



## Application Note

### Modeling Pain with Rat Dorsal Root Ganglion Neurons on MEAs



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## Origin

Axion BioSystems Microelectrode Arrays are manufactured in the United States of America.

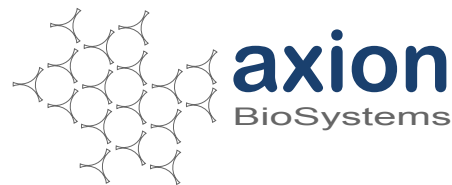
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# Modeling Pain with Dorsal Root Ganglion Neurons on MEAs



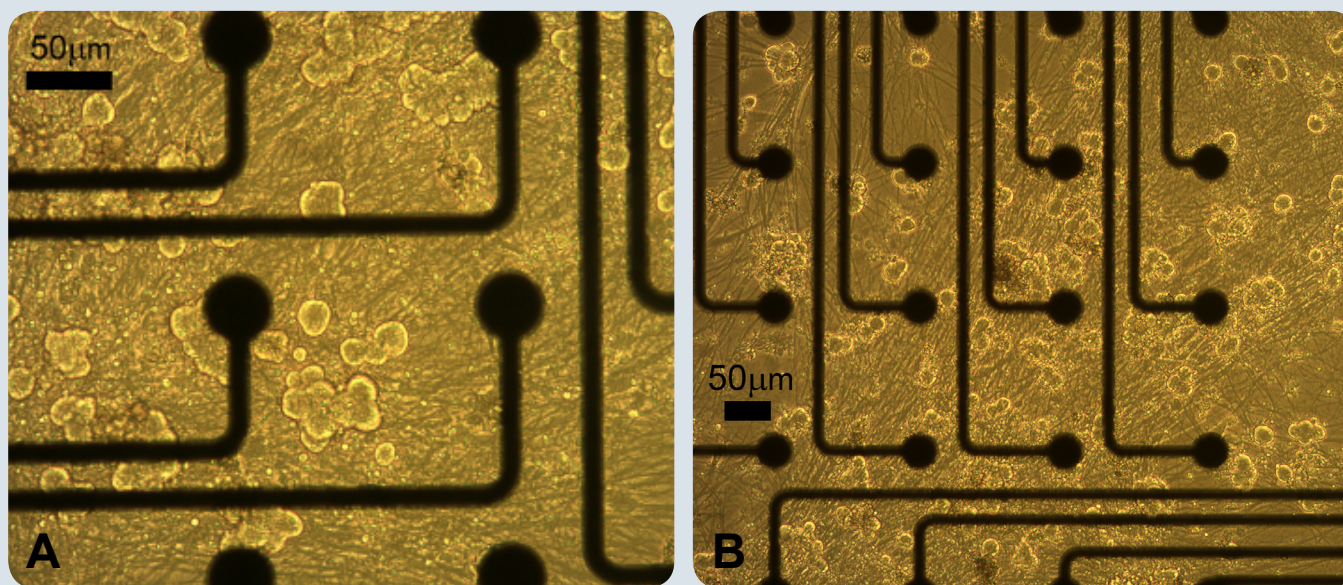
## Introduction

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).<sup>1</sup> Damage to these primary afferents, or defects in the proteins underlying their electrical or sensory function, can cause neuropathic pain, a persistent sensation of pain associated with increased spontaneous firing of DRG neurons.

To date, pain research has been predominantly based on animal models, in part due to a lack of predictive *in vitro* screening methods.<sup>2</sup> A high-throughput *in vitro* assay for pain will significantly contribute to the discovery of therapies for chronic pain, while also reducing the need for research animals. In this application note, we describe an *in vitro* model of pain designed for high-throughput experimentation using the Axion BioSystems Maestro™ platform and commercially-available rat DRG neurons from QBM Cell Science.

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

In combination, DRG neurons and the Maestro MEA system constitute a high-throughput *in vitro* platform for chronic neuropathic pain research.



**Figure 1: Dorsal root ganglion neurons imaged on 12-well MEAs.** A) DRG neurons are shown on DIV6 at  $1 \times 10^4$  cells per well. At 20x magnification, excellent attachment and neurite growth can be seen for small, medium, and large diameter DRG neurons B) 10x magnification illustrates overall cell density.

