In vitro electrophysiological screening with dorsal root ganglion neurons for pain related assays

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I. Introduction

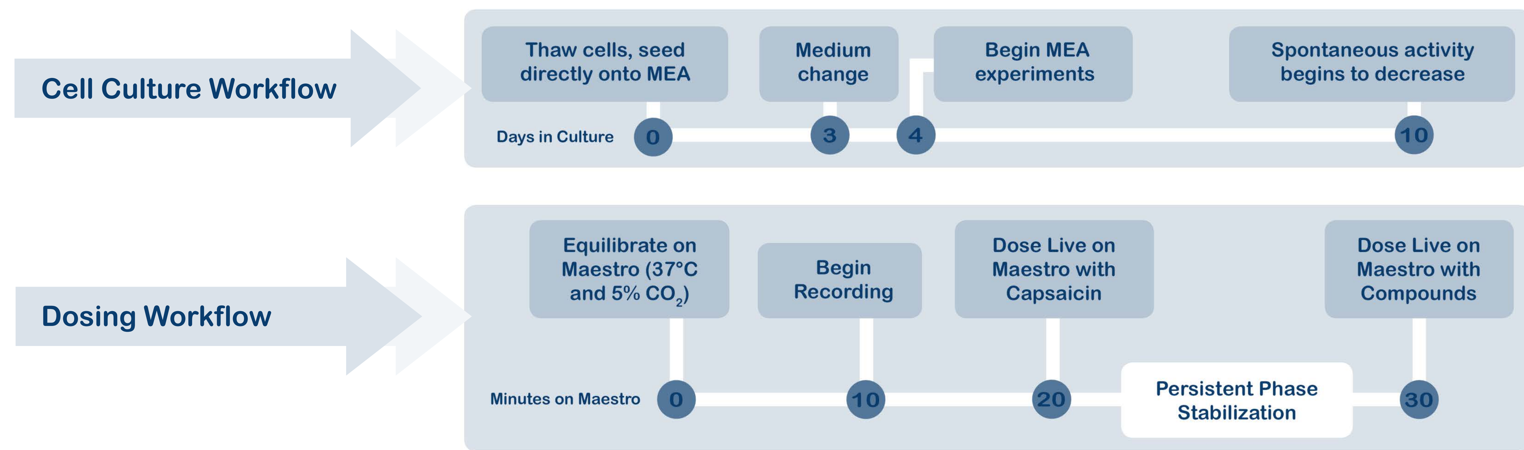
The sensation of pain is transmitted from nociceptive nerve endings to the central nervous system along axons of neurons in the dorsal root ganglion (DRG). Damage to these primary afferents, or inherited defects in the proteins underlying their electrical or sensory function, can cause neuropathic pain, a persistent sensation of pain caused by increased spontaneous activity of DRG neurons. Pain research has thus far been predominantly based on animal models, in part due to a lack of predictive in vitro screening methods. The development of a high throughput *in vitro* assay for pain thus stands to significantly impact the discovery of therapies for chronic pain. Here, we present a non-invasive technique for directly monitoring electrical responses in commercially-available rat primary DRG neurons cultured in 12-, 48- and 96-well microelectrode array (MEA) plates.

