A Validated Human $Na_V1.9$ Screening Cascade for Pain 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, evidencing its potential as a non-opioid pain target[2-4]. High-throughput $Na_V1.9$ drug discovery programmes have been hindered to date by the lack of the cellular tools currently available. Hence, the generation of a robust, stably-expressing $hNa_V1.9$ cell line and high-throughput assay would greatly accelerate the development of selective $Na_V1.9$ modulators without the side-effects associated with current pain treatment options.

Methods

- **Cell culture -** Monoclonal CHO-$hNa_V1.9$/$rNa_V1.9$ cell lines were generated in-house. $hNa_V1.9$-1.5 chimeric channels were expressed using electroporation (MaxCyte).
- **Automated patch clamp electrophysiology –** Recordings were made using multi-hole acquisition on Qube 384 and QPatch 48X platforms (Sophion Bioscience).

Results

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

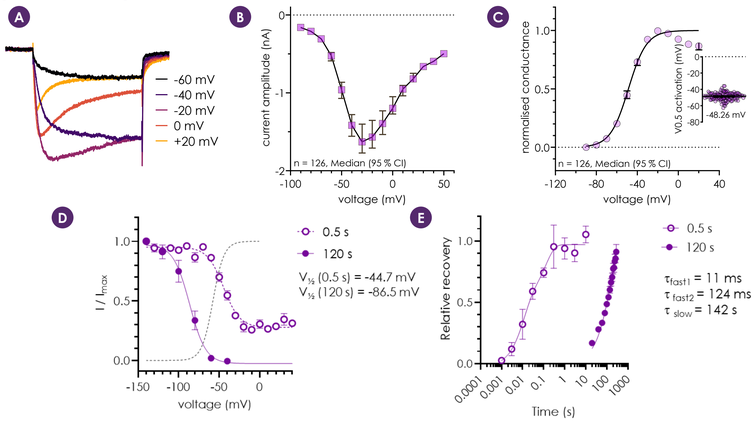


Figure 1 - $hNa_V1.9$ channels have distinct biophysical properties compared to the other Na_V isoforms[1] (A). The IV relationship (B) and conductance (C) of $hNa_V1.9$ currents recorded from Qube 384. Voltage dependence of fast and slow Inactivation displayed in D. Recovery from fast and slow inactivation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

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

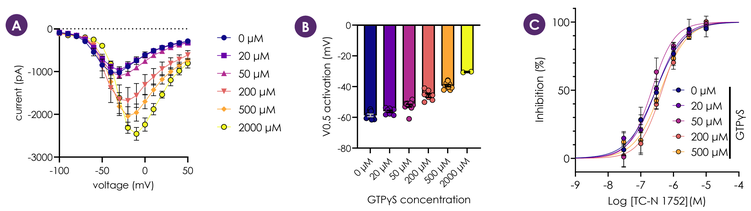
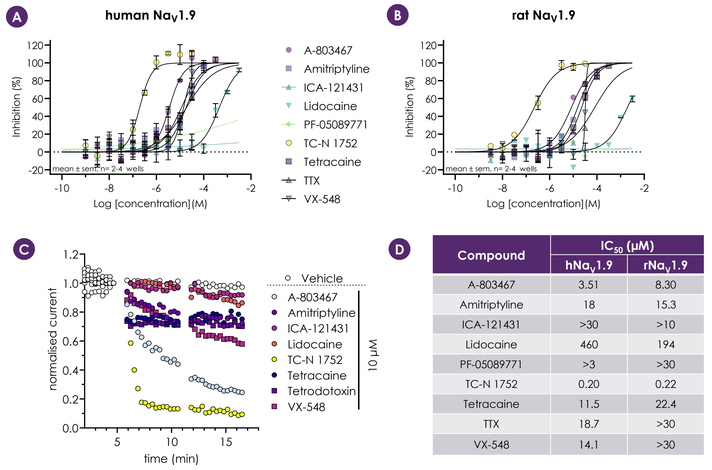


Figure 2 - Enhanced G-protein signalling has been shown to potentiate $Na_V1.9$ current amplitudes[6]. Addition of up to 2000 µM intracellular GTPγS resulted in larger $hNa_V1.9$ currents, with a depolarising shift $V_{0.5}$ of activation (A,B). Importantly, GTPγS concentration did not alter $hNa_V1.9$ pharmacology (TCN-1752 IC_{50} (µM) was 0.29, 0.29, 0.22, 0.45, 0.41 for 0, 20 µM, 50 µM, 200 µM, and 500 µM GTPγS, respectively (C). 200 µM used henceforth.

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



D

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 3 – 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 $hNa_V1.9$ shown in C. Table with calculated IC_{50} values (D).