## Materials and Methods

### Cell Culture

12- and 48-well MEA plates were prepared for cell plating by “dotting” the electrode array in each well with polyethylenimine (PEI), followed by laminin. Cryopreserved DRG neurons were thawed at 37°C for 2 minutes, washed with medium, centrifuged at 1000 rpm, counted, and plated on the electrode array in 3  $\mu$ L drops containing  $1 \times 10^4$  to  $5 \times 10^4$  cells. 1 hour later, when the cells had settled onto the MEA surface, complete medium containing neural growth factor (NGF), was added to the wells. After 4 hours, the antimetabolic agents 5’fluoro- 2’deoxyuridine and uridine were added to stop the proliferation of Schwann cells. Half of the medium in each well was refreshed with new medium every 3 days. This workflow is depicted in Figure 2, and the complete protocol can be found in the [QBM Dorsal Root Ganglion Culture Protocol](#).

### MEA Recording and Analysis

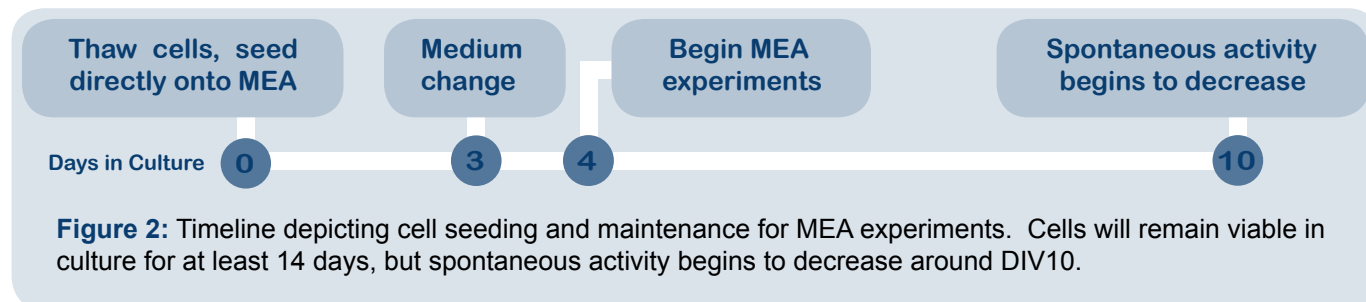
Recording of neural spikes from DRG neurons in multiwell MEA plates was performed using the AxIS software suite for the Maestro system. Voltage data was acquired at 12.5 kHz in neural spikes mode (1200 X Gain with 200-5000 Hz bandpass filtering), and additional high-frequency noise was removed using a software-based 200-3000 Hz bandpass filter. For each electrode, the threshold for spike detection was set at 6x the standard deviation of the voltage. Post hoc analysis was performed using several software packages including NeuroExplorer (NEX Technologies), Offline Sorter (Plexon Technologies) and MATLAB (The Math-Works, Inc). These were used to generate raster plots, sort waveforms, and analyze various metrics, respectively. Only wells containing more than one active electrode (defined as having greater than

5 spikes/min) were included for this work.<sup>3</sup> Due to variability in the phenotypic expression of DRG neurons and generally low spontaneous activity, we applied the active electrode criterion to the induced response (i.e. capsaicin, temperature).

### Chemical and Thermal Stimuli

Due to the transient nature of changes in spike activity in DRG neurons, compounds such as capsaicin were added during recording. The exact time of compound addition was located by the recording artifact caused by the pipette tip. False positive spike events related to these artifacts were excluded from rate calculations. For experiments where sterility was important, the plate lid was replaced with a pierceable microplate sealing film (VWR, cat. 60941-116) with pre-formed openings for drug delivery via pipette. Alternately, sterility has been maintained by operating the Maestro inside a sterile cell culture hood. During extended recordings (longer than 15 minutes) pH and humidity were maintained by placing the Maestro in an enclosure filled with pre-mixed CO<sub>2</sub> gas.

Temperature stimuli were applied using the Maestro MEA system’s built-in heater. A gradual ramp from low to high temperature was achieved by cooling the MEA to 24°C in a freezer (2 minute incubation). The cooled plate was then placed in the Maestro, the recording was started and the temperature was set to 37°C. After holding at 37°C for 2 minutes, the temperature was set to 46°C to reach noxious levels of heat.



**Figure 2:** Timeline depicting cell seeding and maintenance for MEA experiments. Cells will remain viable in culture for at least 14 days, but spontaneous activity begins to decrease around DIV10.

