Development, validation and functional characterisation of primary cultures of sensory neurons derived from adult human dorsal root ganglia

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Introduction

There is a critical need for the establishment, validation and characterisation of human cell-based models in which to study nociceptive processing to aid in the development of novel pain therapeutics, increase the translatability of animal models and ultimately reduce animal use. We have therefore developed techniques for the ethical acquisition, culturing and functional analysis of human dorsal root ganglion (DRG) neurons. To date, we have successfully retrieved whole DRGs from organ donors, dissociated and established primary cultures of DRG cells, shipped cultured cells to both national and international consortium member laboratories, and electrophysiologically characterised DRG neurons in culture.

Methods

Ethics:

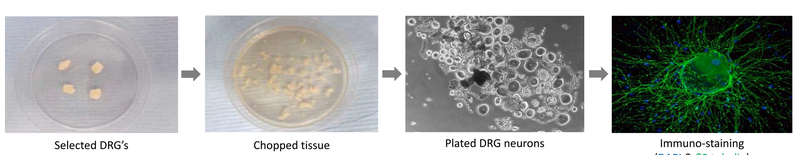
Ethical permission to access organ donors for the purpose of obtaining DRGs, was granted by the West of Scotland Research Ethics Service (Research Ethics Committee ref: 13/WS/0089; NHS GGC Integrated Research Application System ID: 129533).

DRG extraction and dissociation:

DRGs were extracted immediately following organ retrieval and transferred to Hibernate™ media for transfer to the laboratory. Ganglia were dissected into smaller pieces and then underwent enzymatic digestion (Krebs solution supplemented with 0.1% dispase II and 0.25% collagenase Type V), sieving (300µm cell strainer then 26% Percoll solution) and centrifugation. Cells were seeded on 13mm diameter glass coverslips coated with PLL and laminin at 500-1000 cells/15mm diameter well.

Immunohistochemistry:

Immunohistochemical labelling was conducted on 60µm slices of human DRGs prepared using a vibratome. Antibodies used included: chicken anti-neurofilament-200 (NF200; AbCam [ab4680], 1:10000); mouse anti-calcitonin gene related peptide (CGRP; AbCam [4901], 1:100); rabbit anti somatostatin (SOM; Insight Bio [SantaCruz], 1:100); and mouse anti-parvalbumin (PV; Millipore [MAB1572], 1:500).

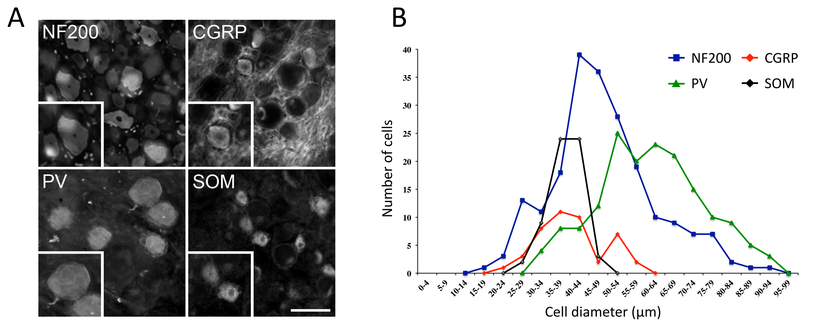


Selected DRG's | Chopped tissue | Plated DRG neurons | Immuno-staining (DAPI & β3 tubulin)

Electrophysiology:

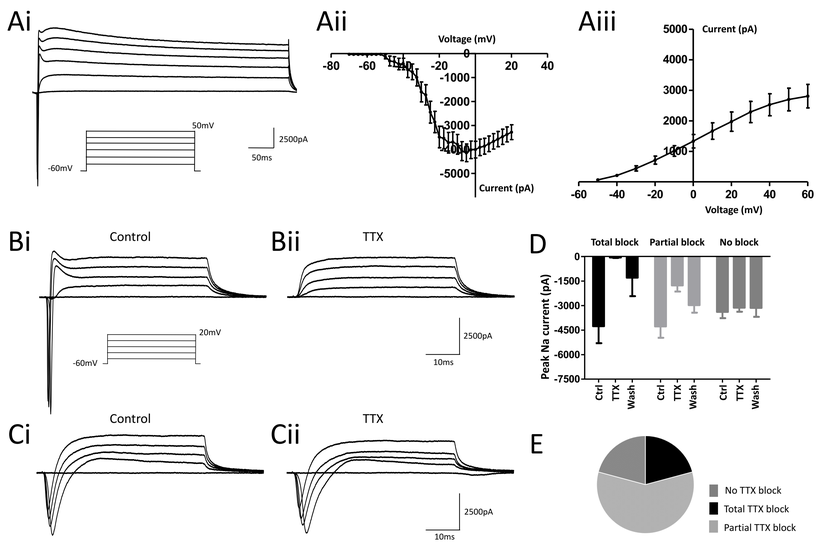
Whole-cell patch-clamp recordings were obtained from cells visualised under infrared differential interference contrast microscopy (IR-DIC). Current-clamp mode recordings were utilised to study the output of hDRG neurons. Voltage-clamp mode recordings were used to study voltage-gated currents and responses to receptor-specific pharmacological agents. Successful recordings were performed across three different sites: St Andrews, Grünenthal and Metrion.

