Application Note

Channel: $rNa_V1.8$
Cells: ND7-23
Tools: SyncroPatch®96

Characterization of $rNa_V1.8$ (ND7-23) on Nanion's SyncroPatch®96

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Cells were supplied by Millipore, USA.

Summary

The $Na_V1.8$ gene (originally named PN3 or SNS; gene symbol SCN10A) encodes a voltage-gated sodium (Na_V) channel, selectively expressed in dorsal root ganglion (DRG) neurons. DRGs transmit peripheral stimuli to the central nervous system and are involved in nociception.

Different Na_V channels play a key role in modulation of DRG action potentials. 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]). $Na_V1.8$ is a TTXr channel. Compared with other Na_V channels, $Na_V1.8$ has slow activation and inactivation kinetics and is opened at relatively high voltages[1]. It is an interesting drug target for inflammatory and neuropathic pain, because modulation of $Na_V1.8$ by inflammatory mediators seems to be a key mechanism of DRG nociceptor sensitization and activation[2]. Interestingly, $Na_V1.8$ has been reported to play an important role in the perception of cold pain[3].

In this Application Note we present data from 1 exemplary run (96-well plate) on the SyncroPatch®96 characterizing ND7-23 cells (a rat DRG/mouse neuroblastoma hybrid) stably transfected with rat $Na_V1.8$. All experiments were performed in the presence of 100 nM TTX to block the endogenous TTXs Na^+ current present in these cells. The $Na_V1.8$ activation and inactivation properties, tetracaine and lidocaine sensitivities recorded on the SyncroPatch®96 were consistent with those reported in the literature[1-7].

Results

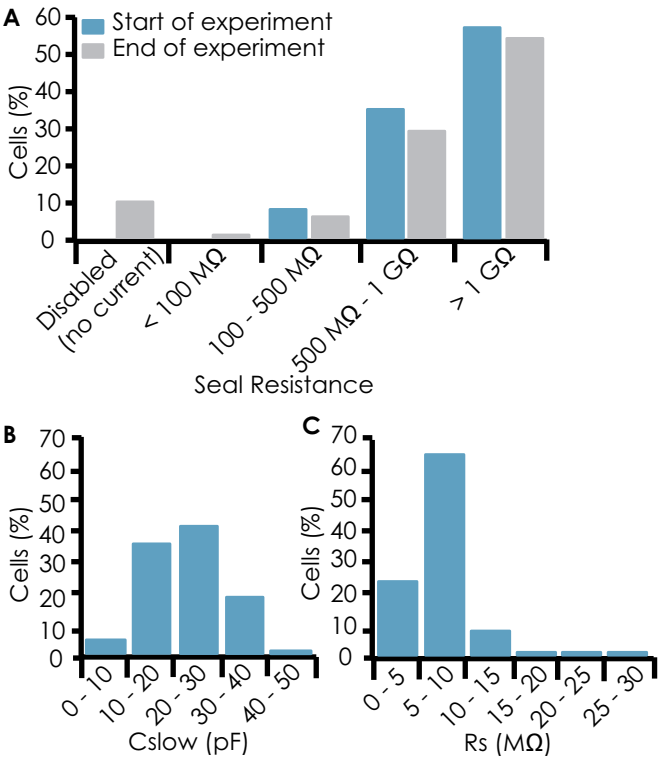

Figure 1:
A Success rate (seal resistance) of ND7-23 cells on the SyncroPatch®96. Shown is a bar graph of seal resistances on the SyncroPatch®96 at the start (blue) and end (grey) of the experiment. **B** Bar graph of cell capacitance (Cslow) of ND7-23 cells. Mean Cslow = 22.6 ± 0.8 pF (n = 88). **C** Bar graph of series resistance (Rs) values for ND7-23 cells on the SyncroPatch®96. Mean Rs = 7.1 ± 0.4 MΩ (n = 88).

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Figure 1 shows seal resistance, Cslow and series resistance values for ND7-23 cells recorded on the SyncroPatch®96. The percentage of cells which had a seal resistance > 500 MΩ was 92% at the start of the experiment and 83% at the end of the experiment. A total of 86 cells were recorded during 1 run, Figure 2 shows current responses to increasing voltage steps for an exemplar ND7-23 cell expressing $Na_V1.8$ and the corresponding activation and inactivation curves for an average of 32 cells. $Na_V1.8$ currents started to activate at about -30 mV, peak response was elicited between 20 and 30 mV and Vhalf of activation was 12 mV. The Vhalf of inactivation was -27 mV in good agreement with the literature[5,6].