## Results

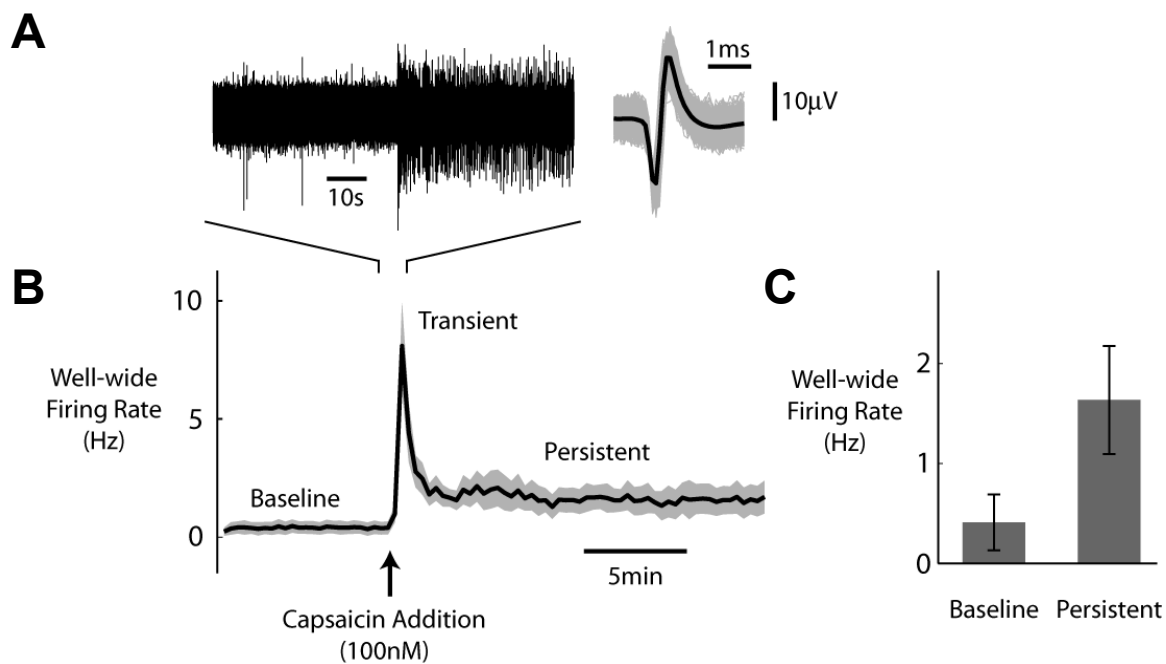
Sensitivity of DRG neurons to noxious stimuli is largely caused by the TRP family of ionotropic receptors, which display differing sensitivity profiles for stimuli including chemical agents<sup>4-11</sup>, noxious heat and cold<sup>12-15</sup>, and changes in pH due to inflammation.<sup>5,7,16</sup> Of particular relevance is the TRPV1 channel, a primary receptor for noxious heat and pH implicated in hyperthermia and inflammatory pain.<sup>11</sup> DRGs linked to sites of spinal cord injury (SCI) show increased TRPV1 expression, leading to hyperexcitability. 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) condi-

tions, providing a tool for screening compounds that inhibit the excitability of DRG neurons.

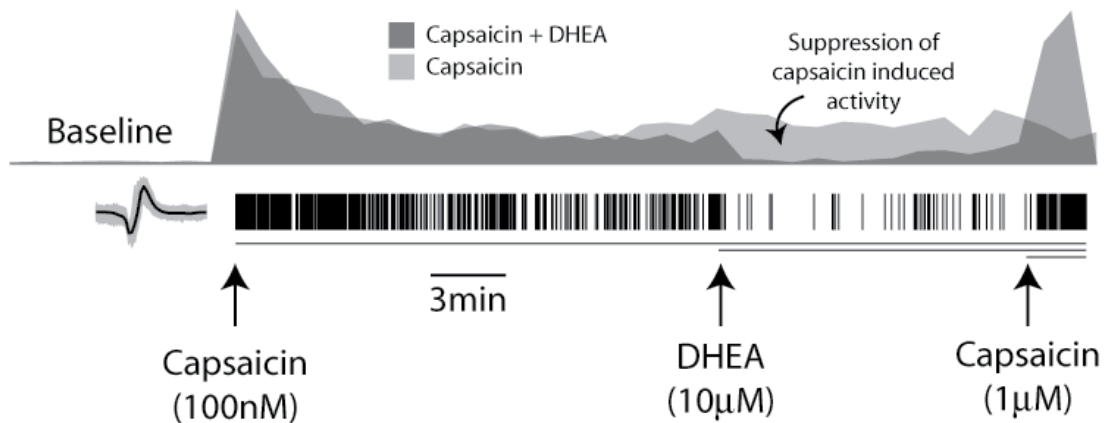
### Culture and Electrical Characterization

As shown in Figure 1, DRG neurons displayed excellent adhesion to multiwell MEA Plates. Clear neurite outgrowth was evident, and healthy cultures were maintained and recorded for at least 10 days through the use of antimetabolic agents (5'-fluoro-2'-deoxyuridine and uridine) to prevent Schwann cell overgrowth.

