Profiling two-pore domain potassium (K2P) channel gene expression in pain-relevant cells

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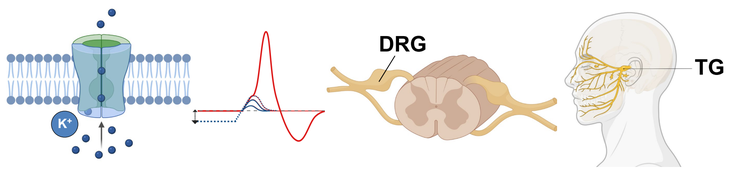
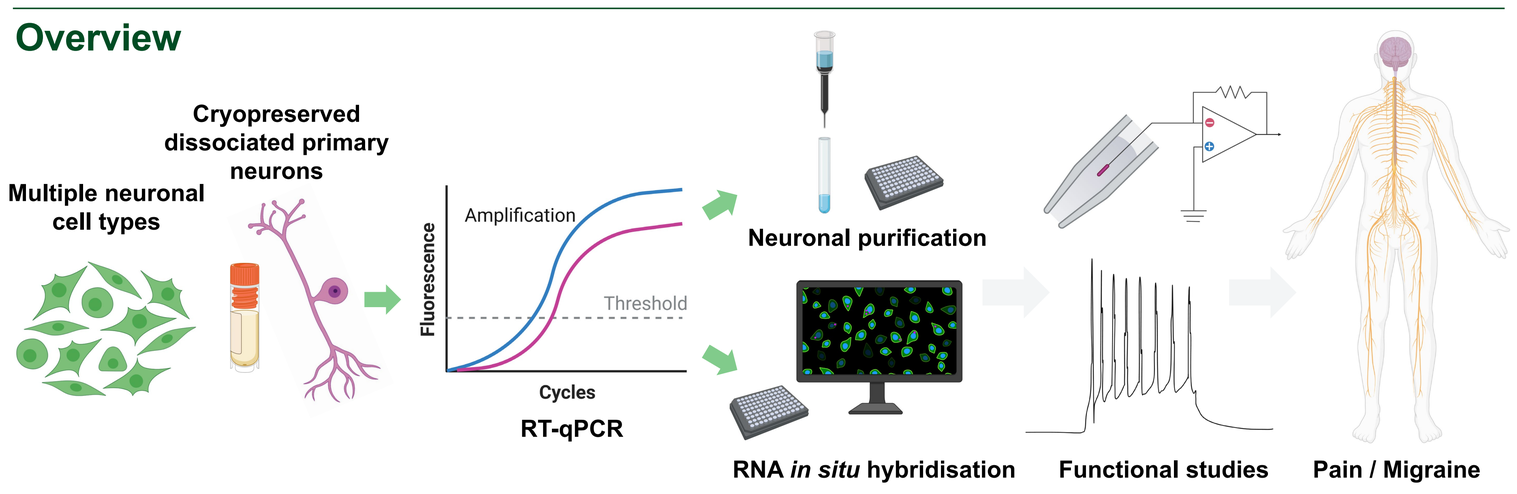
Introduction

- K2Ps carry background (or leak) potassium current and contribute to the maintenance of resting cell membrane potential.
- While genetic and functional evidence points to K2P role in pain and migraine, more extensive target validation is required.
- Dorsal root ganglia (DRG) and trigeminal ganglia (TG) are important structures in these pathophysiologies, as they contain cell bodies of peripheral sensory neurons.
- Gene expression analysis was used to identify cells that would enable more physiologically relevant assay development and screening, in order to support the development of novel K2P-based analgesics.