II. Methods

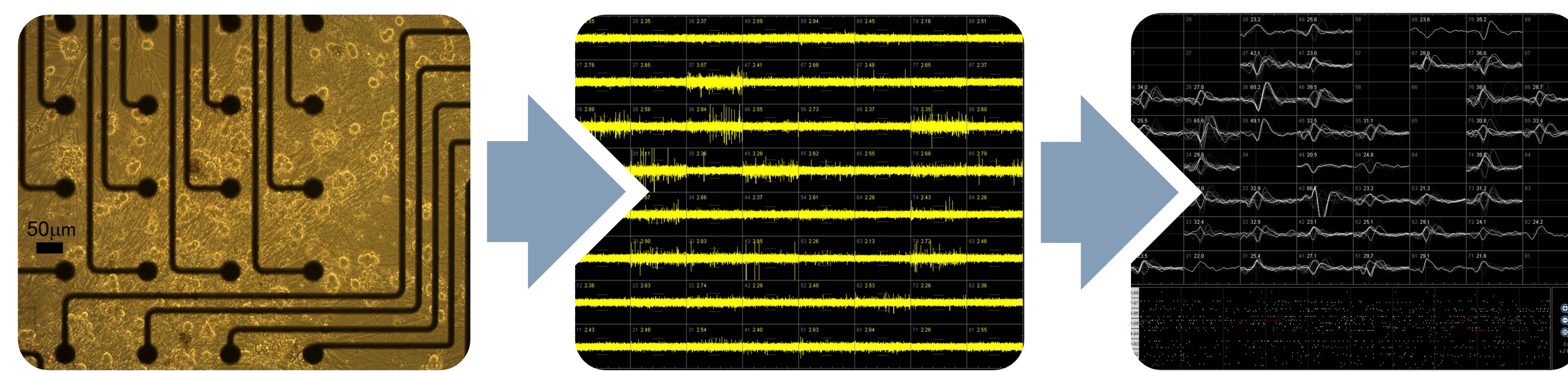
Process
- **Cell Source –** Rat DRG Neurons (QBM Cell Science)
- **Cell Density –** 1x10^4 to 5x10^4 cells per well
- **Surface Coating –** Polyethylenimine (Sigma-Aldrich), Laminin (Sigma-Aldrich)
- **MEAs –** 12-, 48- and 96-well (Axion BioSystems)

Acquisition
- **Inclusion Criteria –** Active Channel (>5 spikes/min), Active Well (>1 Active Channel)
- **Settings –** Signals acquired from 200-3000Hz, Spike detected at 6 x Std. Dev. Of noise
- **Analysis –** MatLAB, Plexon Offline Sorter

Application
- **Compound Sensitivity –** Capsaicin (100nM, 1μM), DHEA (10μM)
- **Thermal Sensitivity –** exposure to temperature ranging from 24-45°C
- **Electrical Stimulation –** ±750mV or ±50mV biphasic stimulus, 250μs duration, 5x repeat