Spontaneous action potentials in plated DRG neurons were clearly detectable as early as 3 days post-plating using the AxIS software, and persisted through day 10. As expected, higher numbers of plated cells yielded higher spontaneous firing rates, but steady baseline rates were recorded using as few as  $1 \times 10^4$  cells per well.



**Figure 3: 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 (N=6,  $p = 0.0313$ , Wilcoxon Signed Rank Test, error bars represent standard error of the mean).



**Figure 4: TRPV1 inhibitors modulate the effect of capsaicin on DRG neuron activity.** DRG neurons at  $1 \times 10^4$  cells per well were exposed to 100 nM capsaicin, followed by the addition of  $10 \mu\text{M}$  DHEA (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 ( $1 \mu\text{M}$ ) 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.

### Capsaicin Sensitivity

Activation of DRG neurons was conducted using capsaicin, an agonist of the TRPV1 temperature and pH receptors. The effect of 100 nM capsaicin on mean firing rate (MFR) of DRG neurons ( $1 \times 10^4$  cells per well) was measured on DIV 7, and is shown in Figure 3. The raw voltage trace shows the increased firing resulting from capsaicin addition, with the waveform of each detected spike plotted to the right (gray), along with the mean spike waveform (black). Three separate stages of the capsaicin response are apparent in the plot of well-wide firing rate (N=6 wells, mean – black, +/- standard error of the mean – gray): baseline spontaneous firing, transient capsaicin-induced firing, and a persistent elevated firing that lasts for tens of minutes following the capsaicin addition. The bar chart represents an average over a 3 minute period for both the baseline and the persistent phases of the capsaicin response, where the firing rate was higher in the persistent phase (N=6,  $p = 0.0313$ , Wilcoxon Signed Rank Test, error bars represent standard error of the mean).

