

Characterization of hNa_v1.7 on Nanion's SyncroPatch® 384PE

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Summary

The Na_v1.7 gene (SCN9A) encodes a voltage-gated sodium (Na_v) channel, primarily expressed in the peripheral nervous system. It has been isolated from rat dorsal root ganglion (DRG) neurons¹, human medullary thyroid cancer cells (hNE-Na)² and PC12 cells^{3,4}.

Different Na_v channels play a key role in modulation of action potentials in the central and peripheral nervous systems. In particular, the fast upstroke of the action potential is mediated by Na_v channels. Na_v channels are in part characterized by their TTX-sensitivity (TTX-resistant [TTXr], TTX-sensitive [TTXs]). Na_v1.7 is a TTXs channel and is sensitive to TTX in the nanomolar range^{1,2}. The role of hNa_v1.7 has yet to be fully elucidated but is proposed to play an important part in nociception and pain sensing. Na_v1.7 has been implicated to play a role in disease pain states, in particular inflammatory pain⁵ and hypersensitivity to heat following burn injury⁶. Common to many of the voltage-gated ion channels, a number of compounds exhibit both state- and use-dependence. For this reason, it is important to be able to reliably measure the effects of compounds using different voltage protocols to investigate state and use-dependency.

In this Application Note we present data using the SyncroPatch® 384PE characterizing CHO cells stably expressing hNa_v1.7. The current-voltage relationship and the state- and use-dependence effects of the sodium channel blocker, tetracaine, are shown.

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Results

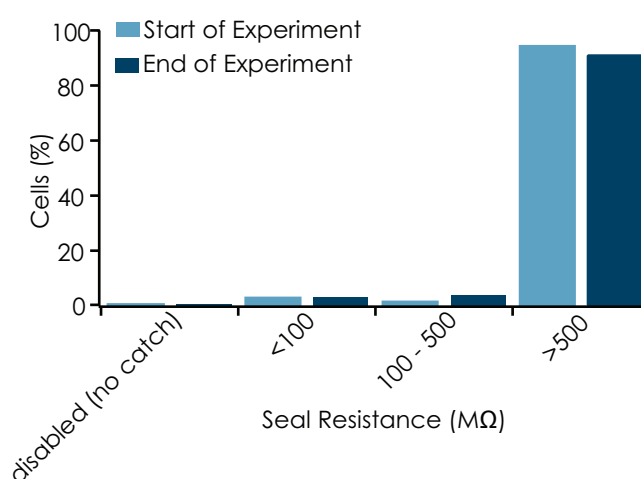
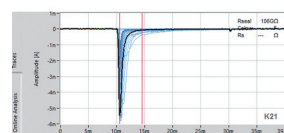


Figure 1:

Success rate (seal resistance) of CHO cells expressing hNa_v1.7 on the SyncroPatch® 384PE. Shown is a bar graph of seal resistances on the SyncroPatch® 384PE at the start (light blue) and end (dark blue) of the experiment. Over 95% of the cells had a seal resistance >500 MΩ at the start of the experiment and 91% at the end of the experiment.

A



B

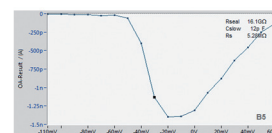


Figure 2:

A Raw traces from an exemplar cell expressing hNa_v1.7 recorded on the SyncroPatch® 384PE. Shown are current responses to increasing voltage steps from -70 to +50 mV. **B** Current-voltage plot for an example cell.

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Figure 1 shows seal resistance values for CHO cells expressing hNa_v1.7 recorded on the SyncroPatch® 384PE. The percentage of cells which had a seal resistance >500 MΩ was 95% at the start of the experiment and 91% at the end of the experiment. In this experiment, 356 cells were used for a pharmacological experiment resulting in a success rate of 92% for completed experiments. Figure 2 shows current responses to increasing voltage steps for an exemplar CHO cell expressing Na_v1.7 and the corresponding current-voltage plot. Na_v1.7 currents started to activate at about -50 mV and peak response was elicited at around -20 mV.

