Phenotypic Profiling of Human Dorsal Root Ganglion Neurons

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Introduction

We have recently developed a novel preclinical discovery strategy, which relies on the isolation and interrogation of human sensory neurons from organ donors (Davidson et al. 2014). In the current study, we report on a new approach for studying the phenotypic profile of human DRG neurons in culture, based on the responses to electrical field stimulation, chemical irritants (capsaicin and AITC) and cold buffer. We then provide an example of how this approach can be used to monitor the phenotypic changes associated with an inflammatory challenge. This methodology provides valuable new tools for studying the properties of human sensory neurons and for investigating the activity of new analgesic drugs in the context of pathological states.

Methods

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. For calcium imaging, cells were loaded with Fluo-8-AM and images (485 nm Ex and 530 nm Em) were acquired at 100 Hz frame rate. Cells were activated by electric field potential stimulation (EFS) using bipolar pulses delivered at 5Hz at 2-8 V/cm. Activation of the different subtypes of VGSC was obtained by the presence or absence of TTX and varying the voltage amplitude of the EFS .
Individual cells' voltage gated sodium channel (VGSC) current was recorded in whole cell voltage clamp mode using a Cs^+-based internal solution.

Results

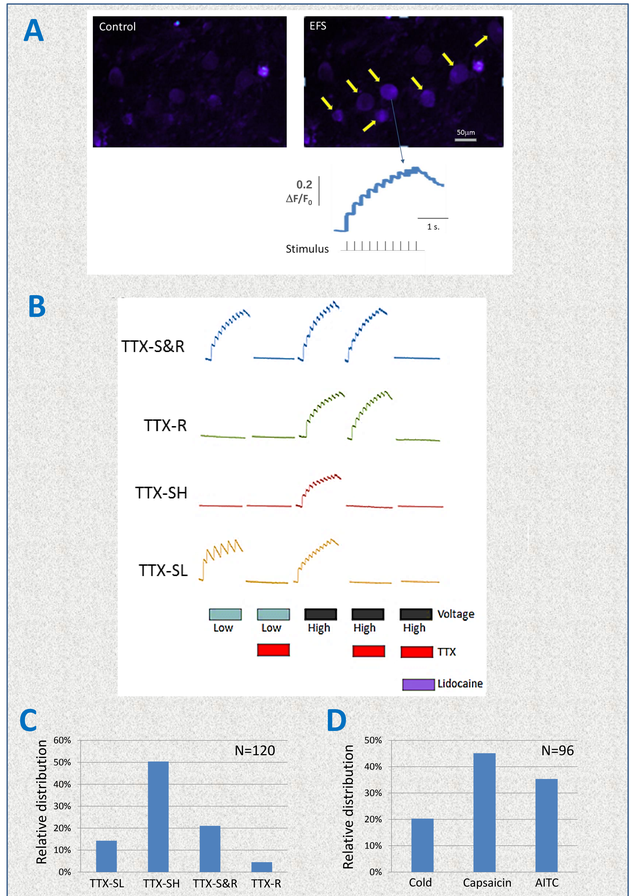

Figure 1. Phenotypic Profiling Human DRG Cells in Culture. **A**. Human DRG neurons in culture exhibit EFS-induced intracellular calcium increase.
B. The EFS-induced calcium signal is dependet upon the activity of VGSC (as shown by the inhibitory effect of 200µM lidocaine) Based on the response threshold and TTX sensitivity, four classes of cells can be identified.
C. Percentage frequency distribution of the 4 cell classes defined in (B).
D. The same population of cells studied in (C) was sequentially challenged with 12°C buffer, 100nM capsaicin and 50µM AITC. The relative distribution is reported.

