Application Note

Channel: rNa$_V$1.7
Cells: ND7-23
Tools: SyncroPatch 96

Pharmacology on rNa$_V$1.7 performed on Nanion's SyncroPatch 96

The electrophysiology team at Nanion Technologies GmbH, Munich.
Cells were supplied by EMD Millipore, USA

Summary

Na$_V$1.7 is an increasingly interesting target for drug development due to its role in pain. In this Application Note measurements are presented to demonstrate the accuracy and robustness of pharmacological Na$_V$1.7 data that can be generated with Nanion's SyncroPatch 96.

Results

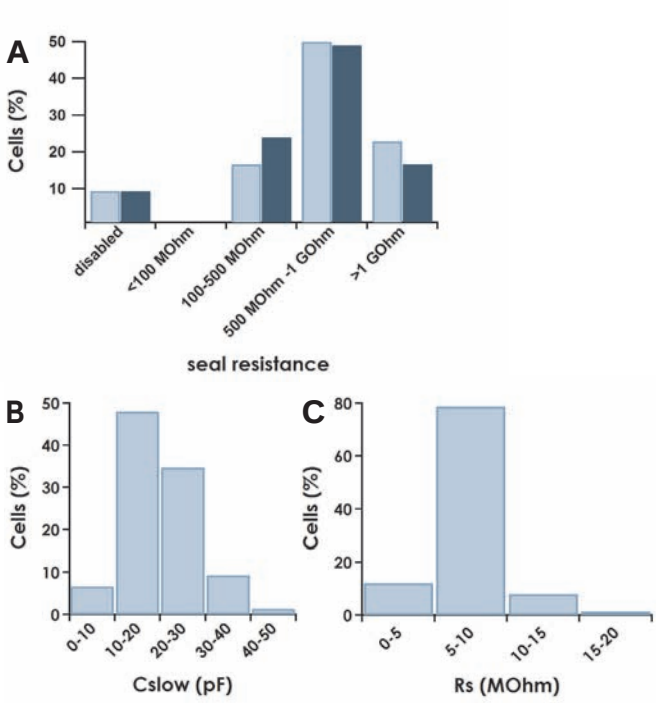

Figure 1:
A Success rate (seal resistance) of ND7-23 cells on the SyncroPatch 96. Shown is a bar graph of seal resistances at the start (light blue) and end of the experiment (dark blue). **B** Bar graph of cell capacitance (Cslow) of ND7-23 cells. The mean Cslow was 19.9 ± 0.8 pF (n = 75). **C** Bar graph of series resistance (Rs) values for ND7-23 cells. The mean Rs was 9.1 ± 1.3 MΩ (n = 75).

Figure 1 shows seal resistance, Cslow and series resistance values for ND7-23 cells recorded on the SyncroPatch 96. A total number of 72 cells was used for Cslow and Rseries analysis from this single run.

To confirm that the biophysics of the channels is correct in the measurements on the SyncroPatch 96, activation and inactivation curves were recorded previous to

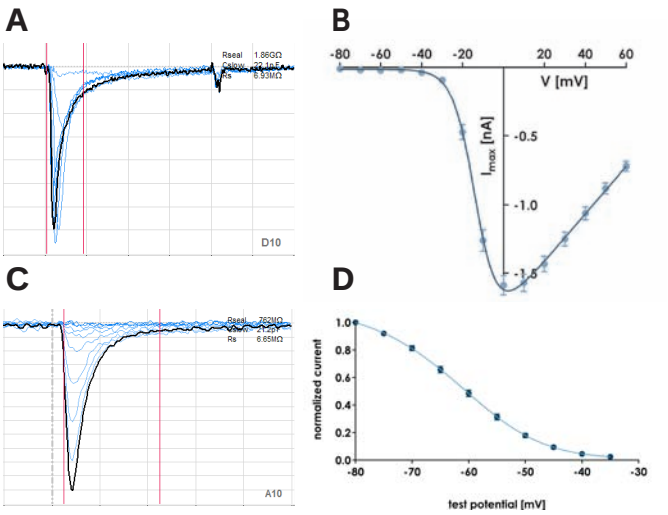

Figure 2:
rNaV 1.7 activation and inactivation curves. **A** Examplary current responses of an individual cell to single voltage step protocol. Grid x: 5 ms, y: 200 pA **B** Average current-voltage relationship for 82 cells recorded on a single chip. Vhalf was determined as -13 mV. Grid x: s ms, y: 100 pA **C** Examplary current responses of an individual cell to inactivation voltage protocol. **D** Average inactivation curve for the same 82 cells as shown in B. The inactivating potential was -63 mV.

