B'SYS GmbH

HEK $Na_V1.9$ Assay

Specification Sheet

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1 B'SYS Nav1.9 CELL LINE

1.1 The voltage gated sodium channels Nav1.9

SCN11A encodes Nav1.9, a member of the sodium channel alpha subunit gene family which is preferentially expressed in peripheral nociceptive neurons. Due to its unique biophysical properties (activation at hyperpolarized membrane potentials, slow activation and inactivation kinetics), Nav1.9 is thought to play a role in regulating the resting membrane potential, amplifying sub-threshold stimuli leading to action potential bursts and increases repetitive firing.
Nav1.9 has been reported to play a role in pain signaling pathways by regulating the excitability of sensory neurons and has been shown to play a role in inflammation-induced hyperalgesia. This makes it an interesting target for the development of novel pain therapeutics.

1.2 B'SYS' HEK Nav1.9 cells

B'SYS has designed a HEK Nav1.9 cell line with an inducible expression of human Nav1.9 sodium channels. The human codon optimized Nav1.9 cDNA (NP_001336182) was cloned and transfected into HEK cells and then the functional properties of the Nav1.9 channels validated using manual and automated (QPatch™) patch-clamp recordings. Results are outlined in section 2.

2 VALIDATION OF HEK NAV1.9 CELLS

2.1 Biophysical characterization

Nav1.9 currents were measured by means of the patch-clamp technique in the whole-cell configuration. The bath solution contained: NaCl 137 mM KCl 4 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 1 mM, HEPES 10 mM, D-Glucose 10 mM, pH (NaOH) 7.4. The pipette solution consisted of: CsF 135 mM, NaCl 10 mM, HEPES 10 mM, EGTA 5 mM, GTPγS 500 µM, pH (KOH) 7.3. After formation of a GΩ seal between the patch electrodes and individual Nav1.9 stably transfected HEK cell, the cell membrane across the pipette tip was ruptured to assure electrical access to the cell interior. All solutions applied to cells were continuously perfused and maintained at room temperature. To determine the voltage dependence of activation, cells were held at a membrane potential of -140 mV and currents were measured during a 200 ms test pulse to potentials of -120 mV to +40 mV in 10 mV increments. The voltage dependence of steady-state inactivation was determined by clamping the cells at -140 mV followed by a 500 ms conditioning prepulse to potentials of -140 mV to +10 mV in 10 mV increments and then a 40 ms test pulse to -40 mV. The voltage pulses were run at intervals of 20 s.

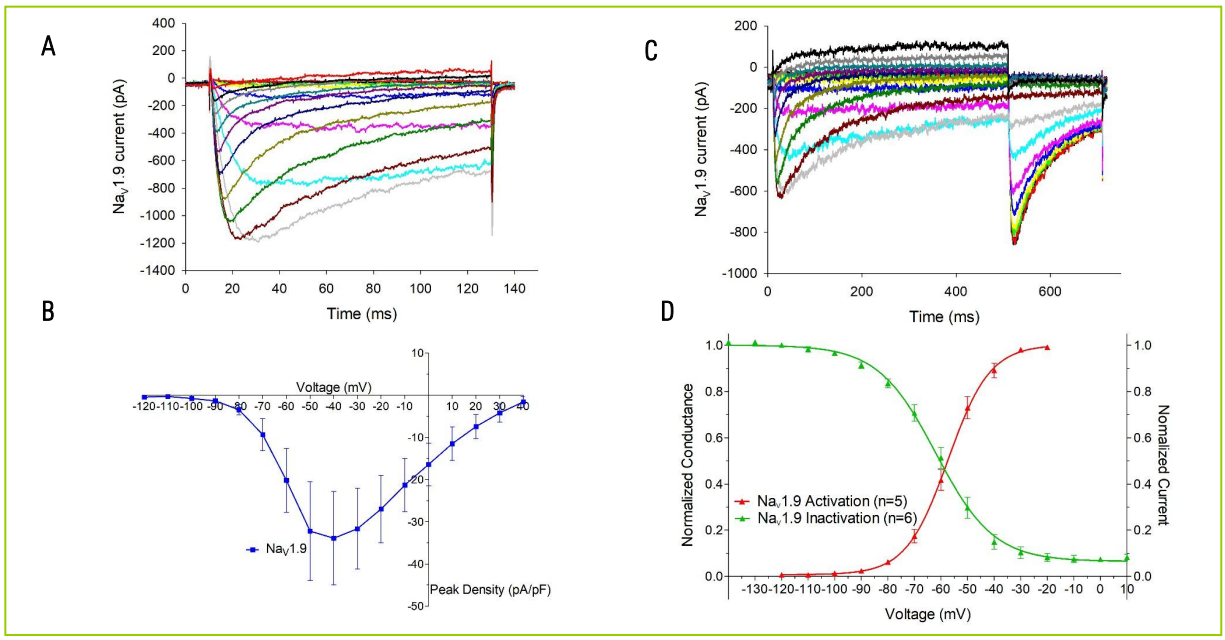

Fig. 1: Biophysical characterization of stably transfected HEK Nav1.9 cells. A) Representative current recordings for test potentials ranging from -140 mV to +40 mV. B) Current density voltage relationship of Nav1.9 cells. The mean current density at -40 mV was -33.90 ± 11.09 pA/pF (n=5). C) Representative current recordings for test potentials ranging from -140 mV to +10 mV for the voltage dependence of inactivation. D) Activation and inactivation curves of Nav1.9 channels. Data were fit by a Boltzmann equation. The $V_{0.5}$ value for the voltage dependence of activation was -57.29 ± 1.43 mV (n=5). The $V_{0.5}$ value for the voltage dependence of inactivation was -61.68 ± 2.00 mV (n=6).