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

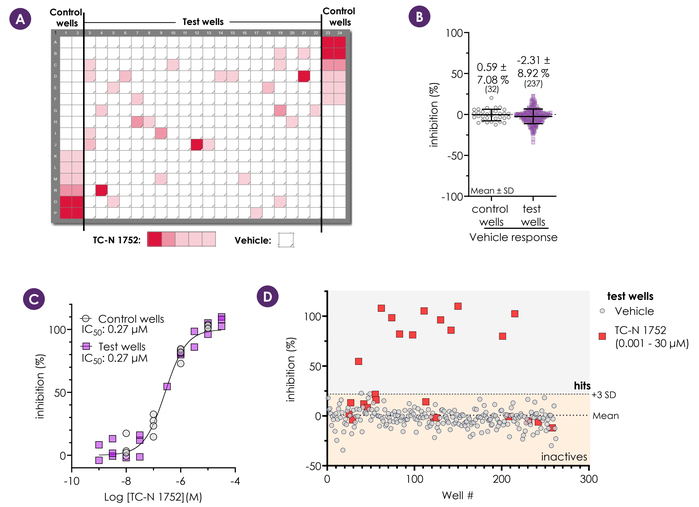
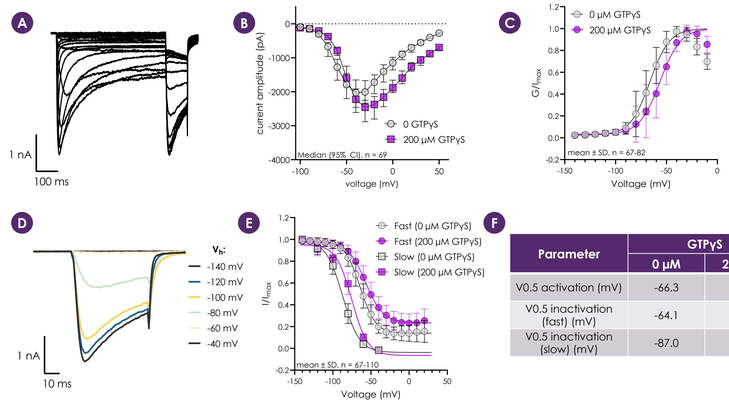


Figure 4 - The robustness of the Qube 384 assay was further validated by assessing the potency of TC-N 1752, using a randomised spiked plate approach (plate map - A). Vehicle response and TC-N 1752 potency correlated well between control and test wells (B, C). In test wells, the vehicle response displayed low variability with the TC-N 1752 response (at >0.1 µM) easily discernible above the mean vehicle response + 3 SD threshold (D).

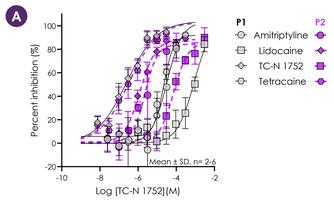
5. Biophysical assessment of $hNa_V1.9$-5C chimera



Parameter	GTPγS	
	0 µM	200 µM
V0.5 activation (mV)	-66.3	-56.3
V0.5 inactivation (fast) (mV)	-64.1	-57.1
V0.5 inactivation (slow) (mV)	-87.0	-75.0

Figure 5 – Biophysical assessment of $hNa_V1.9$-5C chimeric channel. The previously reported $hNa_V1.9$-5C chimera has demonstrated similar voltage dependencies to wt $hNa_V1.9$, yet with larger current amplitudes, broadening the range of screening options for $Na_V1.9$[7] (A) Representative trace of a CHO-$hNa_V1.9$-5C cell stimulated for 500 ms at increasing voltages (-140 to +40 mV) before a 100 ms hold at -40 mV. (B) activation I-V relationship, (C) G-V relationship and (E) voltage dependence of fast and slow inactivation of $hNa_V1.9$-5C chimera channels. (D) Representative trace of a CHO-$hNa_V1.9$-5C cell held at increasing holding voltages (Vh) before a 50 ms step to -40 mV. (F) Quantification of activation and inactivation parameters.

6. State-dependent pharmacological assessment of $hNa_V1.9$-5C chimera



B

Compound	IC_{50} (µM)	
	P1	P2
Amitriptyline	31.2	2.3
Lidocaine	1050	733
TC-N 1752	0.32	0.4
Tetracaine	21.7	1.28

Figure 6 – Pharmacological assessment of the $hNa_V1.9$-5C chimera. (A) Concentration-response curves of four tool compounds using a 2-pulse voltage protocol (2.5 s hold at -40 mV, followed by a 10 ms repolarising step at -140 mV, preceded P2). Leveraging the larger current amplitudes of the $hNa_V1.9$-5C chimeric channel, compared to wt $hNa_V1.9$, allows for typical two-pulse, Na_V state-dependent protocols. (B) Quantification of compound potency. Large P2 potency shifts demonstrated for state-dependent molecules, but not TC-N1752 (state-independent).

Conclusions

- $hNa_V1.9$ biophysics align with reported values.
- Human and rat $Na_V1.9$ channels can be assessed pharmacologically using automated patch clamp. Pharmacology aligned between species for tested compounds.
- Successful development and validation of a $hNa_V1.9$ Qube 384 automated patch clamp assay suitable for high-throughput screening of compounds to accelerate the development of selective $Na_V1.9$ modulators for utility in the treatment of pain.
- $hNa_V1.9$-5C chimera displays similar biophysical properties to wt $hNa_V1.9$ but is suitable for more complex voltage protocols to assess state-dependence of inhibition.

References

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