

High Throughput Pharmacology of Na_v1.9 Channels on Nanion's SyncroPatch 384

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Cells kindly provided by Icagen, Inc., USA.



Summary

The SCN11A gene encodes the voltage-gated sodium channel Na_v1.9 which is predominantly expressed in small-diameter sensory neurons of dorsal root ganglia (DRG) and trigeminal ganglia¹⁻³. Na_v1.9 is characterized by slow activation with little depolarization near the resting membrane potential generating a persistent, tetrodotoxin (TTX)-insensitive current which inactivates only slowly². These properties suggest that the conductance mediated by Na_v1.9 mainly contributes to amplification of depolarizing responses to sub-threshold stimuli leading to lower action potential (AP) firing thresholds and increase in AP firing frequency³⁻⁵.

The role of hNa_v1.9 has yet to be fully elucidated but is proposed to be involved in nociception of inflammatory and neuropathic pain⁶. Several gain-of function mutations in SCN11A have been identified which result in either painful neuropathy⁷ or an insensitivity to pain^{8,9}. Given its proposed role in pain perception, Na_v1.9 has gained some attention as a potential target for the development of novel pain therapeutics.

Here we present high quality data with reliable pharmacology on hNa_v1.9 expressing HEK293 cells at a high throughput collected on the SyncroPatch 384. Biophysical properties of Na_v1.9 expressed in HEK cells (cells kindly provided by Icagen, Inc., USA) and concentration response curves for three Na_v channel blockers are shown, including use-dependence of tetracaine.

Results

Currents mediated by hNa_v1.9 could be reliably recorded on the SyncroPatch 384. Figure 1 shows the biophysical properties of hNa_v1.9 expressed in HEK293 cells. An average activation and inactivation IV plot is shown in panel C and D, respectively. The V_{half} of activation was -43 mV (n = 341) and for inactivation was -41 mV (n = 240), both in good agreement with literature values^{2,3,7,10,11}.

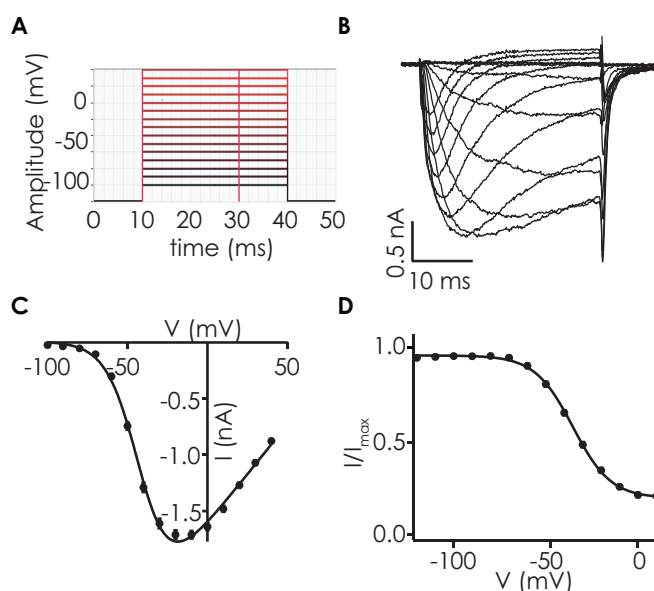


Figure 1: Biophysical properties of Na_v1.9 recorded on the SyncroPatch 384. Currents were obtained by application of a voltage step protocol from a V_{hold} of -120 mV. Voltage steps started at -100 mV to 40 mV increasing in 10 mV increments. **B** Na_v1.9-mediated current responses to the voltage step protocol shown in **A**. **C** Average current voltage plot of 341 cells. The points were fit with a Boltzmann equation and the V_{half} of activation was -43 mV. **D** Average inactivation plot for 240 cells. The points were fit with a Boltzmann equation and the V_{half} of inactivation was -41 mV.

Application Note

Pharmacology of the hNa_v1.9 channel could be performed on the SyncroPatch 384 with success rates of up to 83% for completed experiments. Figure 2 shows the concentration response curves for three Na_v channel blockers on Na_v1.9-mediated currents. In these experiments holding potential was -120 mV. The IC₅₀ values are summarised in Table 1.

