Evaluation of hNav1.9 Screening Cascade for Analgesic Drug Discovery

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Introduction

Encoded by the *SCN11A* gene, $Na_V1.9$ is a voltage-gated sodium (Na_V) channel highly expressed in trigeminal ganglion neurons and small-diameter nociceptors in the dorsal root ganglion. $Na_V1.9$ acts as a threshold channel with a lower activation threshold, slower biophysical properties and a large window current compared to the other Na_V isoforms[1]. These characteristics are important for its role in the regulation of neuronal excitability and the modulation of inflammatory and neuropathic pain. Clinically, $Na_V1.9$ dysfunction has been implicated in altered pain perception in humans (Figure 1), evidencing its potential as a non-opioid pain target[2-4].

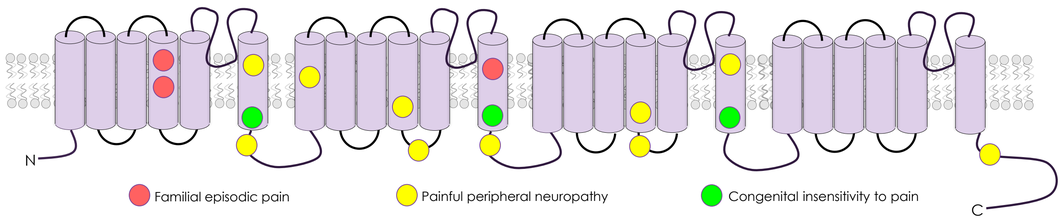


Figure 1 – Schematic of pain-related mutations in $hNa_V1.9$ adapted from Kabata *et al.*[5]

High-throughput $Na_V1.9$ drug discovery programmes have been hindered to date by the lack of the cellular tools and screening assays. Hence, the generation of a robust Nav1.9 screening cascade would greatly accelerate the development of selective $Na_V1.9$ modulators without the side-effects associated with current pain treatment options.

Methods

- **Cell culture -** A stably-expressing monoclonal CHO-$hNa_V1.9$ cell line was generated in-house.
- **Automated patch clamp electrophysiology –** For biophysical assessment, cells were held at -140 mV with 100 ms steps from -100 mV to +50 mV in 10 mV increments. For compound screening, cells stepped to -40 mV (50 ms) from -140 mV (0.05 Hz). IV stimulation was the same as manual patch, except 100 ms steps. Recordings were made using multi-hole on a Qube 384 (Sophion Bioscience).

