High-content functional imaging reveals differential effects of inflammation on two distinct populations of human nociceptive neurons

AnaBios
Early Human Insights

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Introduction

The etiology of chronic neuropathic and inflammatory pain remains incompletely understood, creating serious challenges to the development of new, non-addictive medications. Pain drug discovery has had very limited success in translation from animal models to humans. We have developed a novel preclinical discovery strategy, which relies on human sensory neurons isolated from organ donors. In the current study, we describe the high content imaging-based profiling of human sensory neurons under normal as well as inflammatory conditions using a combination of electrical field stimulation (EFS) and fluorescence-based calcium imaging.

Methods

Cell isolation & culture. Human DRGs were collected within 2 hrs. post-mortem and immediately transferred to AnaBios' cold plegic solution to preserve viability. Cells were enzymatically dissociated and plated on PDL-coated glass coverslips. Cells were cultured in DMEM-F12 in the presence of 10ng/mL NGF and 10ng/mL GDNF, at 37°C with 5% CO_2 .

Fluorescence calcium imaging. Human DRG neurons were loaded with Fluo-8-AM and were excited at 480nm while emission was collected at 100 Hz at 520nm with a pcoEDGE sCMOS camera (PCO) mounted on an inverted microscope (Olympus IX71).

Electric field stimulation (EFS). A pair of carbon fiber rods was used as stimulating electrodes; stimulation was delivered in trains of biphasic pulses, each with a duration of 10 ms at a frequency of 5 or 20 Hz using a Master 8 electronic stimulator (AMPI). Stimulus intensity was set at 7.5V/cm (low voltage) or 15V/cm (high voltage). The two different stimulation intensities were set after determining that in our experimental chamber the low voltage stimulation was at threshold for generating responses from a subset of cells, while the high voltage consistently provided supra-threshold stimulation with the maximal response to EFS that still exhibited sensitivity to lidocaine (200 μM) indicating that the stimulation did not directly activate voltage-gated calcium channels.

Results

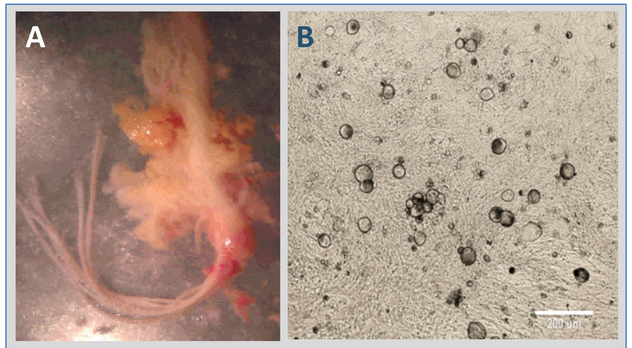

Figure 1. Human dorsal root ganglion neurons in culture.
A. Fresh DRG and efferent nerve fibers dissected from human donor. **B**. hDRG neurons in culture (scale bar 200 μm).

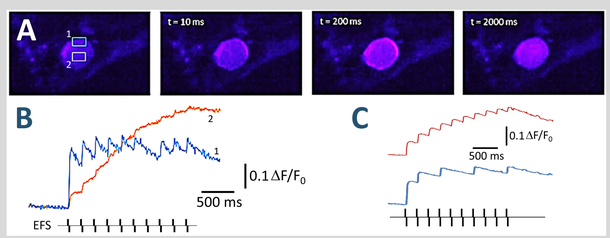

Figure 2. Intracellular calcium transients elicited by EFS in human DRG neurons.
A. Time lapse images from a representative hDRG neuron showing Fluo-8 emission following EFS. **B**. Calcium transients at submembrane location (ROI/Trace #1) or at a cytoplasmic location (ROI/Trace #2) in the cell in (A). **C.** Total whole cells calcium transients measured in 2 hDRG neurons. *Red trace*: a neuron following the entire stimulus train. *Blue trace*: a neuron that could only partially follow the stimulus train.

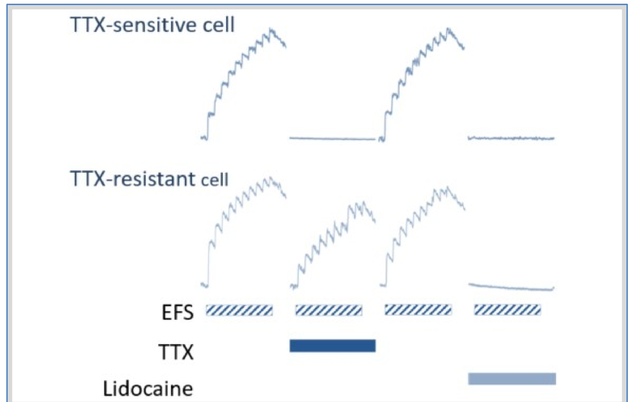

Figure 3. TTX-sensitivity of EFS-induced calcium transients in hDRG neurons.
Examples of a TTX-sensitive (500nM TTX) (top trace) and a TTX-resistant hDRG neuron. All cells exhibited calcium transients that were inhibited by 200μM lidocaine.