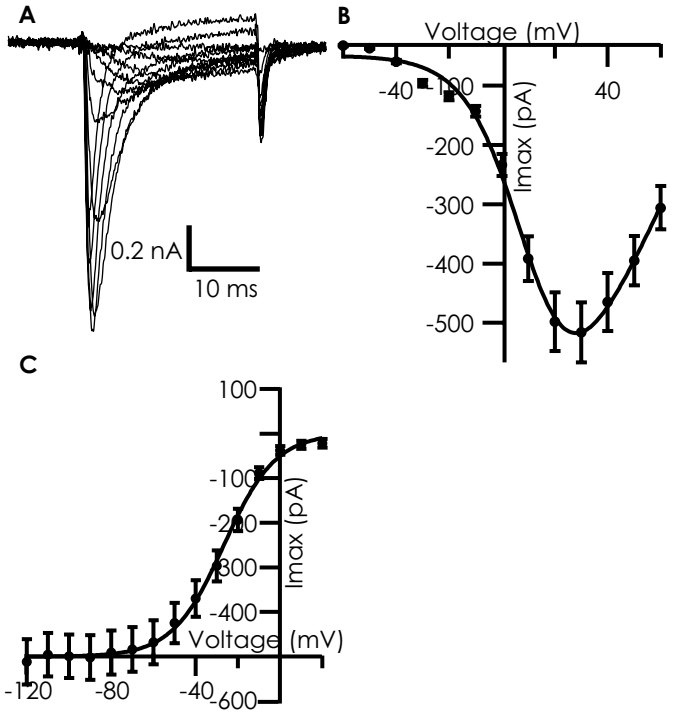

Figure 2:
A Raw traces from an exemplar cell recorded on the SyncroPatch®96. Shown are current responses to increasing voltage steps from -60 to +60 mV. **B** Average current-voltage plot, Vhalf of activation was 12 mV (n = 32). **C** Average inactivation plot, Vhalf of inactivation was -27 mV (n = 32).

Figure 3 shows current responses to a single step protocol to 20 mV and inhibition of the $Na_V1.8$ current by increasing concentrations of lidocaine. The corresponding concentration response curve is also shown revealing an IC_{50} for lidocaine of 178 ± 11 µM (n = 35) in good agreement with the literature[6] (holding potential -120 mV). Figure 4 shows inhibition of $Na_V1.8$ currents by increasing concentrations of tetracaine. The IC_{50} for tetracaine was 71 ± 5 µM (n = 40) in good agreement with the literature[7] (holding potential -120 mV). In both cases (lidocaine and tetracaine) full washout was achieved and currents were fully recovered upon removal of the drug.

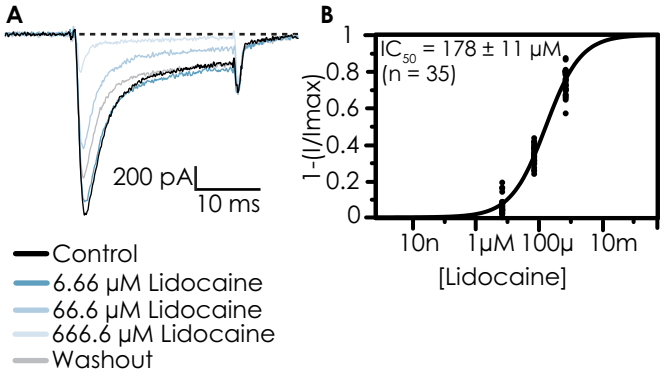

Figure 3:
A Raw traces from an exemplar cell recorded on the SyncroPatch®96 showing inhibition of current by increasing concentrations of lidocaine. Shown are current responses to a single step protocol to 20 mV for 25 ms from a holding potential of -120 mV. Current amplitude was completely recovered upon washout of lidocaine. **B** Average concentration response curve for lidocaine, IC_{50} = 178 ± 11 µM (n = 35).

