Application Note

Channel: $hNa_V1.7$
Cells: CHO
Tools: SyncroPatch® 384PE

Characterization of $hNa_V1.7$ on Nanion's SyncroPatch® 384PE

The electrophysiology team at Nanion Technologies GmbH, Munich, Germany.
Cells were kindly provided by Anaxon.

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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 and has been isolated from rat dorsal root ganglion (DRG) neurons[1], 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 role in nociception and pain sensing. $Na_V1.7$ has been implicated to play a role in disease pain states, in particular inflammatory pain[5] and hypersensitivity to heat following burn injury[6]. 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 properties of the sodium channel blocker, tetracaine, are shown.

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Results

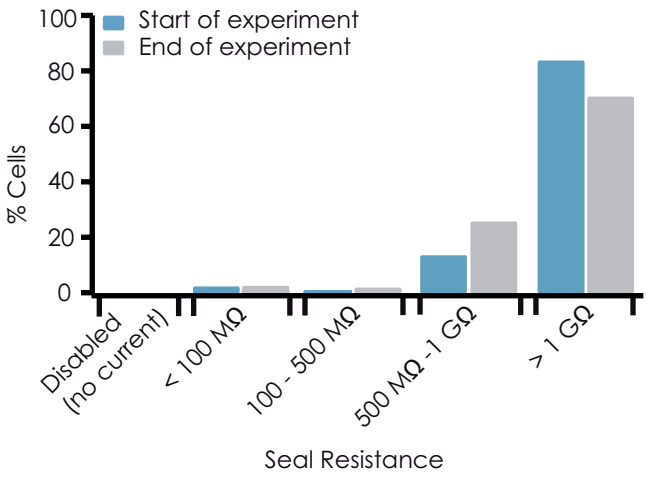

Figure 1:
Success rate (seal resistance) of CHO cells expressing $hNa_V1.7$ on the SyncroPatch® 384PE. Shown is a bar graph of seal resistances on the SyncroPatch® 384PE at the start (blue) and end (grey) of the experiment. Over 80% of the cells had a seal resistance >1 GΩ at the start of the experiment.

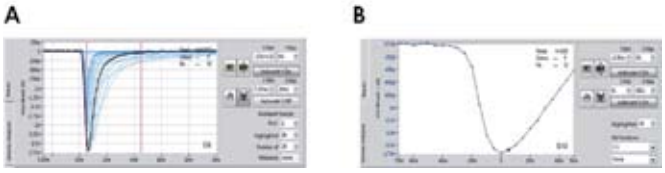

Figure 2:
A Raw traces from an exemplar cell expressing $hNa_V1.7$ recorded on the SyncroPatch® 384PE. Shown are current responses to increasing voltage steps from -70 to +50 mV. **B** Current-voltage plot for the cell shown in Panel A.

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Figure 1 shows seal resistance values for CHO cells expressing hNa$_V$1.7 recorded on the SyncroPatch® 384PE. The percentage of cells which had a seal resistance >1GΩ was 84% at the start of the experiment and 71% at the end of the experiment. In this experiment, 356 cells were used for a pharmacological experiment resulting in a success rate of 92% for completed experiments. Figure 2 shows current responses to increasing voltage steps for an exemplar CHO cell expressing Na$_V$1.7 and the corresponding current-voltage plot for this cell. Na$_V$1.7 currents started to activate at about -35 mV and peak response was elicited at around 0 mV.

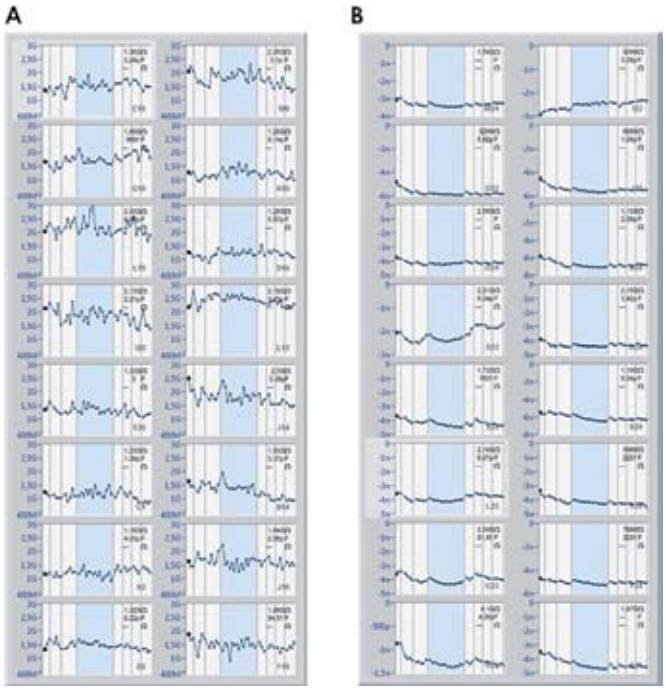

Figure 3:
A Seal stability and **B** current stability for a representative 16 cells during a recording of CHO cells expressing hNa$_V$1.7 recorded on the SyncroPatch® 384PE during an inactivation experiment. Shown is seal resistance (A) or current amplitude (B) versus time during a single point concentration response curve (in this case 0.1% DMSO was added as a control). The seal resistance and current amplitude were stable over the entire 15 minute recording.

