C)** The persistent, elevated firing rate was significantly different from baseline (average over a 3 minute period, n = 6, p = 0.0313, Wilcoxon Signed Rank Test, error bars represent standard error of the mean).

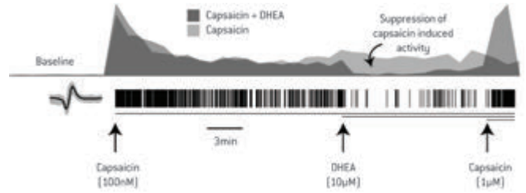


Figure 6: TRPV1 inhibitors modulate the effect of capsaicin on DRG neuron activity. Robust *in vitro* activation of DRG neurons by capsaicin sets the stage for screening assays in which this response is blocked by potential pain therapeutics. DRG neurons at 1×10^4 cells per well were exposed to 100 nM capsaicin, followed by the addition of the TRPV1 competitive inhibitor dehydroandrosterone (DHEA, 10 µM, dark gray), which reduced well-wide firing compared to a control well exposed to capsaicin only (light gray). The histograms represent the well-wide firing rate normalized by the capsaicin induced activity (bin size of 60 secs). Addition with a higher dose of capsaicin (1 µM) rescued the firing activity suppressed by DHEA (dark gray). Throughout the course of the recording, the persistent activity observed following capsaicin treatment stayed relatively stable in the control well.

Discussion and Conclusion

In this application note, we demonstrated the viability and functional activity of Lonza DRG neurons on the Axion BioSystems Maestro™ MEA platform. Shortly after plating, low baseline levels of spontaneous spiking activity were recorded by the non-invasive MEA electrodes, and marked changes in spike rate were observed with chemical perturbations.

Specifically, we characterized the response of the DRG neurons to the TRPV1 agonist capsaicin and the TRPV1 antagonist DHEA. DRG neurons exhibited a capsaicin-evoked response on multiple timescales: 1) a rapid transient increase in firing rate, and 2) a persistent elevation in spontaneous firing above pre-dose baseline. Furthermore, the firing rate during this sustained phase could be suppressed by TRPV1 antagonists, demonstrating the utility of the preparation for screening candidate pain inhibitors.

In summary, commercially-available DRG neurons exhibit electrophysiological responses on the Axion BioSystems Maestro™ MEA that are consistent with *in vivo* function, providing a high-throughput *in vitro* assay for addressing pain-related neurobiology, and ultimately for identifying compounds of therapeutic value.

References

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