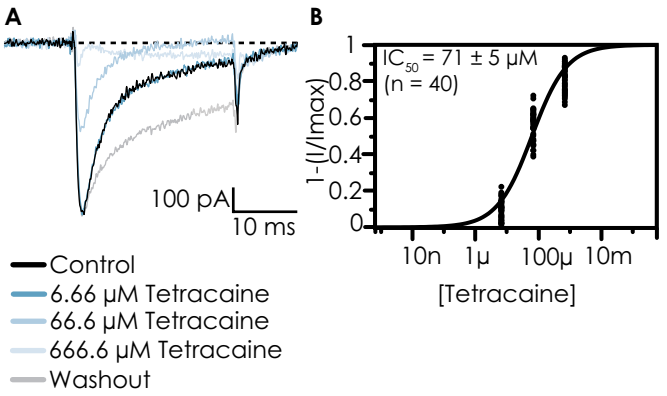

Figure 4:
A Raw traces from an exemplar cell recorded on the SyncroPatch®96 showing inhibition of current by increasing concentrations of tetracaine. Shown are current responses to a single step protocol to 20 mV for 25 ms from a holding potential of -120 mV. Current amplitude was completely recovered upon washout of tetracaine. **B** Average concentration response curve for tetracaine, IC_{50} = 71 ± 5 µM (n = 40).

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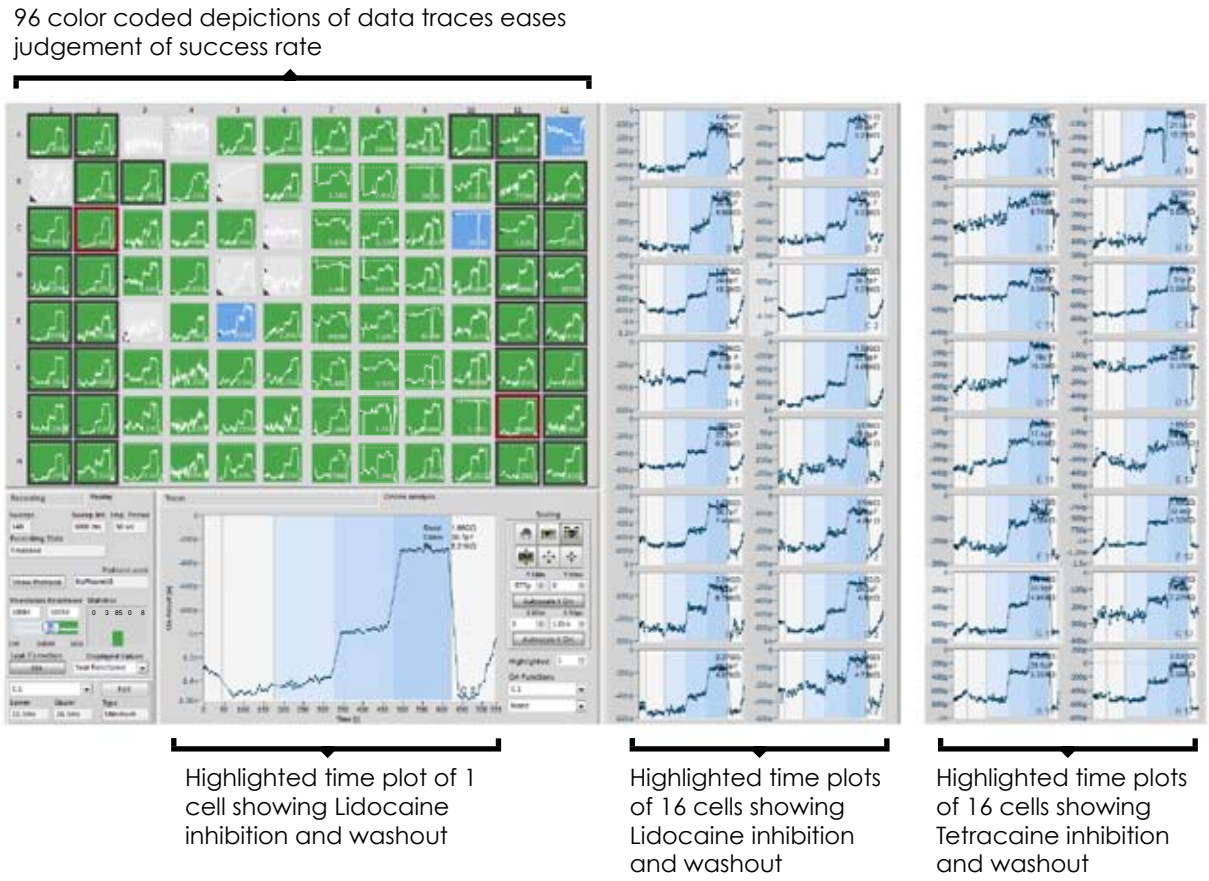