Figure 3 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.

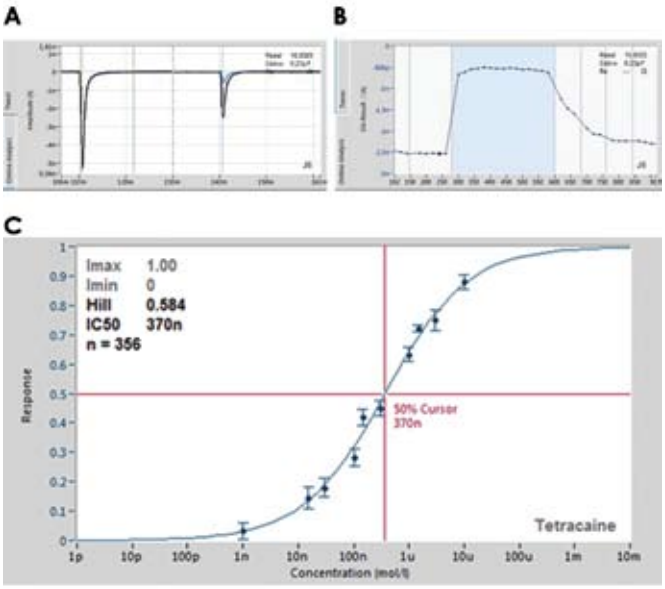

Figure 4:
A Current traces of an exemplar cell using an inactivation protocol to inactivate 70% of channels in the 2nd peak. **B** Online analysis showing peak current of the 2nd peak versus time. Blue area indicates application of tetracaine. **C** Concentration response curve for tetracaine for an average of 356 cells using a voltage protocol where 70% of Na$_V$1.7 channels are inactivated. The IC$_{50}$ for tetracaine on the inactivated state of the receptor is 370 nM (n = 356).

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Figure 4 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 (IC_{50} = 370 nM, n = 356) compared with the open state (IC_{50} = 41.8 µM, n = 355, Figure 5, 1st pulse).