Results

1. Biophysical assessment of $hNa_V1.9$ using QPatch and Qube automated patch clamp

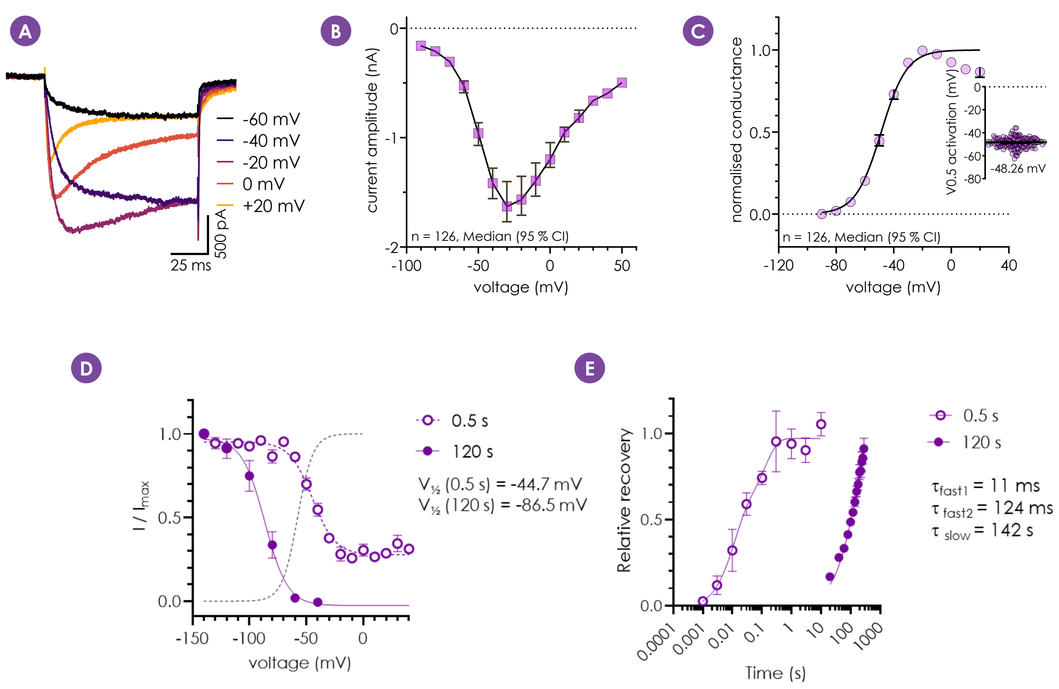
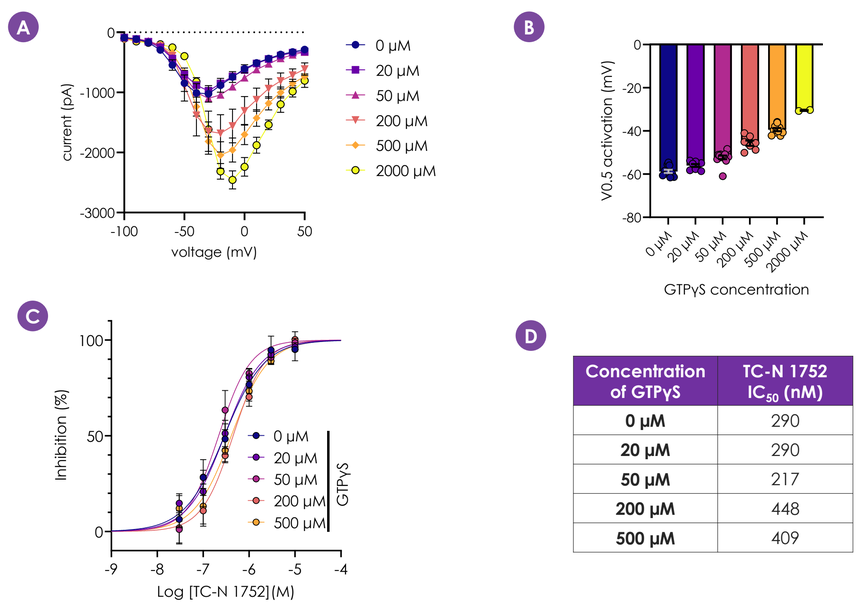


Figure 2 - $hNa_V1.9$ channels have distinct biophysical properties compared to the other Na_V isoforms[1] (A). The IV relationship (B) and conductance (C) of $hNa_V1.9$ currents recorded from 126 Qube 384 multi-hole wells. Voltage dependence of fast and slow Inactivation displayed in D. Recovery from fast and slow inactivation shown in E. Data for inactivation kinetics obtained using QPatch48.