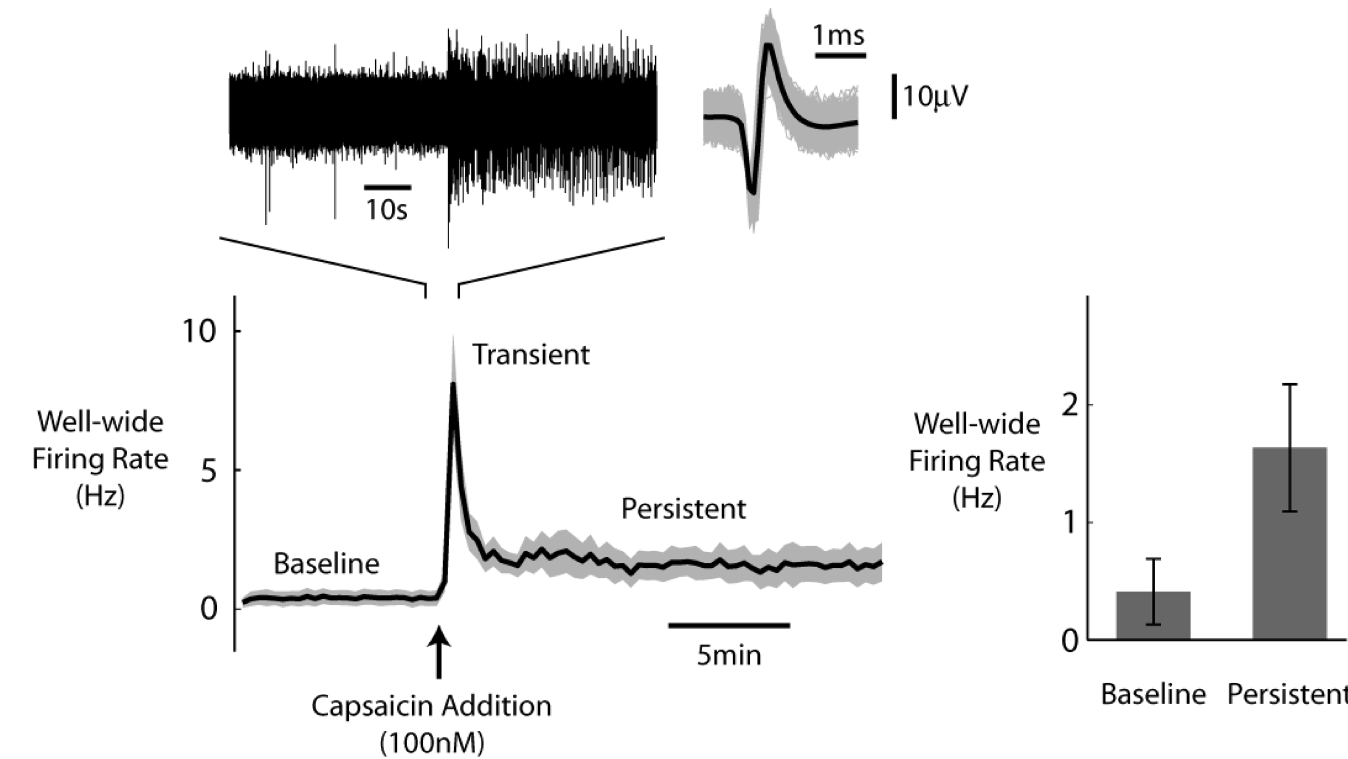


III. Characterization



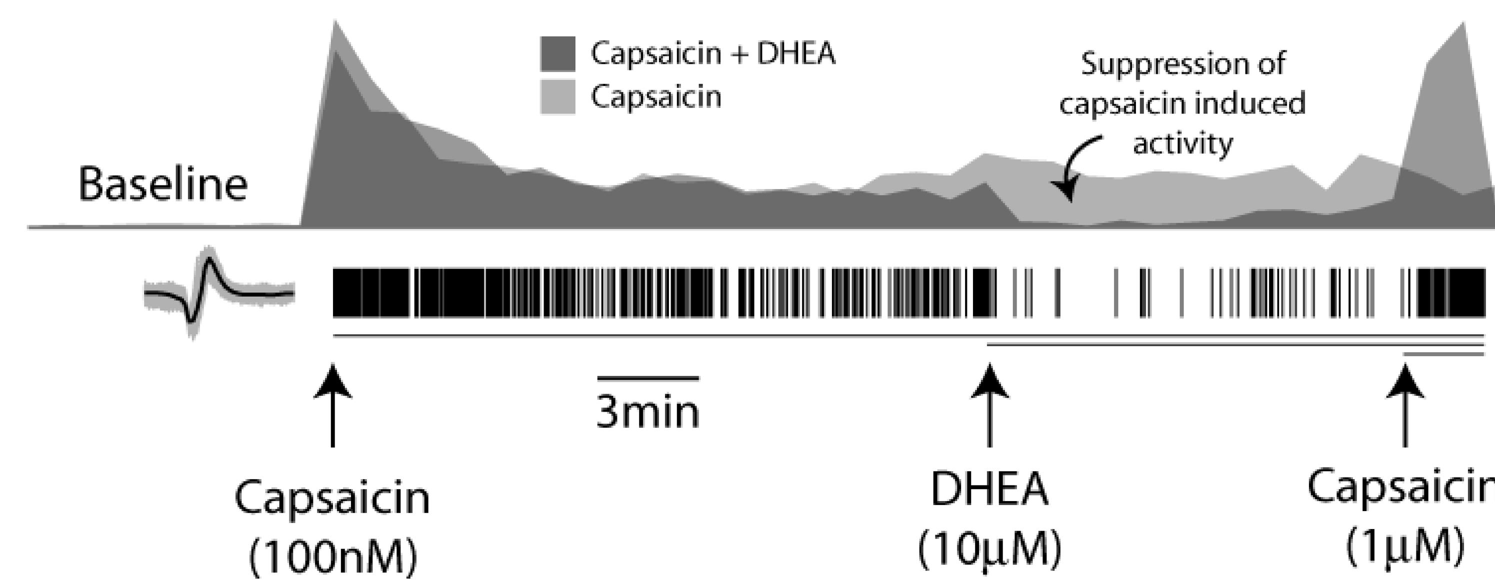
DRG neurons displayed excellent adhesion to multiwell MEA Plates. (Left) Clear neurite outgrowth was evident and healthy cultures were maintained for at least 10 days. **(Middle)** Spontaneous action potentials in plated DRG neurons were clearly detectable as early as 3 days post-plating using the AxIS software and persisted though day 10. As expected, higher numbers of plated cells yielded higher spontaneous firing rates, but steady baseline rates were recorded using as few as 1x10^4 cells per well. **(Right)** Spike and raster plot data taken from AxIS displays the number of active channels in one well following treatment with capsaicin.