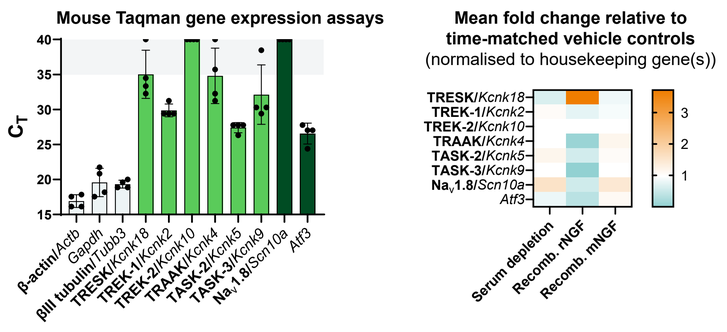
Overview



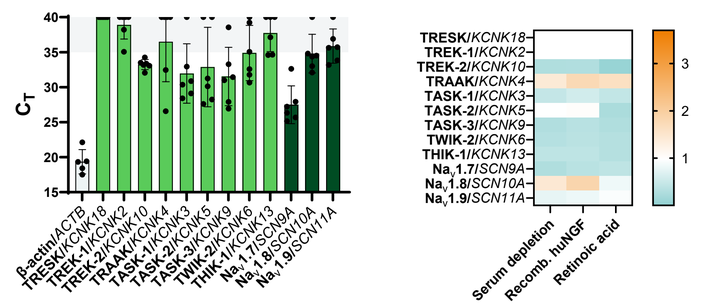
Neuronal cell lines

ND7/23 and SH-SY5Y cell lines display suboptimal K2P gene expression profile

ND7/23 - mouse neuroblastoma and rat DRG neuron hybrid



SH-SY5Y - human neuroblastoma cell line

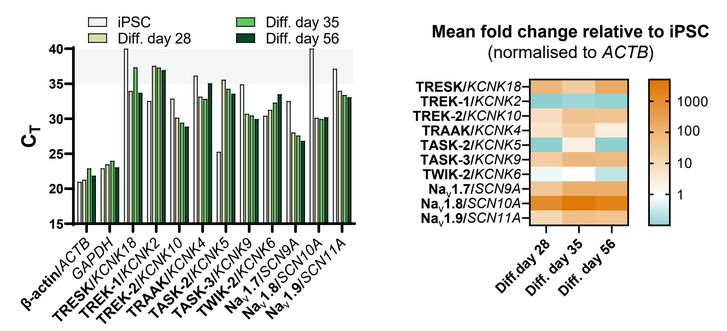


Relative gene expression levels were measured by RT-qPCR. Lower C_T values correspond to higher gene expression. Treatments involved exposure to 0.5% FBS, 100 ng/ml recombinant nerve growth factor (NGF), or 10 µM retinoic acid for either 3 days (ND7/23) or 6 days (SH-SY5Y).

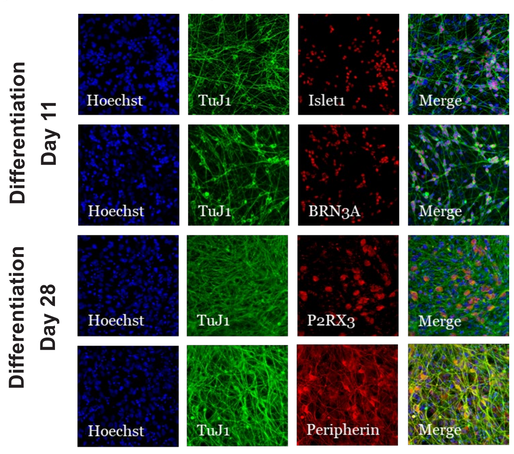
Human iPSC-derived sensory neurons

TRESK, TREK-2, TRAAK, and TASK-3 genes are expressed in human iPSC-derived sensory neurons

K2P upregulation in iPSC-derived sensory neurons compared to iPSCs



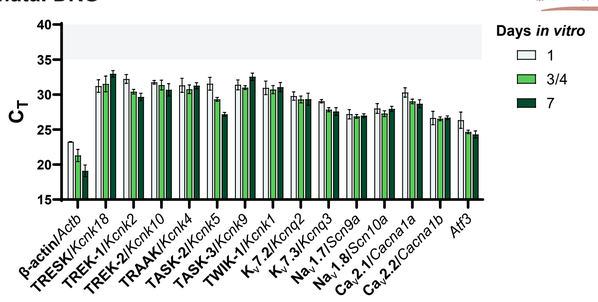
Sensory neuron markers expressed in differentiated hiPSC cells



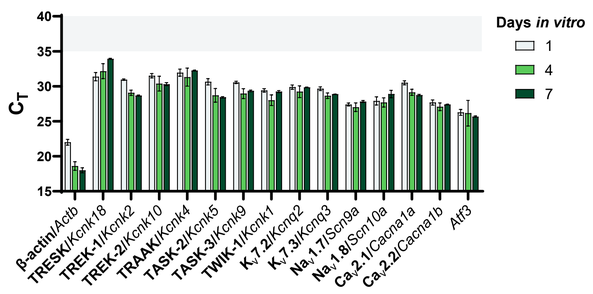
Primary rodent DRG and TG cells

Multiple K2P genes are consistently expressed in primary DRG and TG cell cultures