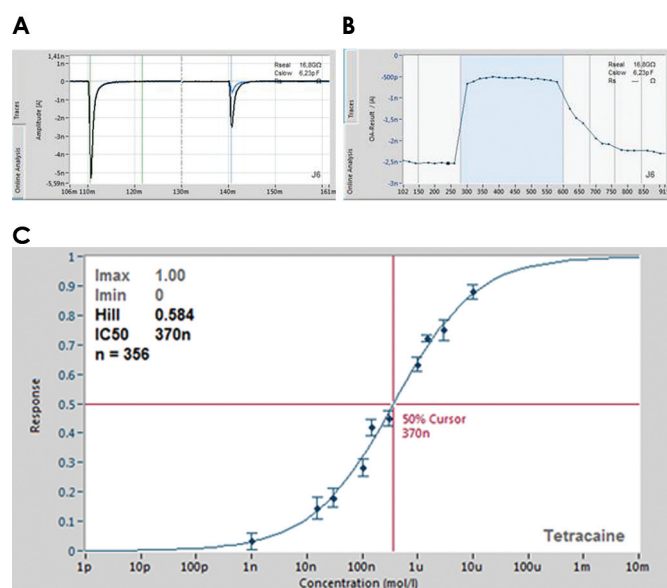


Figure 3:
A Current traces of an exemplar cell using an inactivation protocol to inactivate 70% of channels in the 2nd peak. **B** Online analysis showing peak current of the 2nd peak versus time. Blue area indicates application of tetracaine. **C** Concentration response curve for tetracaine for an average of 356 cells using a voltage protocol where 70% of Na_v1.7 channels are inactivated. The IC₅₀ for tetracaine on the inactivated state of the receptor is 370 nM (n = 356).

Figure 3 shows a recording from an exemplar cell

using an inactivation voltage protocol. Tetracaine had a much larger effect on the 2nd (70% inactivated channels) peak shown in Panel A as compared with the first peak. This indicates that tetracaine exhibits a preference for the inactivated state of Na_v1.7 which is confirmed by the concentration response curve. Tetracaine exhibits a much higher affinity for the inactivated state of the receptor (IC₅₀ = 370 nM, n = 356) compared with the open state (IC₅₀ = 41.8 μM, n = 355, Figure 5, 1st pulse). In this experiment a control wash step is followed by addition of a single concentration of tetracaine and the concentration response curve is calculated across the whole plate. Additionally, after tetracaine incubation, control solution is added in several wash steps and the effect of tetracaine could be completely reversed.

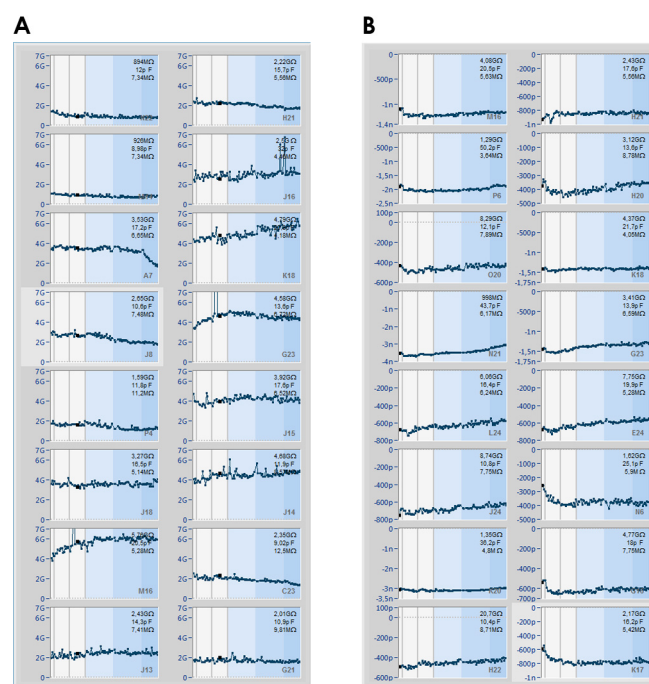


Figure 4:
A Seal stability and **B** current stability for a representative 16 cells during a recording of CHO cells expressing hNa_v1.7 recorded on the SyncroPatch® 384PE during an inactivation experiment. Shown is seal resistance (A) or current amplitude (B) versus time during a cumulative (3-point concentration response curve (in this case 0.1% DMSO was added three times as a control). The seal resistance and current amplitude were stable over the entire 18 minute recording.

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Figure 4 shows seal stability and current stability over the course of a 15 minute experiment. The current response is shown during application of control (0.1% DMSO) solution during an inactivation experiment. Both seal resistance and current amplitude were stable over the entire 15 minute recording.