IV. Capsaicin Sensitivity



Capsaicin induces transient and persistent increases in the firing rate of DRG neurons. Raw voltage trace from a single electrode in response to capsaicin addition, including the accompanying spike waveforms (mean – black, individual spikes – gray). 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. 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).

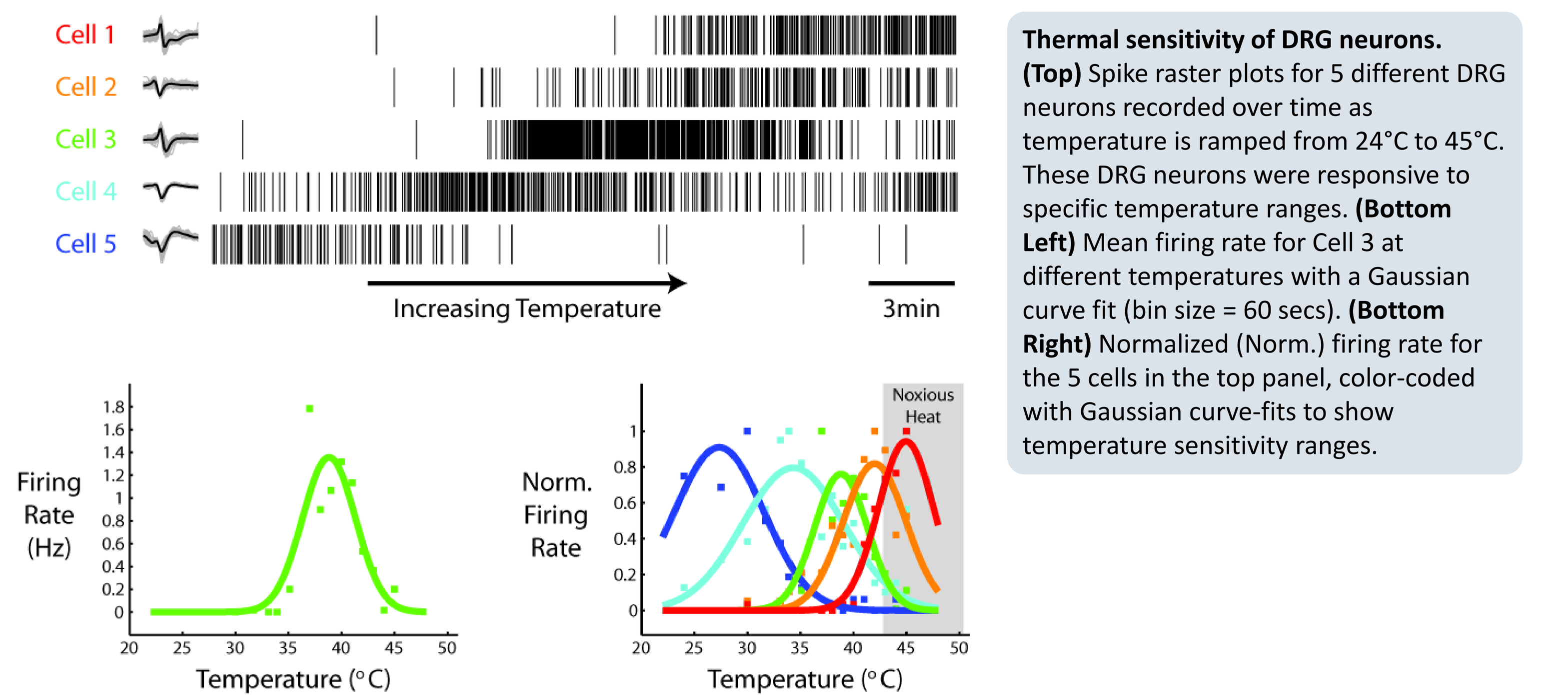
- **Activation of DRG neurons was conducted using capsaicin, an agonist of the TRPV1 temperature and pH receptors.** In the above figure, the raw voltage trace shows the increased firing resulting from capsaicin addition, with the waveform of each detected spike plotted to the right (gray), along with the mean spike waveform (black).
- **DRG neurons exhibit a transient and persistent response to capsaicin.** In the figure above, the plot of well-wide firing rate: 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.
- **Robust *in vitro* activation of DRG neurons by capsaicin sets the stage for screening assays.** The figure below illustrates how the capsaicin response can be blocked by potential pain therapeutics. To explore this concept, we employed the TRPV1 competitive inhibitor dehydroandrosterone (DHEA).[1,2,3]