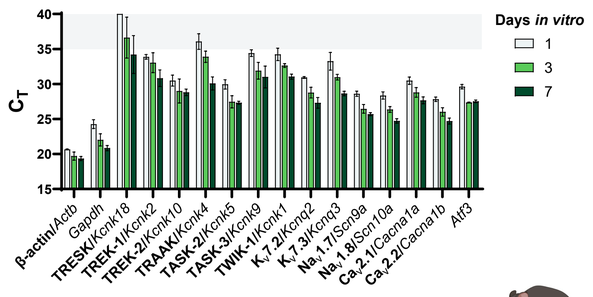
Rat neonatal DRG



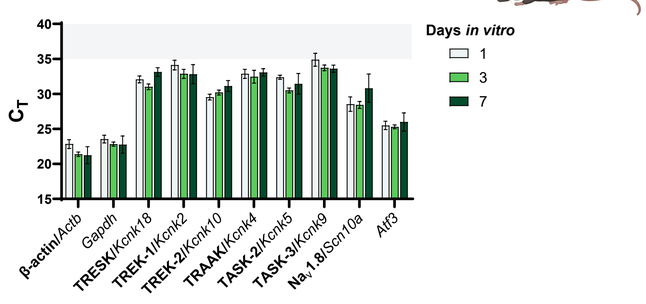
Rat neonatal TG



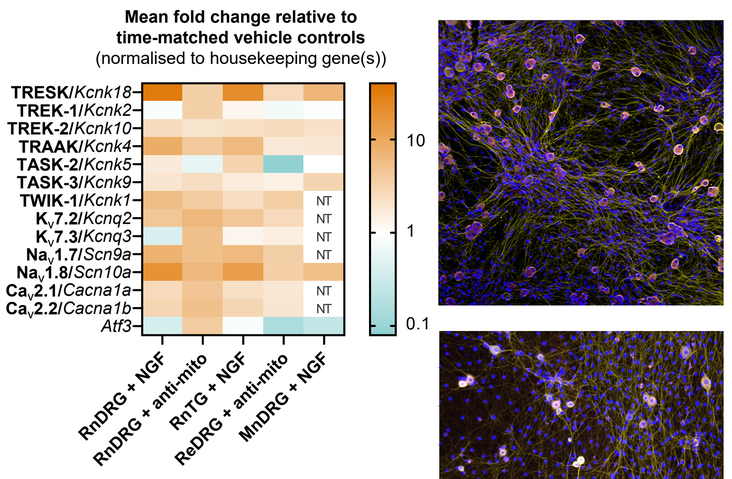
Rat embryonic DRG



Mouse neonatal DRG



Upregulation seen in response to nerve growth factor (NGF) or anti-mitotic agents suggests neuronal K2P expression



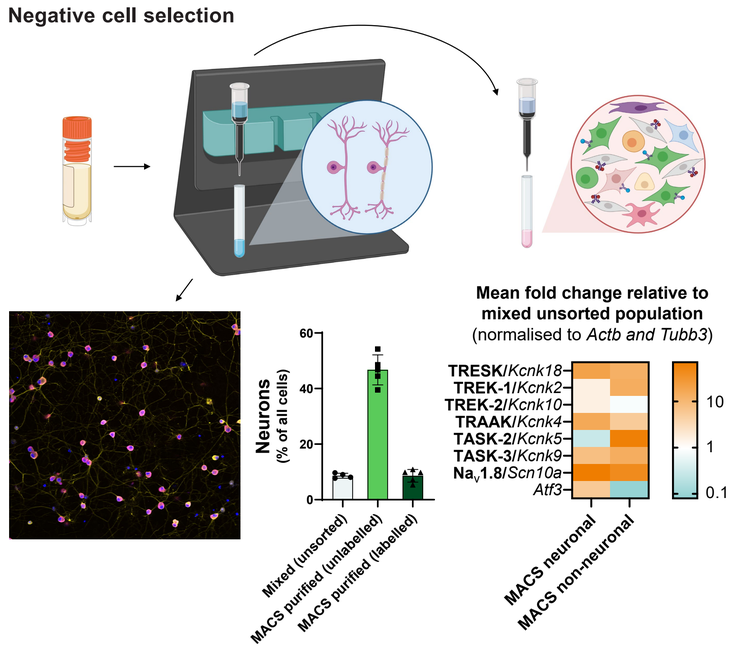
Cells were treated for 7 days with NGF (100 ng/ml), a combination of 7.5 µg/ml 5-fluoro-2'-deoxyuridine and 17.5 µg/ml uridine, and/or 1 µM Ara-C. NT, not tested.