Figure 5:
Graphical user interface of the screening and data analysis software used on the SyncroPatch 96. Screenshot of depiction of online analysis data of $Na_V1.8$ expressing cells as recorded on one NPC-96 patch clamp chip. Ninety-six small color-coded pictures as seen in the upper left part display 96 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 below, 16 other selected experiments are displayed on the right. Graphs show current amplitudes plotted against time of individual cells which were inhibited by three lidocaine (left half of the 96-well plate) or tetracaine (right half; excerpt of only the 16 highlighted traces) concentrations (6.66, 66.6, and 666.6 µM). One wash step with control solution prior to compound application and one washout step after the application of all concentrations was performed. For highlighted experiments, note the different shades of blue overlaid on the curves, representing the three increasing concentrations. The white color represents the wash steps.

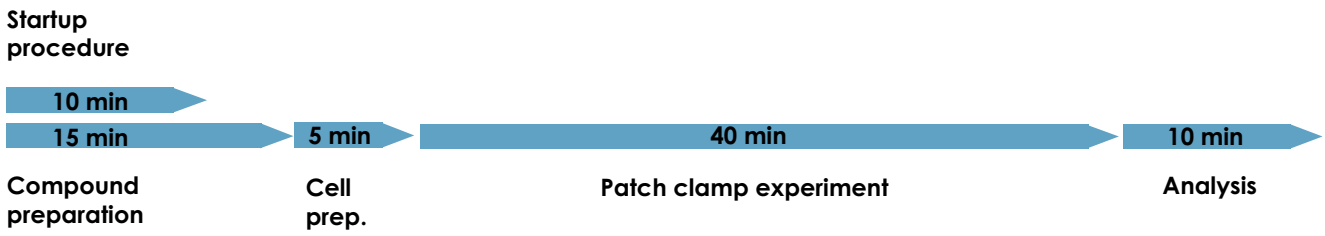

Figure 6:
The completion of 1 experiment on the SyncroPatch®96 patch clamp chip (96 wells) for a 3 point concentration response curve plus control and washout at the end of the experiment on $Na_V1.8$ took 70 mins. In this time we completed 75 concentration response curves giving a total of 300 data points (including control) in 70 mins at a consumable cost of < $0.75 per data point.

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Figure 5 shows a screenshot of the SyncroPatch®96 software during an experiment. A color-coded overview (based on seal resistance in this case) of all 96 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 plotted against time. An individual well can be highlighted to monitor the progression of the experiment. The online analysis shows the timepoint at which the different concentrations of compound were applied, indicated by the different shades of blue.

Figure 6 offers a visual representation of a typical experiment on the SyncroPatch®96, from startup of the system, execution of the patch clamp experiment and analysis of the data. In the case of the ND7-23 cells, 75 concentration response curves (control plus 3 compound concentrations) for either lidocaine or tetracaine were completed and analysed in 70 minutes. Importantly, the ability to perform cumulative concentration response curves on single cells drastically reduces the consumable cost per data point to < $0.75.

In conclusion, $Na_V1.8$ expressed in ND7-23 cells can be recorded on the SyncroPatch®96 with a high success rate (92% with a seal > 500 MΩ). During the experiment, 10% of cells were disabled as the current amplitude was too small to measure, giving a final success rate of 83% at the end of the experiment. The activation and inactivation properties of $Na_V1.8$ recorded on the SyncroPatch®96 are in excellent agreement to those reported in the literature[5,6], confirming the reliability of the data. In addition, the concentration response curves for lidocaine and tetracaine revealed IC_{50} values in good agreement with the literature[6,7] (N.B: holding potential -120 mV).

The SyncroPatch®96 is a high throughput and highly reliable automated patch clamp device for recording $Na_V1.8$. User-friendly software, excellent success rates, multiple additions of compound to each cell and easy analysis result in high quality, reliable data at an increased throughput with an economical cost per data point.

References

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Methods

Cells

ND7-23 cells stably expressing rat $Na_V1.8$ were supplied by Millipore.

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®96. All recordings were made in the presence of 100 nM TTX to block endogenous TTXs Na_V currents. Current-voltage recordings were made using voltage steps from -60 mV to 60 mV for 25 ms increasing in 10 mV steps, from a holding potential of -120 mV. Inactivation protocol used a 100 ms pre-pulse to the voltage indicated followed by a step to 20 mV for 25 ms. Pharmacology experiments used a single voltage step protocol to 20 mV for 25 ms from a holding potential of -120 mV, repeated every 5 s.

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