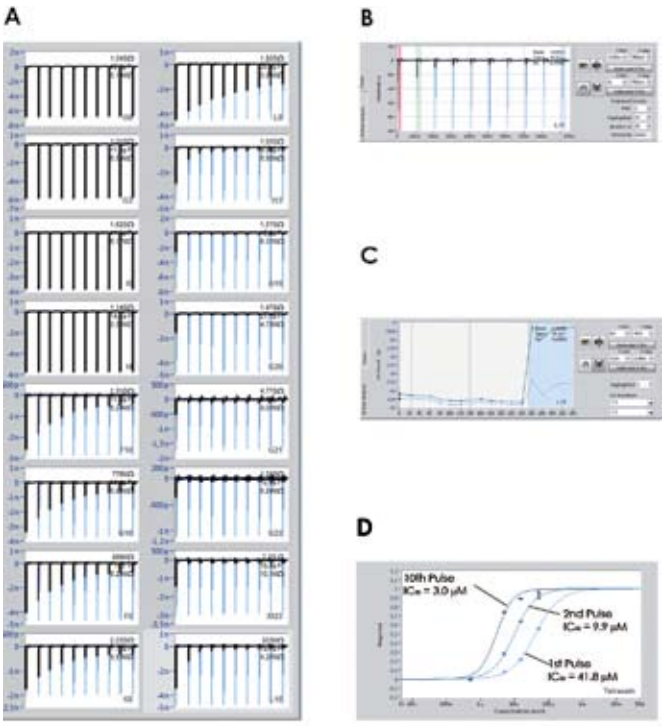

Figure 5:
A Current responses to a 10-pulse voltage protocol from 16 exemplar cells expressing $Na_V1.7$ recorded on the SyncroPatch® 384PE. The responses are in the presence of control solution (top left), 5 µM tetracaine (bottom left), 15 µM tetracaine (top right) or 50 µM tetracaine (bottom right). 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 and 10th 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 $Na_V1.7$ 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, 2nd pulse was 9.9 µM and for the 10th pulse 3.0 µM (n = 355) 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 and 10th 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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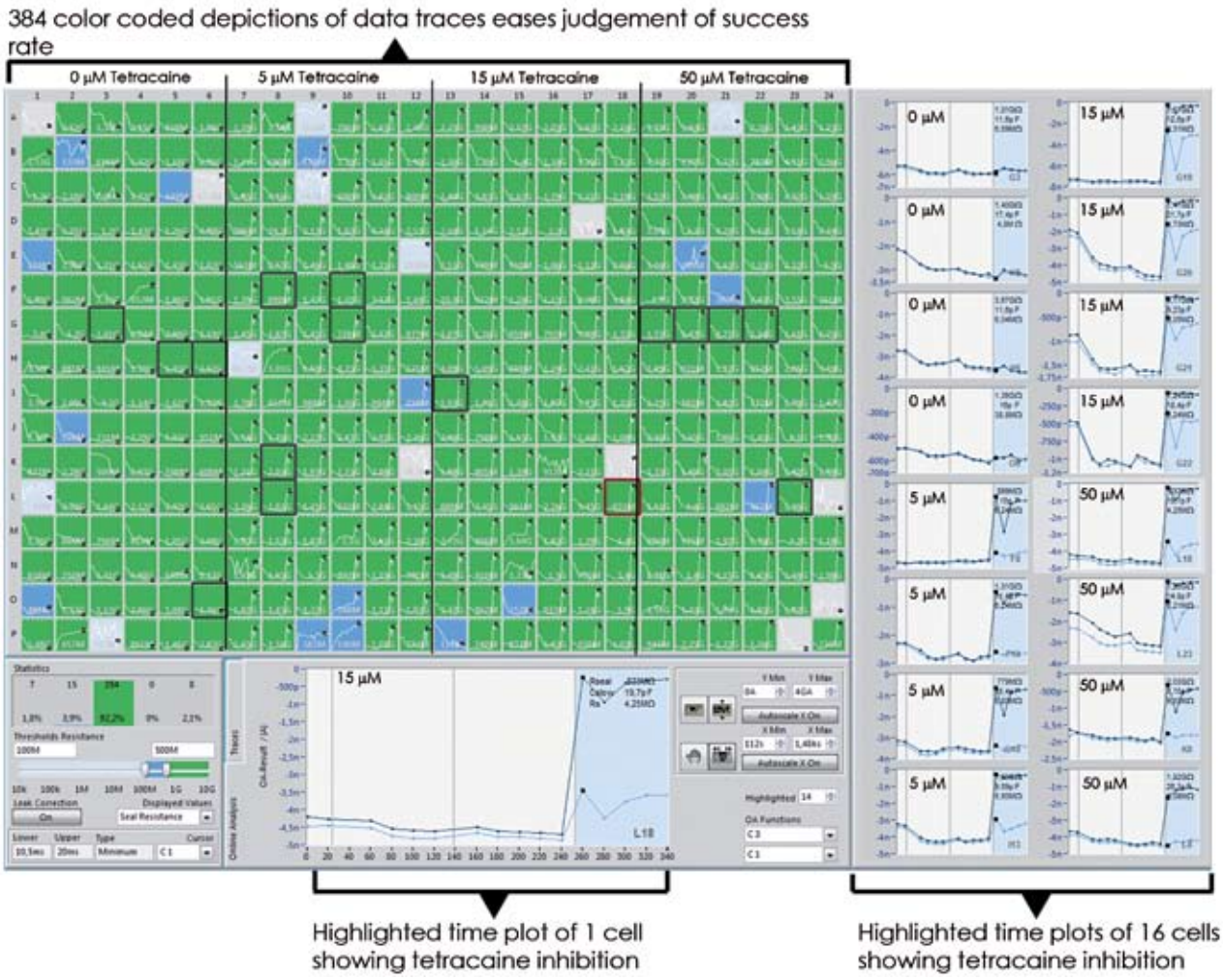

Figure 6:
Graphical user interface of the screening and data analysis software used on the SyncroPatch® 384PE. Screenshot of depiction of online analysis data of $Na_V1.7$ expressing CHO cells as recorded on one NPC-384 patch clamp chip. Three hundred and eighty-four small color-coded pictures as seen in the upper left part display 384 recordings. Depending on the seal resistance, pictures are green (Rmemb > 500 MΩ), blue (Rmemb = 100–500 MΩ), light blue or grey (Rmemb < 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 and 10th 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.

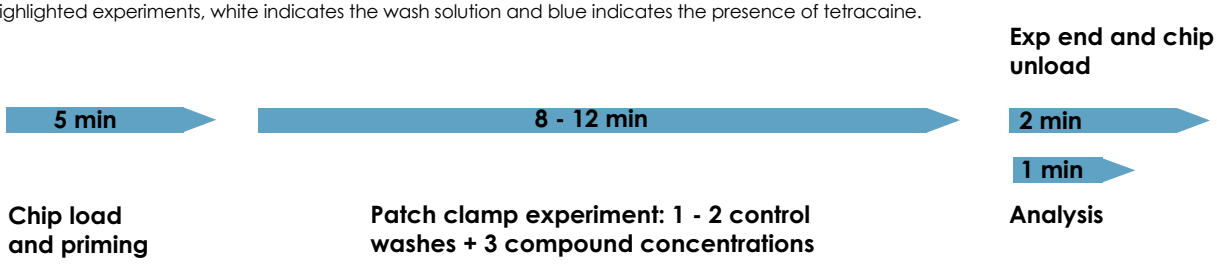

Figure 7:
The completion of 1 experiment on the SyncroPatch® 384 patch clamp chip (384 wells) for a 3-point concentration response curve plus 1 - 2 control washes at the beginning of the experiment on $Na_V1.7$ took approximately 15 min.

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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.

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