Rat neonatal DRG culture (4 days *in vitro* with NGF; *top*) and mouse neonatal DRG culture (7 days *in vitro* with NGF; *bottom*) images. Blue – Hoechst (nuclei), magenta – Nissl (neuron cell bodies; *top*) or isolectin B4 (IB4; *bottom*), yellow – anti-TuJ1 (neuron-specific βIII tubulin).

Magnetic-activated cell sorting (MACS)

Successful enrichment, but not complete purification, of cryopreserved dissociated mouse neonatal DRG neurons

Negative cell selection

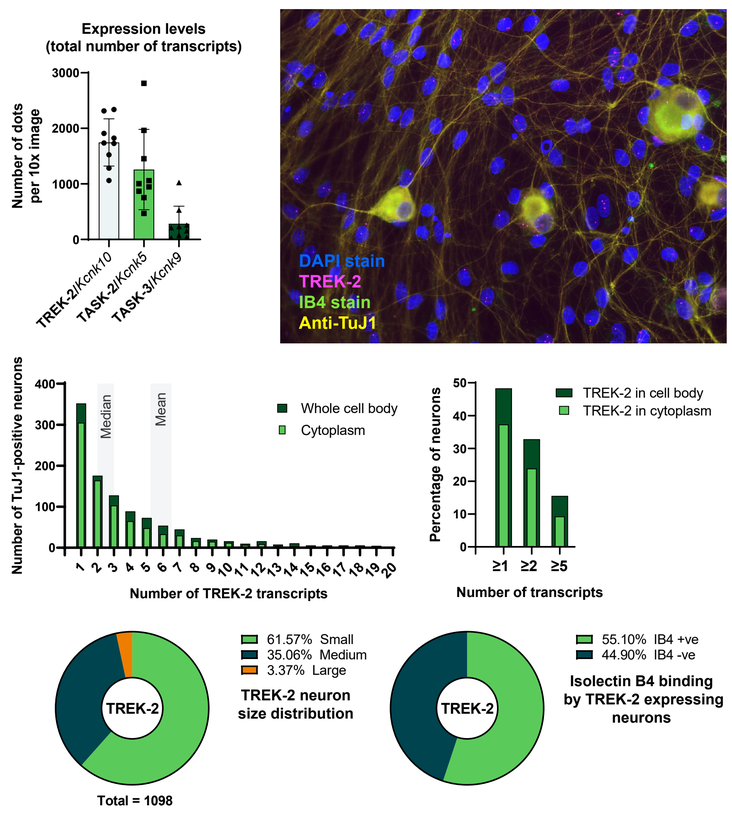


Non-neuronal cells are tagged and given a magnetic charge, which when passed through a column in a magnetic field allows for all the untagged neuronal cells to flow through, while the tagged non-neuronal cells are retained within the column. Immunocytochemistry image shows MACS-purified mouse DRG neuron population after 1 day *in vitro*.

RNA fluorescence *in situ* hybridisation

Visualising K2P localisation without anti-K2P antibodies

ViewRNA Cell Plus Assay



Combined immunocytochemistry and viewRNA image shows rat neonatal DRG neurons (7 days *in vitro*, exposed to NGF). Punctate magenta signal is produced by TREK-2/*Kcnk10* viewRNA probes. Each dot represents a single TREK-2 transcript. Image quantification was performed using CellProfiler software. IB4, isolectin B4.

Future directions

- Neuronal purification will enable further interrogation into the role of K2Ps in regulating neuronal excitability.
- Visualising K2P mRNA localisation in different sensory neuron subsets will aid functional studies in these cells.
- Assays in (patho)physiologically relevant cells will contribute to the development of compounds with more predictable analgesic effects.

Illustrations created with BioRender.com