TRPV1 inhibitors modulate the effect of capsaicin on DRG neuron activity. DRG neurons at 1x10^4 cells per well were exposed to 100 nM capsaicin, followed by the addition of 10 μM 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). Higher doses of capsaicin (1μM) 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.

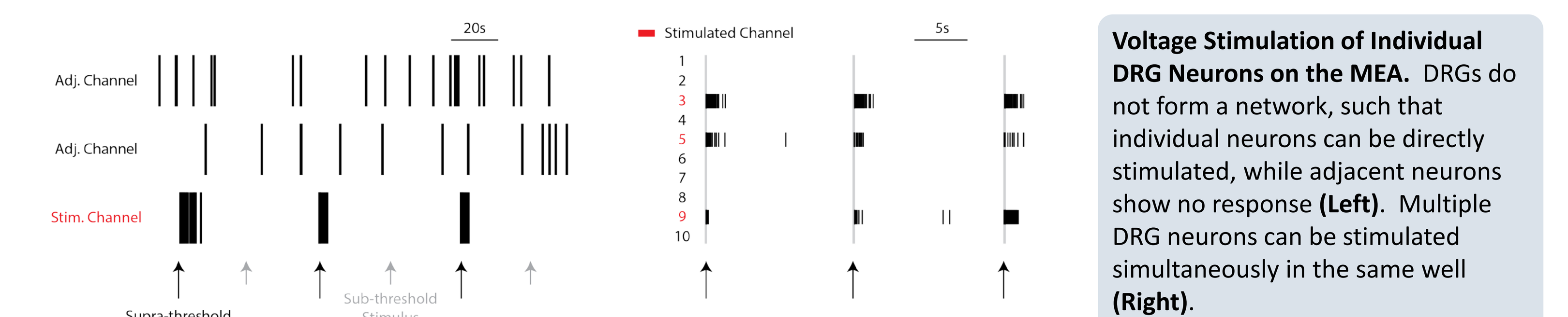
V. Thermal Sensitivity

- **Separate populations of DRG neurons are sensitive to cold, ambient, and hot temperatures.**[4,5] 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.[3,6]
- **The thermal response of DRG neurons can be evaluated on the Maestro**. Using the integrated temperature control, the DRG neurons are directly heated through the MEA plates. The figure below illustrates the response of 5 individual DRG neurons to increasing temperature.



Thermal sensitivity of DRG neurons. (Top) 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. **(Bottom Left)** Mean firing rate for Cell 3 at different temperatures with a Gaussian curve fit (bin size = 60 secs). **(Bottom Right)** Normalized (Norm.) firing rate for the 5 cells in the top panel, color-coded with Gaussian curve-fits to show temperature sensitivity ranges.

VI. Electrical Stimulation



Voltage Stimulation of Individual DRG Neurons on the MEA. DRGs do not form a network, such that individual neurons can be directly stimulated, while adjacent neurons show no response **(Left)**. Multiple DRG neurons can be stimulated simultaneously in the same well **(Right)**.

VII. Conclusion

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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