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compound application. Figure 2 shows the average activation and inactivation curve. The activating and half inactivating potential of -13 mv and -62 mV respectively, correspond well to the literature values[1].

After running the activation and inactivation voltage protocols, 16 cells each were exposed to increasing concentrations of one of four different compounds (tetracaine, propafenone, mexilitine, amitriptyline) or negative control (32 cells). The holding potential in this experiment was -100 mV. Currents were elicited by 20 ms voltage steps to 0 mV. Figure 3 shows the current responses of an individual cell in the presence of increasing tetracaine concentrations. The average dose response curve for block of $rNa_V1.7$ by tetracaine reveals an IC_{50} of 12.5 µM[2]. This is in good agreement with the literature. Figure 4, 5 and 6 show equivalent data for propafenone, mexilitine, and amitriptyline, respectively.

IC_{50}s for each compound are shown on the average dose response graphs and are listed in the appropriate figure legend text. In all cases the determined IC_{50}s agree very well with the literature values[2,3,4]. The negative controls showed stable currents with each addition of external solution (see Figure 7).

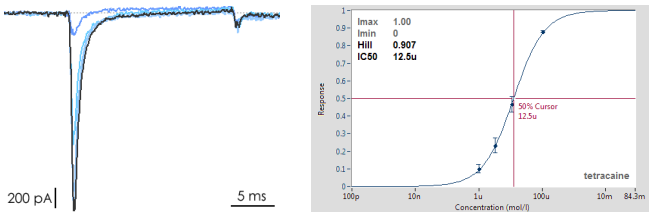

Figure 3:
Shown on the left are raw current traces from an individual cell expressing $rNa_V1.7$ under control conditions (black) and at increasing concentrations of tetracaine (1 µM, 3.3 µM, 11.1 µM and 103.7 µM). The Hill fit reveals an IC_{50} of 12.5 µM (n = 13/16).

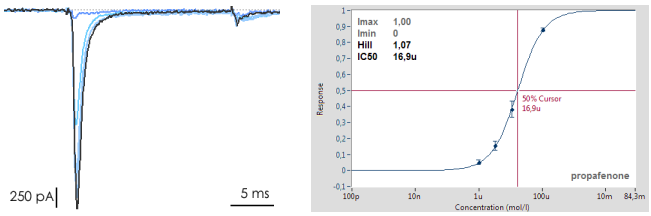

Figure 4:
Shown on the left are raw current traces from an individual cell expressing $rNa_V1.7$ under control conditions (black) and at increasing concentrations of propafenone (1 µM, 3.3 µM, 11.1 µM and 103.7 µM). The Hill fit reveals an IC_{50} of 16.9 µM (n = 12/16).

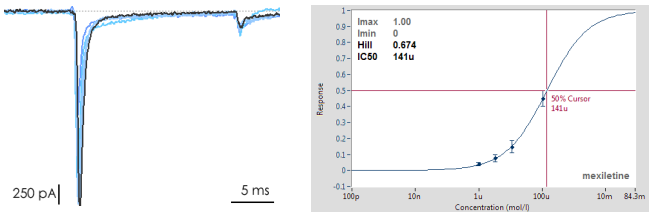

Figure 5:
Shown on the left are raw current traces from an individual cell expressing $rNa_V1.7$ under control conditions (black) and at increasing concentrations of mexiletine (1 µM, 3.3 µM, 11.1 µM and 103.7 µM). The Hill fit reveals an IC_{50} of IC50 = 141 µM (n = 12/16).

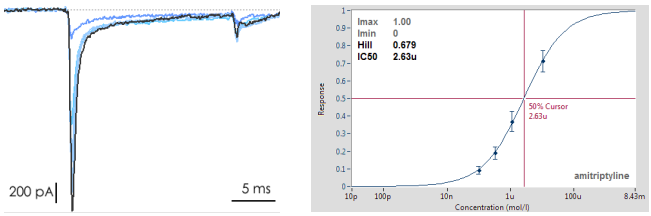

Figure 6:
Shown on the left are raw current traces from an individual cell expressing $rNa_V1.7$ under control conditions (black) and at increasing concentrations of amitriptyline (100 nM, 333 nM, 1.1 µM and 10.4 µM). The Hill fit reveals an IC_{50} of 2.6 µM (n = 13/16).