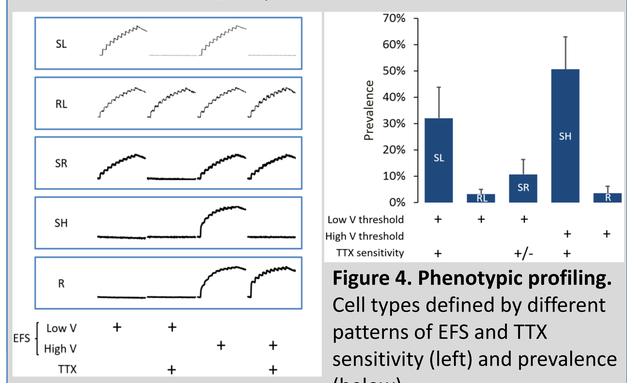

Figure 4. Phenotypic profiling. Cell types defined by different patterns of EFS and TTX sensitivity (left) and prevalence (below).

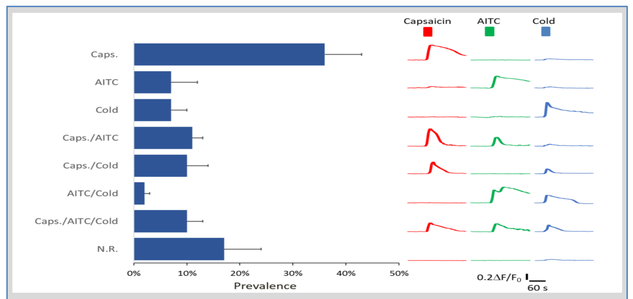

Figure 5. Phenotypic profiling. Prevalence of different phenotypic types identified in hDRG neurons based on responses to Capsaicin (100nM), AITC (50μM) and cold buffer.

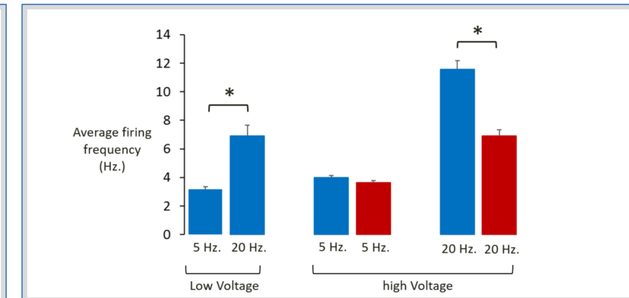

Figure 6. EFS-based identification of two cell classes. Class 1 cells (blue bars) exhibit lower activation threshold and higher transient frequency at 20Hz stimulation, compared to Class 2 cells (red bars).

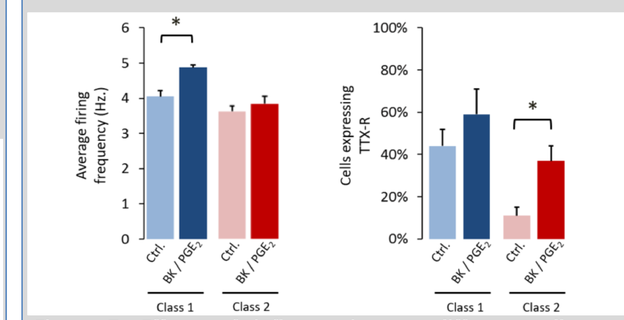

Figure 7. Effects of inflammation on different cell types. Following incubation with BK 100nM and PGE_2 1μM for 2 hrs., Class 1 cells exhibited increased transient frequencies, while Class 2 cells exhibited higher percentage of TTX-R transients.

Conclusion

1- EFS / fluorescent-based Ca^{2+} imaging enables the efficient profiling of the phenotype and pharmacological sensitivity of hDRG neurons.

2- Two classes of cells can be detected, based on the EFS activation threshold and Ca^{2+} transient frequency.

3- The two classes of cells exhibit different responses to inflammatory agents.

This approach allows the human-specific profiling of new drug candidates in functionally defined sub-populations of cells, improving the ability to select human-active drugs for clinical trials.