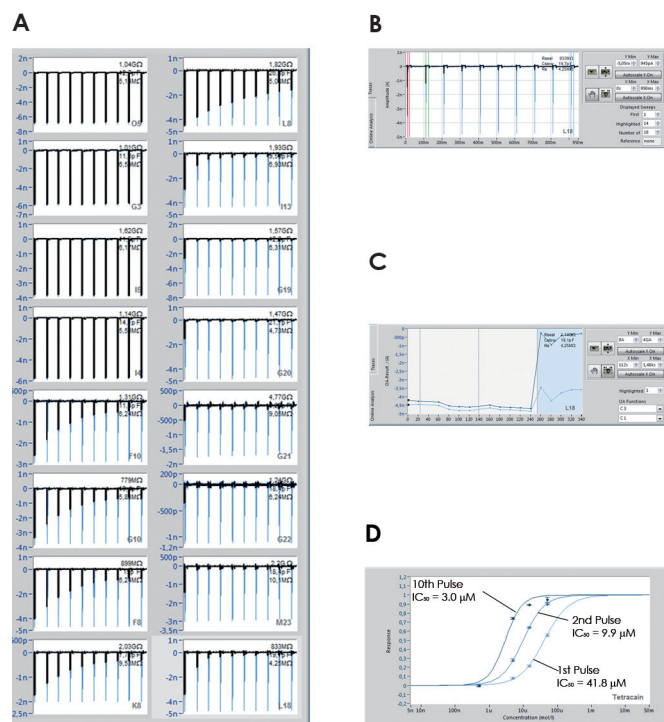


Figure 5:

A Current responses to a 10-pulse voltage protocol from 16 exemplar cells expressing $\text{Na}_v1.7$ recorded on the SyncroPatch® 384PE. The responses are in the presence of control solution (top left), 5 μM tetracaine (bottom left), 15 μM tetracaine (top right) or 50 μM tetracaine (bottom right). The peak current responses of the 1st, 2nd and 10th pulses were used for analysis to investigate use-dependence. In this experiment, each cell received a single concentration of tetracaine and the concentration response curve was calculated across the whole plate. **B** Responses to a 10-pulse voltage protocol of an exemplar cell in the presence of 15 μM tetracaine. **C** Current amplitude of the 1st (light blue) and 10th (dark blue) peaks plotted against time. **D** Concentration response curves to tetracaine for the 1st, 2nd and 10th pulses. Average IC_{50} 's are given.

Figure 5 shows current responses of 16 cells to a 10-pulse voltage protocol and inhibition of $\text{Na}_v1.7$ currents by

tetracaine. In this experiment, a single concentration of tetracaine was applied to each cell and the concentration response curve calculated across the whole plate. The current amplitude of the 1st, 2nd and 10th pulses were used for analysis. The IC_{50} for tetracaine for the 1st pulse was 41.8 μM (light blue), 2nd pulse (mid-blue) was 9.9 μM and for the 10th pulse (dark blue) was 3.0 μM ($n = 355$). The concentration response curve of the 2nd and 10th pulses is shifted to the left compared with the first pulse showing that tetracaine exhibits use-dependence. The success rate for completed experiments was 92%.

Figure 6 shows a screenshot of the SyncroPatch® 384PE software (PatchControl 384) 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. In this case, online analysis is chosen and the graphs represent current amplitude of the 1st (light blue) and 10th (dark blue) voltage pulse plotted against time. An individual well can be highlighted to monitor the progression of the experiment. The online analysis shows the timepoint at which either control solution (white) or tetracaine was applied (blue).