Neurochemical subtypes of hDRG neurons



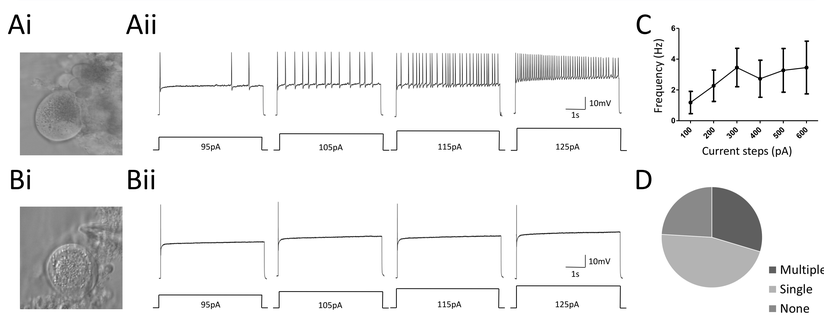
Neurochemical subtypes and size profiles of neurons within slices of isolated human DRGs. **A**, immunohistochemical staining of hDRG slices revealed subtypes of neurons expressing neurofilament-200 (NF200), calcitonin gene-related peptide (CGRP), parvalbumin (PV) and somatostatin (SOM). Scale bar = 100µm. **B**, analysis of soma size of subpopulations of hDRG neurons revealed size distributions similar to other species (e.g. rodents, pigs) with smaller, likely nociceptive CGRP$^+$ and SOM$^+$ neurons, and larger, likely proprioceptive PV$^+$ neurons.

Voltage-gated currents of hDRG neurons



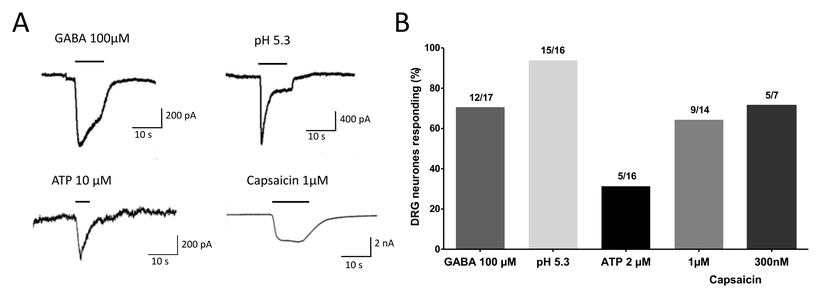
Properties of voltage-gated sodium and potassium currents in hDRG neurons. **Ai**, example of fast, inactivating Na$^+$ currents and persistent K$^+$ currents recorded from a hDRG neuron in response to depolarising voltage steps. **Aii**, voltage-current relationship of fast, inactivating Na$^+$ currents in hDRG neurons (n=54). **Aiii**, voltage-current relationship of persistent K$^+$ currents in hDRG neurons (n=23). **Bi & Bii**, TTX-sensitive Na$^+$ currents in a hDRG neuron. **Ci & Cii**, TTX-insensitive Na$^+$ currents in a hDRG neuron. **D**, magnitude of Na$^+$ currents with varying sensitivities to TTX. **E**, Proportion of hDRG neurons exhibiting Na$^+$ currents with either total, partial or no sensitivity to TTX (n=24).

Firing properties of hDRG neurons



hDRG neurons produce varying patterns of action potential output.
Ai & Aii, a hDRG neuron generating repetitive firing in response to current injection.
Bi & Bii, a hDRG neuron that only generates single action potentials in response to stimuli.
C, average frequency–current relationship for repetitively firing hDRG neurons (n=8).
D, proportion of hDRGs exhibiting different firing patterns (n=54).

Receptors expressed by hDRG neurons



Responses of hDRG neurons to a range of receptor–specific pharmacological agents. **A**, current responses recorded from hDRGs in response to: GABA (100µM); low pH (5.3); ATP (10µM); and capsaicin (1µM). **B**, the proportion of hDRG neurons that respond to various receptor agonists.

Summary

- We have produced primary cultures of functionally viable human DRG neurons that can be shipped internationally and survive for weeks in culture.
- Functional analyses reveal a heterogeneous population of hDRG neurons in cultures – including likely nociceptive neurons.
- Cultured hDRG neurons are suitable for functional & pharmacological studies.

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