To demonstrate the reproducibility and robustness of the data, the same experiment was repeated with -100 mV holding potential. A set of experiments with a two pulse protocol stepping once from -130 mV and once after 1 s at -65 mV to 0 mV to elicit the current was also perfomed. Determined IC_{50}s for the different compounds and different experiments are shown in the table below. Results demonstrate a run to run comparison of less than 3-fold!

	hp	Chip1	Chip2	Chip3
Tetracaine	**-130 mV**	52.9 µM	76.4 µM	51.6 µM
	-100 mV	6.8 µM	12.5 µM	
	-65 mV	3.1 µM	2.9 µM	2.2 µM
Propafenone	**-130mV**	15.7 µM	26.4 µM	18 µM
	-100 mV	16.9 µM		
	-65 mV	4.3 µM	8.1 µM	4.6 µM
Mexilitine*	**-130 mV**	>100 µM	>100 µM	>100 µM
	-100 mV	146 µM	141 µM	
	-65 mV	7.3 µM	18.5 µM	6.0 µM
Amitriptyline*	**-130 mV**	>10 µM	>10 µM	>10 µM
	-100 mV	5.5 µM	2.6 µM	
	-65 mV	2.0 µM	3.0 µM	1.3 µM

***** For the measurements with the holding potential of -130 mV the concentrations were not high enough to allow a meaningful fit of the dose response data. At the highest concentration currents were less than 30 % reduced.

Nanion Technologies GmbH
Erzgiessereistr. 4
80335 Munich, Germany

phone +49 89 218997972
fax +49 89 218997960
www.nanion.de • info@nanion.de

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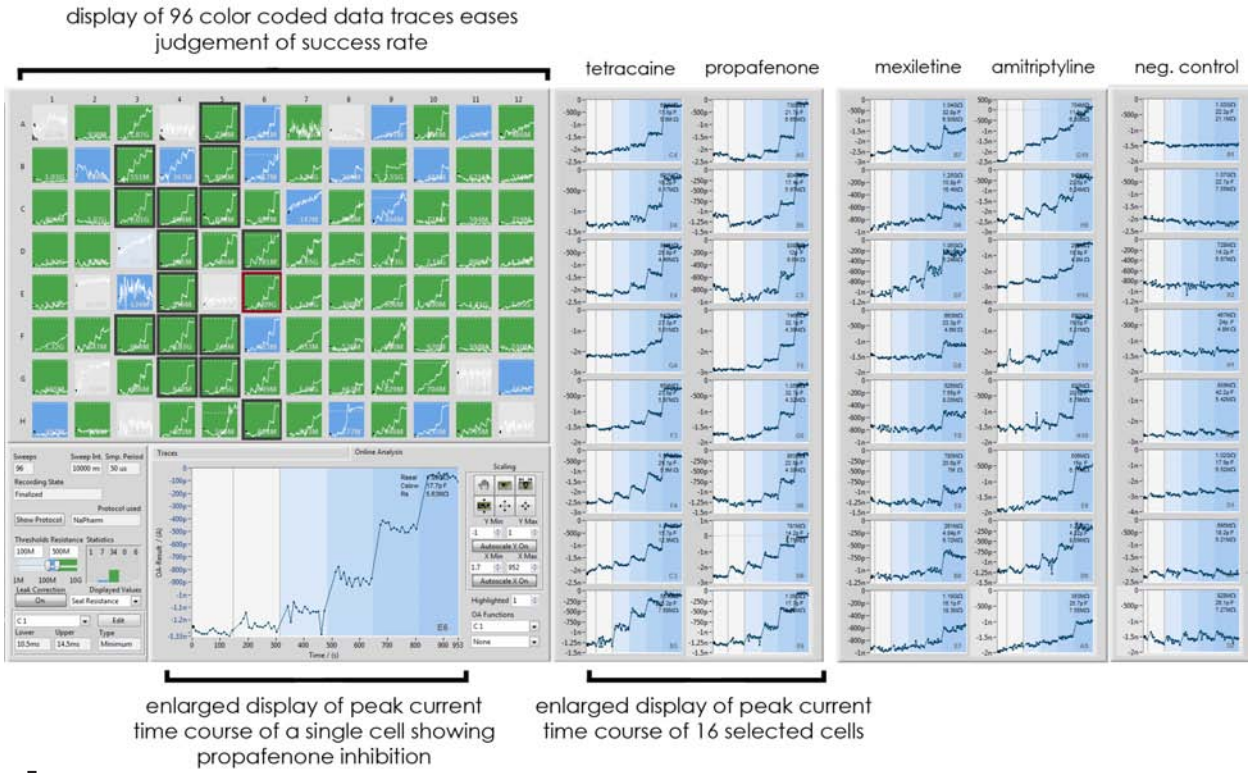