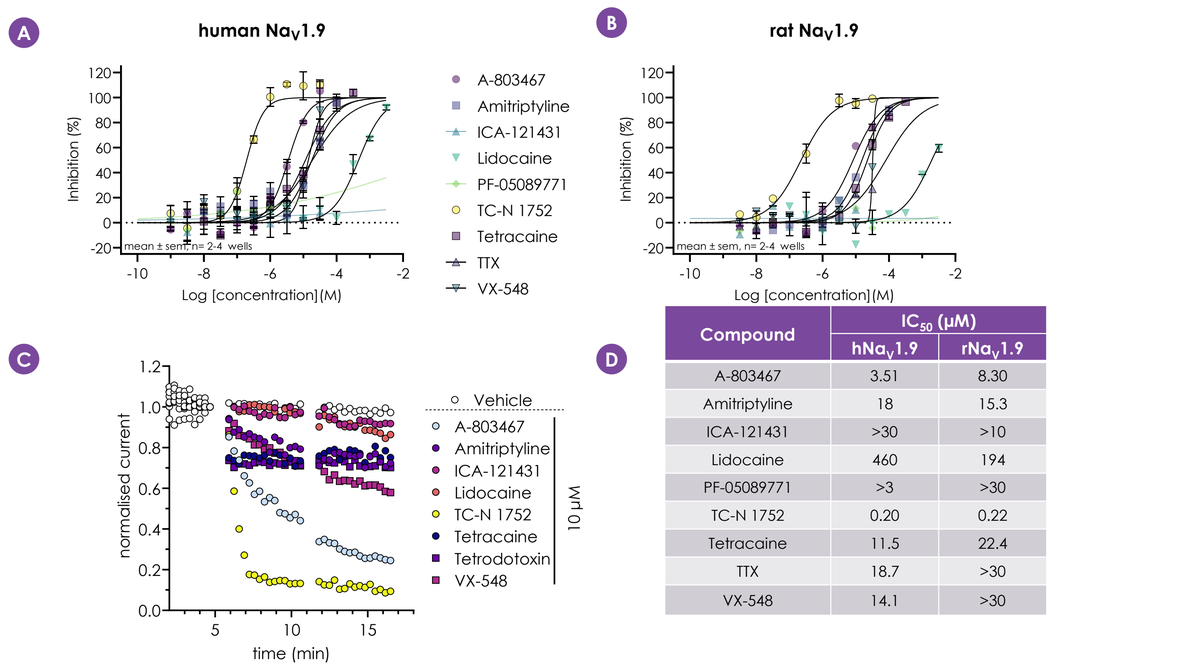
2. Effects of GTPγS on $hNa_V1.9$ on biophysics/pharmacology



Concentration of GTPγS	TC-N 1752 IC_{50} (nM)
0 µM	290
20 µM	290
50 µM	217
200 µM	448
500 µM	409

Figure 3 - Enhanced G-protein signalling has been shown to potentiate $Na_V1.9$ current amplitudes[6]. Addition of up to 500 µM intracellular GTPγS resulted in larger $hNa_V1.9$ currents, with a depolarising shift $V_{0.5}$ of activation (A,B). Importantly, GTPγS concentration did not alter $hNa_V1.9$ pharmacology (C,D). A concentration of 200 µM was used for routine screening.

3. Pharmacological assessment of $hNa_V1.9$ and $rNa_V1.9$ using Qube



Compound	IC_{50} (µM)	
	$hNa_V1.9$	$rNa_V1.9$
A-803467	3.51	8.30
Amitriptyline	18	15.3
ICA-121431	>30	>10
Lidocaine	460	194
PF-05089771	>3	>30
TC-N 1752	0.20	0.22
Tetracaine	11.5	22.4
TTX	18.7	>30
VX-548	14.1	>30

Figure 4 – Validated Qube 384 assays testing a selection of Na_V inhibitors with a range of potencies against $hNa_V1.9$ (A) and $rNa_V1.9$ (B). Representative I-t plots of vehicle or compound (at 10 µM) against $hNa_V1.9$ are shown in C. Table with calculated IC_{50} values (D).