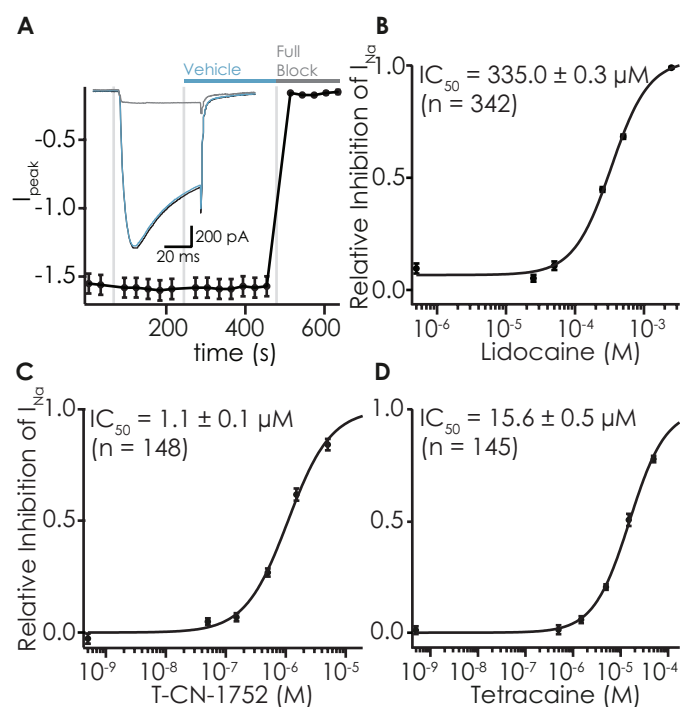


Figure 2: Inhibition of Na_v1.9 by lidocaine, T-CN-1752 and tetracaine recorded on a four-hole NPC-384 patch clamp chip **A** Na_v1.9 mediated currents were activated by depolarizing cells from -120 mV to -30 mV. Average current response after application of vehicle is indicated in blue, grey trace shows the response after 300 μM tetracaine (full block). Average current amplitudes ± SEM of 135 wells were plotted over time. **B-D** The concentration response curves for lidocaine (B), T-CN-1752 (C) and tetracaine (D) were constructed across multiple wells (single concentration per well). The SyncroPatch 384 analysis software (DataControl 384) was used to calculate the average concentration response curves, normalized to maximum block and fitted with a standard Hill-equation.

In the experiment in Figure 3, a 10-pulse voltage protocol was used to investigate use-dependence of inhibition of Na_v1.9 currents by lidocaine and tetracaine. A single concentration of each compound was applied to each well and the concentration response curve calculated across multiple wells. The current amplitude of the first, second and tenth pulses were used for analysis. The IC₅₀ for T-CN-1752 for the first pulse was 0.8 μM (dark blue),

second pulse (mid-blue) was 0.8 μM and for the tenth pulse (light blue) was 0.7 μM (n = 104). The IC₅₀ for tetracaine for the first pulse was 11.7 μM (dark blue), second pulse (mid-blue) was 8.0 μM and for the tenth pulse (light blue) was 2.7 μM (n = 100). While the concentration response curves for T-CN-1752 overlap almost exactly (Fig. 3C), the curves for tetracaine of the second and the tenth pulse are shifted to the left compared to the first pulse (Fig. 3D), showing that tetracaine exhibits use-dependence whereas T-CN-1752 does not. The success rate for completed experiments was around 80%.

