High-content imaging-based profiling of ion channel gene expression and function in primary cultures of rodent dorsal root ganglion and trigeminal ganglion cells

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Abstract

Primary cultures of rodent sensory neurons of the dorsal root ganglia (DRG) or trigeminal ganglia (TG) are commonly used in pain research as a physiologically relevant *in vitro* system. However, the heterogeneous nature of these cultures, containing both post-mitotic neurons and supporting non-neuronal cells, can complicate target validation. Here, we describe high-content imaging-based assays in primary cultures of cryopreserved neonatal rat DRG and TG cells that facilitate investigation of neuronal and non-neuronal ion channel gene expression and function.

Due to a limited availability of validated antibodies for specific labelling of ion channels, the localisation of targets, such as a two-pore domain potassium (K2P) channel TREK-2 and transient receptor potential (TRP) channels TRPV1 and TRPM8, was visualised in different DRG/TG cell types using RNA fluorescence *in situ* hybridisation (ViewRNA Cell Plus Assay, Invitrogen). The use of different combinations of ViewRNA probes and general neuronal markers enabled subsequent development of quantitative image analysis methods.

In addition, FLIPR Membrane Potential Assay (Molecular Devices) that is typically used in populations of recombinant cells was adapted to detect changes in voltage across the cell membranes of individual neurons. This assay was utilised for initial functional small-molecule compound profiling, with depolarising or hyperpolarising effects of retigabine (voltage-activated K_v7 family potassium channel opener), TPA (non-selective potassium channel blocker), BL-1249 (K2P channel activator), and a novel LifeArc TREK-2 activator, given as examples.

Assays described in this poster could be applied to other targets present at various expression levels in diverse (patho)physiologically relevant cells, in order to support identification of compounds with more predictable *in vivo* efficacy.