4. Blinded assessment of $hNa_V1.9$ pharmacology using spiked plated approach

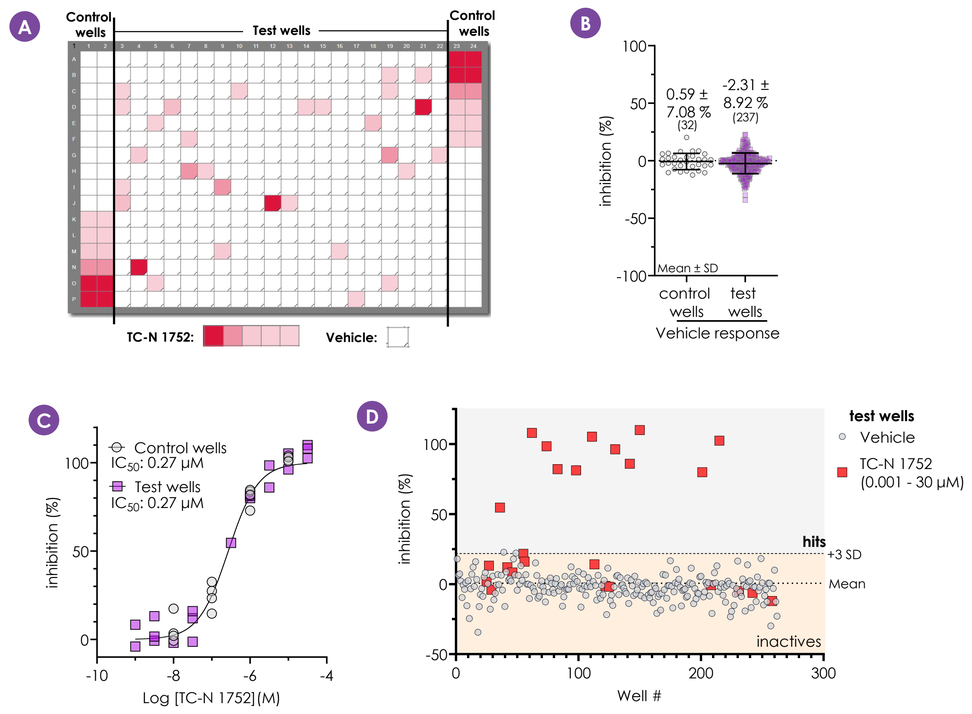
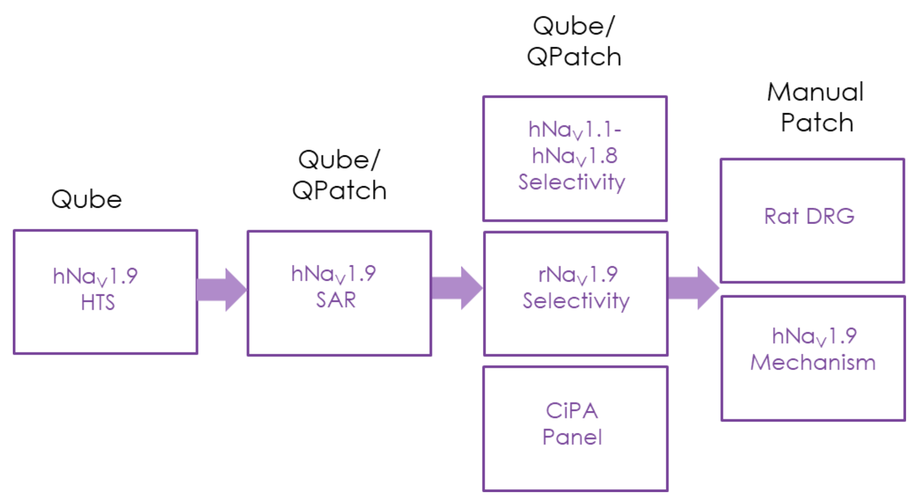


Figure 5 - The robustness of the Qube 384 assay was further validated by assessing the potency of TC-N 1752, using a randomised spiked plate approach (plate map - A). Vehicle response and TC-N 1752 potency correlated well between control and test wells (B, C). In test wells, the vehicle response displayed low variability with the TC-N 1752 response (at >0.1 µM) easily discernible above the mean vehicle response + 3 SD threshold (D).



Conclusions

- $hNa_V1.9$ biophysics from this cell line match the characteristics of native $hNa_V1.9$
- Pharmacology obtained for a variety of known sodium channel inhibitors against both $hNa_V1.9$ and $rNa_V1.9$
- A robust screen sequence has been developed based around $hNa_V1.9$ Qube 384 automated patch clamp assay to accelerate the development of selective $Na_V1.9$ modulators for utility in the treatment of pain

References

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