### Sensitivity to Capsaicin-Inhibitors

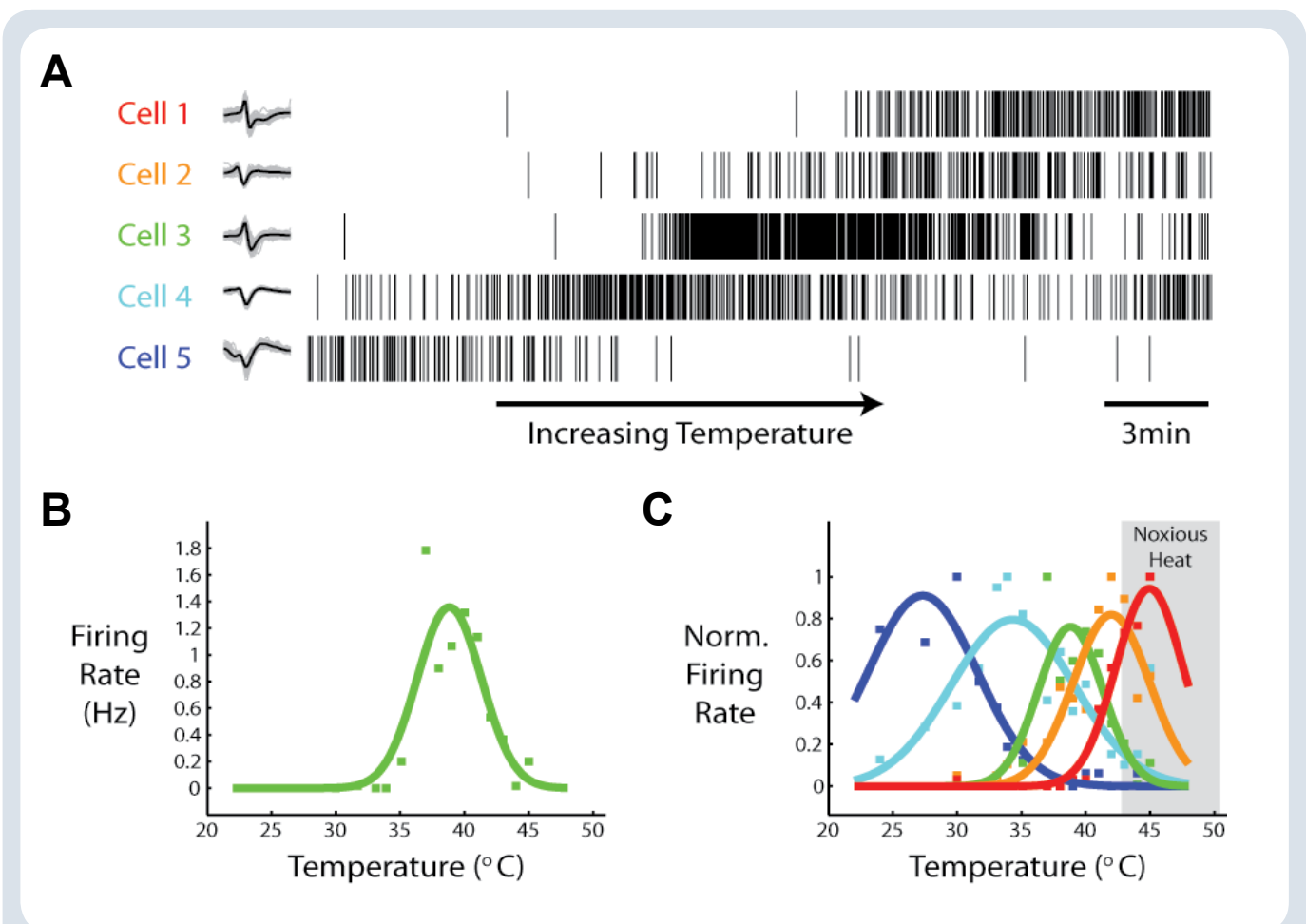
Robust *in vitro* activation of DRG neurons by capsaicin sets the stage for screening assays in which this response is blocked by potential pain therapeutics. To explore this concept, we employed the TRPV1 competitive inhibitor dehydroandrosterone (DHEA).<sup>4,6,15</sup> Figure 4 shows two wells of DRG neurons ( $1 \times 10^4$  cells per well) treated with 100 nM capsaicin at 7 minutes in a live recording, causing an increase in firing rate. After 20 minutes, one of the wells was treated with  $10 \mu\text{M}$  DHEA. The DHEA treated well exhibited a significant reduction in mean firing rate compared to the control well, consistent with previous whole cell patch clamp studies on DRG neurons.<sup>6</sup> After 10 minutes of reduced firing due to DHEA exposure, the effect was markedly reversed by addition of  $1 \mu\text{M}$  capsaicin, demonstrating continued sensitivity of the TRPV1 receptors.

## Results

### Thermal Sensitivity

Separate populations of DRG neurons are sensitive to cold, ambient temperature, and heat, based on different gene expression profiles established in part by the position of the cell body in the ganglion during development.<sup>17,18</sup> In addition, noxious heat is a modulator of TRPV1 channel conformation. Temperatures in excess of 43°C lower its activation threshold, causing increased excitability of the neuron.<sup>14,15</sup> The thermal response of DRG neurons can be evaluated on the Maestro using the integrated temperature control, which directly heats the MEA plates. To demonstrate this process, Figure 5 illustrates the response of 5 individual

DRG neurons to increasing temperature. This was achieved by spike sorting using the Plexon Offline Sorter. The raster plots of the spike trains versus temperature are shown in Figure 5A alongside the recorded waveform. Figure 5B shows the mean firing rate of Cell 3 plotted versus temperature. This data was fit with a Gaussian curve with a bin size of 60 seconds. This particular neuron was spontaneously active at 37°C, but showed little spiking at room temperature or when exposed to noxious heat (>43°C). Figure 5C shows Gaussian fits for all 5 neurons from Figure 5A, illustrating that each cell responds to a different temperature range.



**Figure 5: Thermal sensitivity of DRG neurons.** A) Spike raster plots for 5 different DRG neurons recorded over time as temperature is ramped from 24°C to 45°C. These DRG neurons were responsive to specific temperature ranges. B) MFR for Cell 3 at different temperatures with a Gaussian curve fit (bin size = 60 secs). C) Normalized (Norm.) firing rate for the 5 cells in A, color-coded with Gaussian curve-fits to show temperature sensitivity ranges.

## Discussion and Conclusions

In this application note, we demonstrated the viability and functional activity of QBM Cell Science 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 and thermal perturbations. Specifically we characterized the response of the DRG neurons to the TRPV1 agonist capsaicin, the TRPV1 antagonist DHEA, and noxious heat. 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. Finally, the integrated temperature controls of the Maestro MEA system were used to characterize the thermal sensitivity of DRG neurons.

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.

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