For the IV curve the peak current amplitude of the pulses between -120 mV and -20 mV was plotted versus the applied voltage. This resulted in a bell-shaped curve. The minimum of the IV curve was at -40 mV (Fig 1 B). Results from the IV curve were used to calculate the activation curve. The resulting activation curve was fit with a Boltzmann equation. The $V_{0.5}$ value was -57.29 ± 1.43 mV (n=5), (Fig 1 C, red symbols). For the inactivation curve, currents from steady-state inactivation were plotted as a function of the prepulse potential and were fit with a Boltzmann equation. The $V_{0.5}$ value was -61.68 ± 2.00 mV (n=6), (Fig 1 C, green symbols). For experiments using automated patch-clamp recordings, the $V_{0.5}$ for the voltage dependence of inactivation was -60.73 ± 0.47 mV (n=4). This validation shows the same biophysical characteristics as described in the literature (Lin at al. 2016).

2.2 Pharmacological characterization

For pharmacological characterization of Nav1.9, cells were clamped at -140 mV and currents elicited by 40 ms pulses to -40 mV every 10 s. A single concentration per test item was applied per cell.
To confirm the tetrodotoxin resistance of Nav1.9 channels, a single concentration of 10 µM was tested. As expected, no significant effect was observed on Nav1.9 channels (Fig 2 A, n=3).

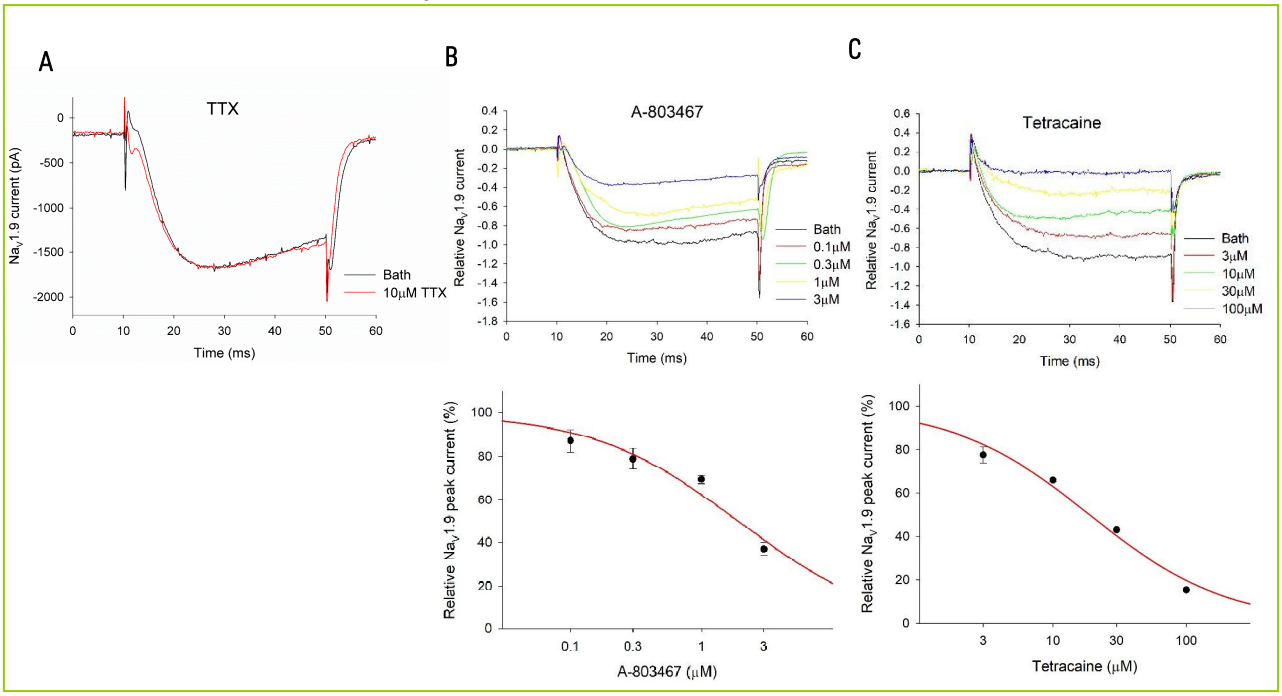

Fig. 2: Pharmacological characterization of stably transfected HEK Nav1.9 cells. A): Representative current traces recorded in the presence of 10 µM TTX. B) Representative current traces and concentration-response curve for A-803467 tested on Nav1.9, the IC_{50} was 1.91 µM (Hill coefficient 0.78). C) Representative current traces and concentration-response curve for tetracaine tested on Nav1.9, the IC_{50} was 18.82 µM (Hill coefficient 0.84). The traces were recorded at a holding potential of -140 mV

The Nav1.8 specific blocker A-803467 was tested at concentrations of 0.1, 0.3, 1 and 3 µM and blocked Nav1.9-mediated currents with an IC_{50} of 1.91 µM, Hill coefficient 0.78 (Fig 2 B, n=4).
The local anaesthetic tetracaine was tested at concentrations of 3, 10, 30 and 100 µM (n=4) and inhibited Nav1.9-mediated currents with an IC_{50} of 18.82 µM, Hill coefficient 0.84 (Fig 2 C).
For automated patch-clamp recordings the IC_{50} value for tetracaine was 27.91 µM, Hill coefficient 0.80.

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