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

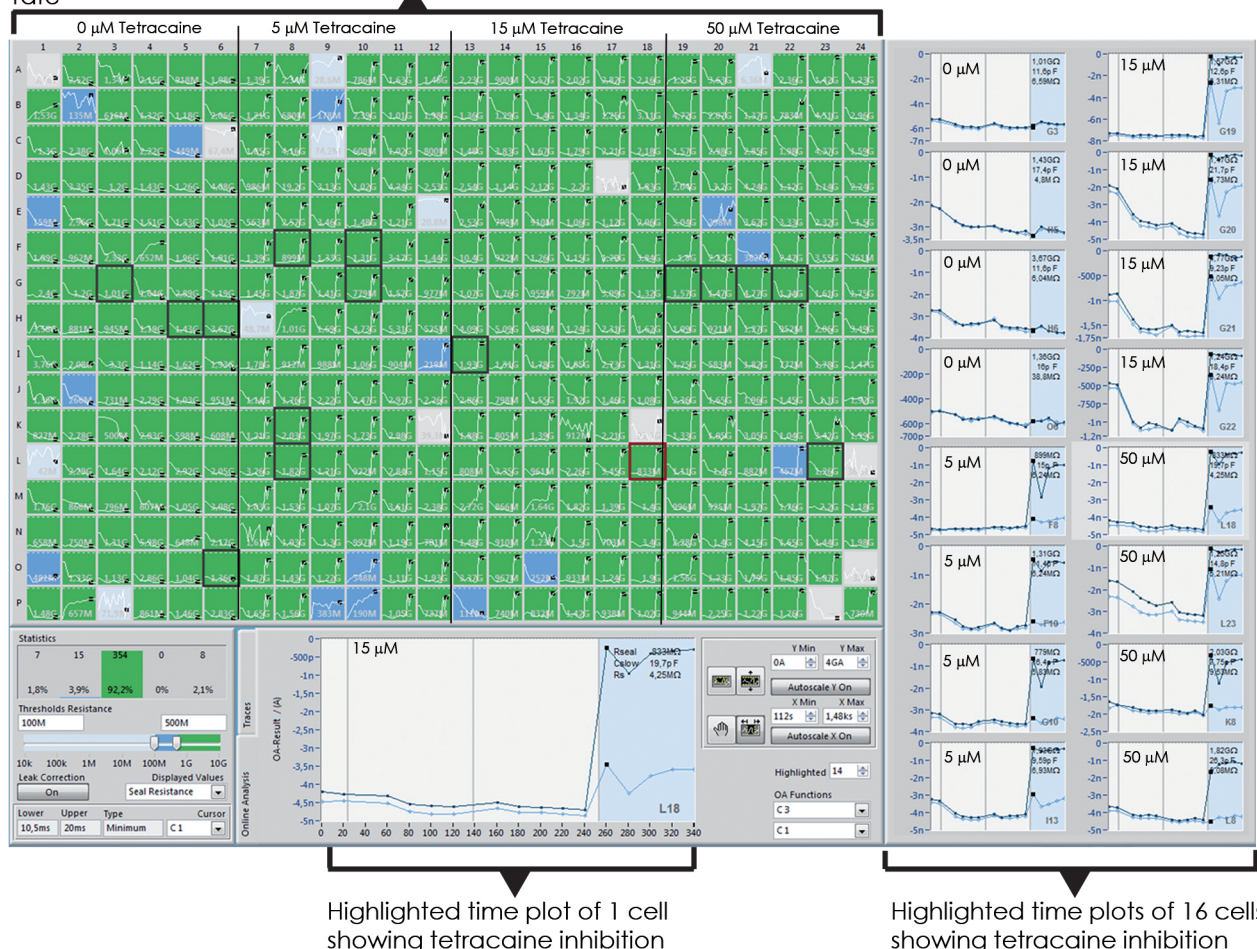


Figure 6:

Graphical user interface of the screening and data analysis software used on the SyncroPatch® 384PE. Screenshot of depiction of online analysis data of Na_v1.7 expressing CHO cells as recorded on one 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_{seal} > 500 MΩ), blue (R_{seal} = 100–500 MΩ), light blue or grey (R_{seal} < 100 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 the 1st (light blue) and 10th (dark blue) pulse of a 10-pulse voltage protocol plotted against time of individual cells in control solution and then differing concentrations of tetracaine, 0, 5, 15 or 50 μM. Two wash steps with control solution prior to compound application were performed. For highlighted experiments, white indicates the wash solution and blue indicates the presence of tetracaine.

Exp end and chip unload

5 min

8 - 12 min

2 min

1 min

Chip load and priming

Patch clamp experiment: 1 - 2 control washes + 3 compound concentrations

Analysis

Figure 7:

The completion of 1 experiment on the SyncroPatch® 384 patch clamp chip (384 wells) for a 3-point concentration response curve plus 1 - 2 control washes at the beginning of the experiment on Na_v1.7 took approximately 15 min.

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Figure 7 offers a visual representation of a typical experiment on the SyncroPatch® 384PE, from loading of the chip and priming of solutions, execution of the patch clamp experiment and analysis of the data. Experiments can be performed either as a single point concentration response curve where a single concentration of compound is applied to each cell and the concentration response curve calculated across the whole plate, or cumulative concentration response curves can be performed on each cell. A complete experiment which includes 1 - 2 control applications, followed by 3 concentrations of compound takes approximately 15 minutes. The SyncroPatch® 384PE generates a throughput of 20,000 data points per day with a competitive consumable cost per data point of ~ €0.20.

In conclusion, hNa_v1.7 expressed in CHO cells can be recorded on the SyncroPatch® 384PE with a high success rate (> 90% for completed experiments). The SyncroPatch® 384PE is a high throughput and highly reliable automated patch clamp device for recording Na_v1.7 and can be used to test compounds for state- and use-dependence. User-friendly software, excellent success rates, multiple additions of compound to each cell and easy analysis result in high quality, reliable data at a high throughput with an economical cost per data point.

References

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Methods

Cells

CHO cells stably expressing hNa_v1.7 were supplied by Anaxon.

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® 384PE. Current-voltage recordings were made using voltage steps from -70 mV to 50 mV for 20 ms increasing in 5 mV steps, from a holding potential of -120 mV (leak subtraction protocol used). Pharmacology experiments used voltage step protocols to mimic state- and use-dependence.