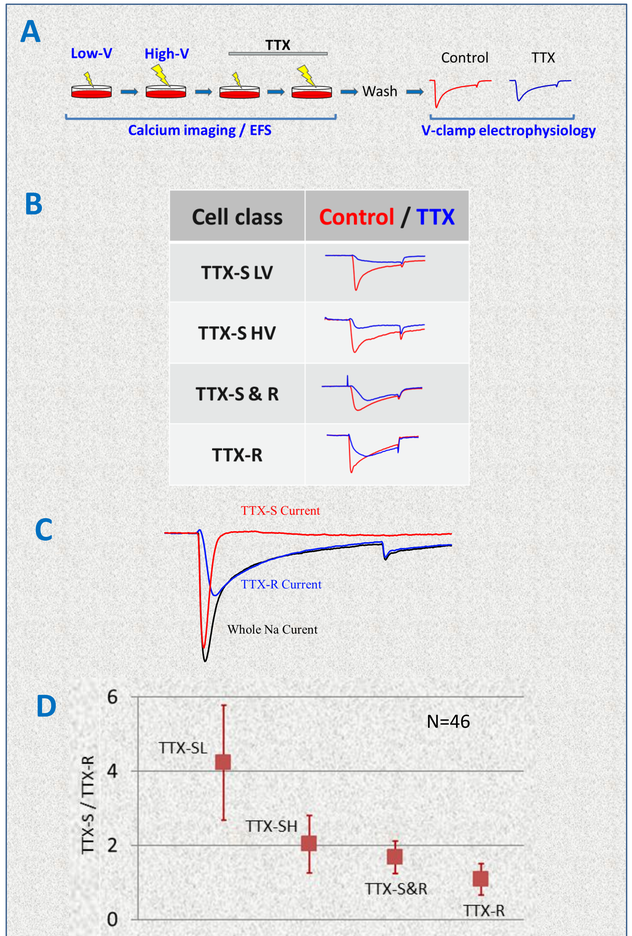

Figure 2. Correlation Between the Properties of EFS–induced Responses and the Expression of Different VGSC Subtypes.
A. A subset of cells was subjected to EFS/ calcium imaging and subsequently impaled with borosilicate electrodes for whole cell voltage clamp-based characterization of the VGSC in each cell.
B. Examples of whole cell sodium channel currents recorded for the different classes of cells defined in Figure 1B.
C. For each cell the contribution of TTX-R VGSC was determined by comparing control vs. 300nM TTX currents. The contribution of TTX-S VGSC was estimated by subtracting the TTX-R current from the control current.
D. The relative proportions of TTX-S and TTX-R for the different classes of cells are shown.

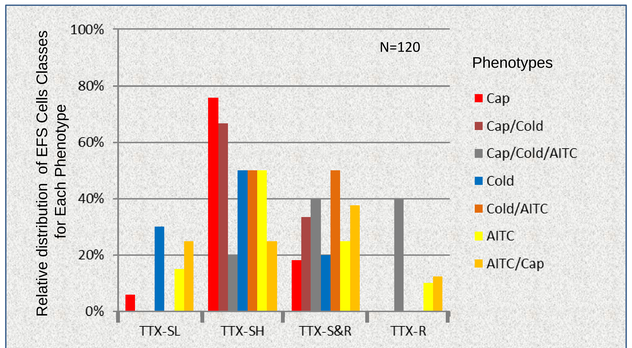

Figure 3. Profiling of Human DRG Neurons by Combining EFS and Chemical and Physical Stimulation. For each phenotype listed on the right, the percentage of cells in the 4 classes of cells defined by EFS is shown.

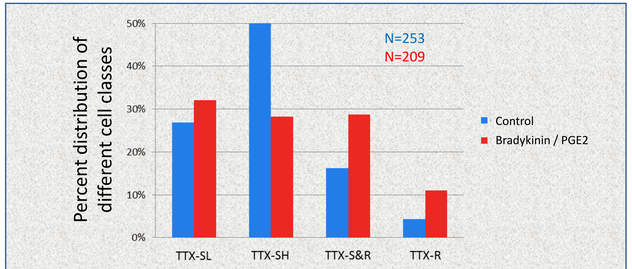

Figure 4. Inflammation-induced Changes in Excitability and TTX Sensitivity of Human DRG Neurons. Cells were profiled by EFS in control conditions (blue bars) or after 2 hrs. treatment with bradykinin (100nM) and PGE_2 (1µM).

Conclusion

- EFS combined with calcium imaging provides a medium throughput tool for investigating the properties of human sensory neurons phenotypes.
- EFS-based cell clustering provides a valuable drug discovery tool that allows rapid identification of VGSC subtype selectivity in human sensory neurons.
- Different sensory neuron phenotypes express different proportion of VGSC subtypes.
- Inflammatory agents induce rapid changes in the VGSC properties of human DRG neurons.