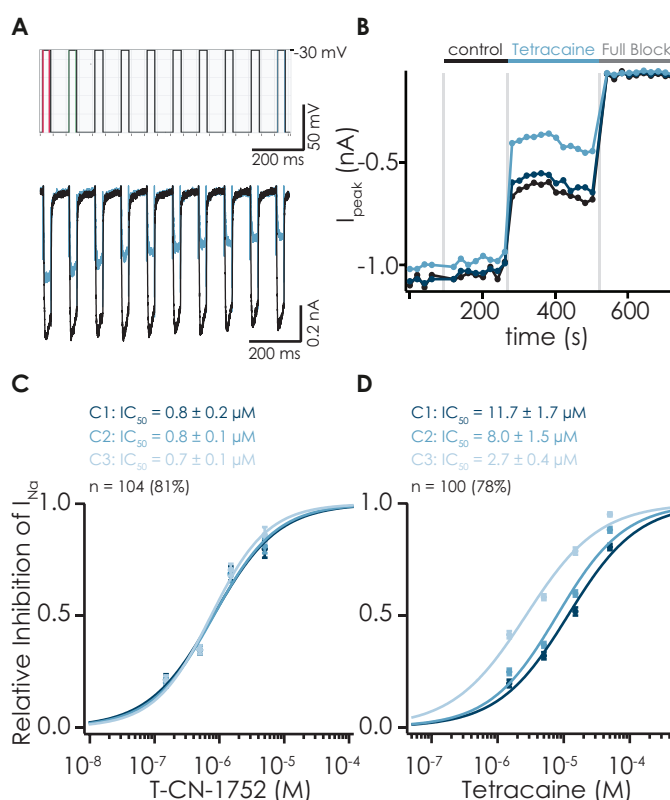


Figure 3: Effect of high frequent stimulation on potency of tetracaine block of Na_v1.9. **A** Current response to a 10-pulse voltage protocol from one exemplar cell expressing hNa_v1.9 recorded on the SyncroPatch 384. The responses are shown in the presence of control solution (black trace) and 5 μM tetracaine (blue trace). The peak current responses of the 1st, 2nd and 10th pulses were used for analysis to investigate use-dependence. In this experiment, each well received a single concentration of tetracaine or T-CN-1752 and the concentration response curve was calculated across multiple wells. **B** Current amplitude of the 1st (black), 2nd (dark blue) and 10th (light blue) peaks plotted against time. **C, D** Concentration response curves for T-CN-1752 (C) and tetracaine (D) for the 1st, 2nd and 10th pulses.

Application Note

384 color coded depictions of data traces eases judgement of success rate

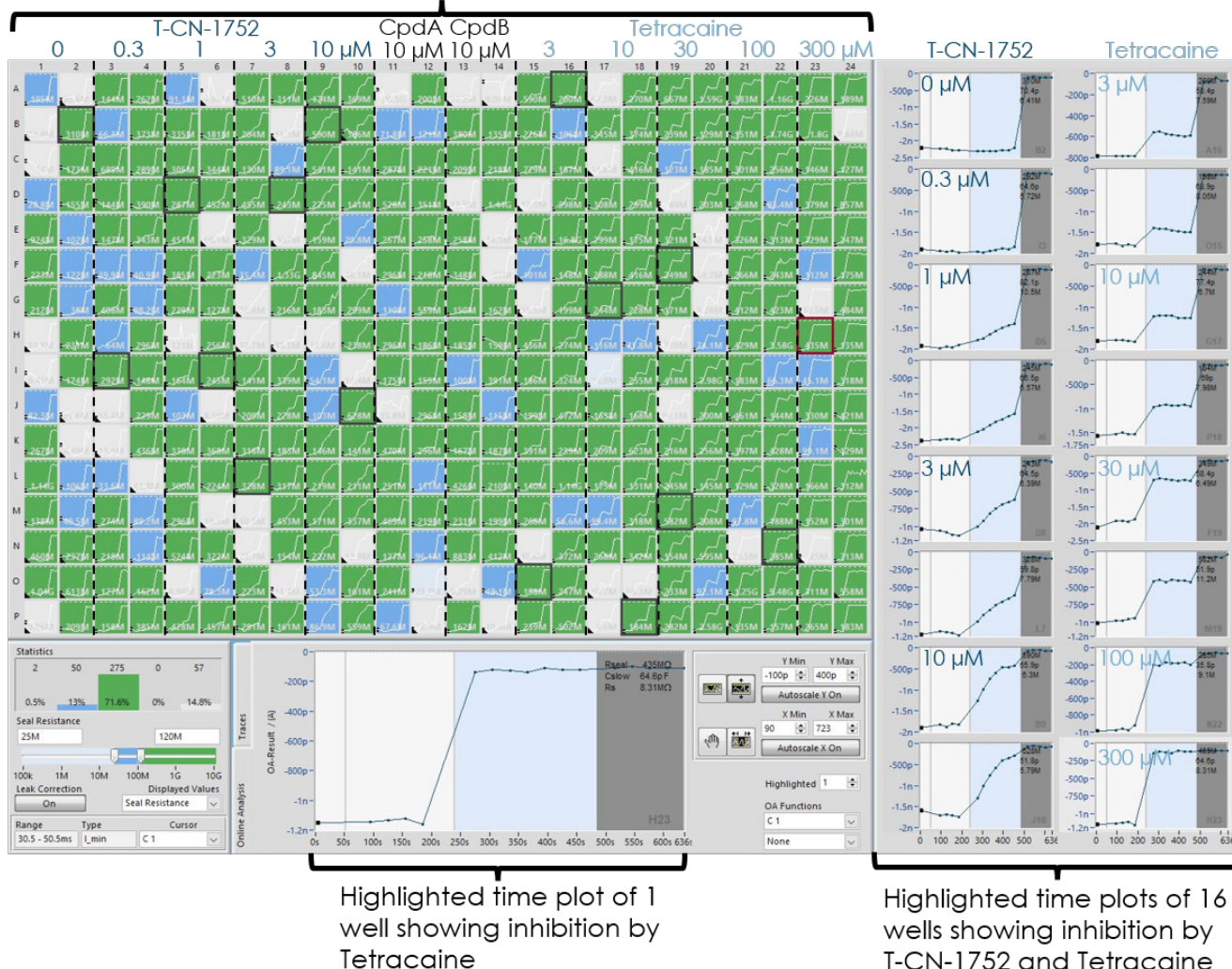


Figure 4: Graphical user interface of the screening and data analysis software used on the SyncroPatch 384. Screenshot of depiction of online analysis data of hNa_v1.9 expressing HEK293 cells as recorded on a multi-hole (4x) NPC-384 patch clamp chip. Three hundred and eighty-four small color-coded pictures as seen in the upper left part display 384 recordings. Depending on the seal resistance, pictures are green (R_{memb} > 120 MΩ), blue (R_{memb} = 25 – 120 MΩ), light blue or grey (R_{memb} < 25 MΩ or cells disabled). One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. Graphs show current amplitudes of Na_v1.9 channels during the voltage step to -30mV during application of control solution (white region), compound (blue region) and full block by 300 µM tetracaine (dark grey region).

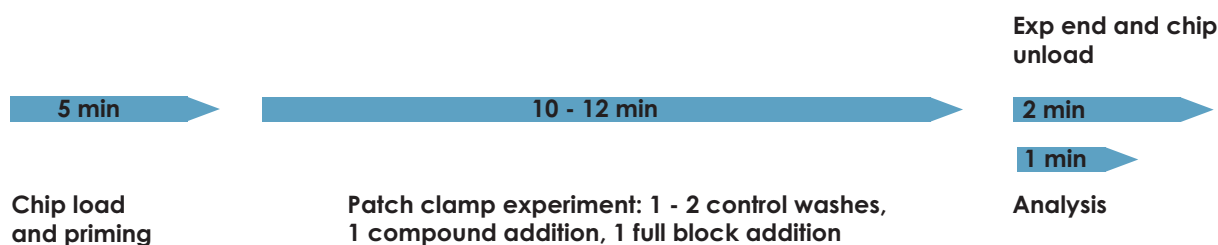


Figure 5: Timeline of an experiment on the SyncroPatch 384. The completion of one experiment on the SyncroPatch 384 patch clamp chip (384 wells) for a single point or cumulative concentration response curve on Na_v1.9-mediated currents took approximately 17-20 min.

Application Note

Compound	IC ₅₀ (μM)	Success rate (%)	Literature value (μM)
T-CN-1752	1.1 (148)	76	1.6 ¹⁰
Tetracaine	15.6 (145)	69	32 ¹⁰
Lidocaine	335 (342)	89	356 ¹⁰

Table 1: IC₅₀ values for T-CN-1752, tetracaine and lidocaine on Na_v1.9-mediated currents recorded on the SyncroPatch 384. Shown are IC₅₀ values (number of wells shown in brackets), success rate for completed experiments and the expected literature IC₅₀ values.

Figure 4 shows a screenshot of the SyncroPatch 384 software during an experiment. A color-coded overview (based on seal resistance in this case) of all 384 wells gives the user a good impression of the success rate of the experiment. The user can choose whether to visualize raw traces or online analysis. Here, online analysis is chosen and the graphs represent current amplitude vs. time. An individual well can be highlighted to monitor progression of the experiment. In the Online Analysis view, the time points at which solution additions have been made are indicated by

vertical lines, as well as different background colors. In this example, white shows incubation in control solution, blue is different concentrations of T-CN-1752 or tetracaine and grey is full block with 300 μM tetracaine.

In conclusion, hNa_v1.9 expressed in HEK293 cells can be recorded on the SyncroPatch 384 with high success rates for completed experiments (>70%). The IC₅₀s of three Na_v channel blockers were in good agreement the literature¹⁰. Furthermore, we observed use-dependent block of tetracaine on Na_v1.9.

The SyncroPatch 384 is a high throughput and highly reliable automated patch clamp device for recording Na_v1.9 currents. User-friendly software, excellent success rates, single additions or multiple additions of compound to each cell, and easy analysis result in reliable high quality data at an increased throughput with an economical cost per data point.

References

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Methods

Cells

HEK293 cells expressing hNa_v1.9 kindly provided by IcaGen, Inc.

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch 384 and multihole (4x) chips. A voltage step protocol from a holding potential of -120 mV to -30 mV was applied to the cells every 20 s for pharmacology experiments. Peak amplitude at -30 mV was examined for analysis (unless otherwise indicated).