Figure 7:
Graphical user interface of the recording and data analysis software used with the SyncroPatch 96. Screenshot of depiction of online analysis data of $rNa_v1.7$ expressing cells as recorded on a single NPC-96 patch clamp chip. Ninety-six small color-coded pictures as seen in the upper left part displaying 96 individual recordings. Depending on seal resistance, backgrounds of the graphs are green (Rmemb > 500 MΩ), blue (Rmemb = 100–500 MΩ), light blue or grey (Rmemb < 100 MΩ or cells disabled). Data of single selected cell can be displayed grately enlarged. This single cell plus fifteen other selected cells are displayed on the right. Graphs show peak current amplitudes plotted against time for individual cells which were inhibited by four increasing compound concentrations. One wash step with control solution prior to compound application was performed. For highlighted experiments, note the different shades of blue in the background of the time courses, depicting the presence of the four different compound concentrations. The white color represents the presence of control solution.
To show peak current time courses for all used compounds used in this study three more columns of eight cells are displayed in the right of the software screenshot. Here time courses for cells exposed to mexiletine, amitriptyline and just external solution (negative control) are shown as well.

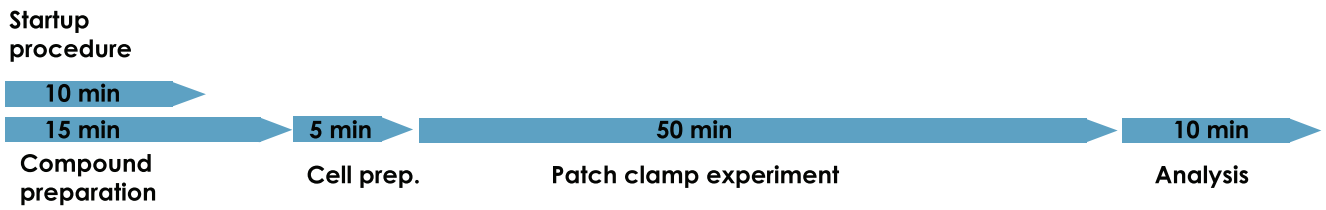

Figure 8:
The completion of 1 experiment on the SyncroPatch 96 patch clamp chip (96 wells) for a 4 point concentration response curve plus control on $rNa_v1.7$ took 80 mins. In this time we completed 73 concentration response curves giving a total of 365 data points (including control) in 80 mins at a consumable cost of < $0.60 per data point.

Nanion Technologies GmbH
Erzgiessereistr. 4
80335 Munich, Germany
phone +49 89 218997972
fax +49 89 218997960
www.nanion.de • info@nanion.de

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Figure 7 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 provides the user with an intuative overview over the whole experiment. The user can choose whether to visualize raw current traces or online analysis time courses. In the shown case, online analysis is chosen. The graphs show peak current amplitude plotted against time. An individual well can be enlarged to view more detail of the data. In the online analysis view the background colors indicate the presence of control solution (white) and increasing concentrations of compound (shades fo blue).

Figure 8 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, 73 concentration response curves (control plus 4 compound concentrations) for either tetracaine, propafenone, mexilitine or amitiptyline were completed and analyzed in 80 minutes. Importantly, the ability to perform cumulative concentration response curves on single cells drastically reduces the consumable cost per data point to < $0.60.

Conclusion

In conclusion, $rNa_V1.7$ expressed in ND7-23 cells can be recorded on the SyncroPatch 96 with a high success rate. Overall, 73 cells (76 %) were included in the analysis.
The activation and inactivation properties of $rNa_V1.7$ recorded on the SyncroPatch 96 are in excellent agreement with those reported in the literature, confirming the quality of the data. In addition, the concentration response curves for the tested compounds revealed IC_{50} values in good agreement with the literature for all holding potentials. Lastly, but just as importantly, repeated experiments give very robust results.
The SyncroPatch 96 is a high throughput and highly reliable automated patch clamp device for recording $rNa_V1.7$ currents. User-friendly software, excellent success rates, 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

1 Patrick L. Sheets et al, J Physiol 581.3 (2007) pp 1019–1031
2 Neil Castle at al, Combinatorial Chemistry & High Throughput Screening 12 (2009) pp107-122
3 Andreas Leffler et al, JPET 320 (2007) pp 354-364
4 Annamarie De Luca et al., JPET 282 (1997) pp 93-100

Methods

Cells

PrecisION - $Na_V1.8$ ND-7 cells (endogenously expressing $rNa_V1.7$, CYL 3050) were supplied by EMD Millipore, USA.

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. For the activation curve voltages were stepped from holding of -120 mV for 20 ms to increasing voltages between -80 mV and +60 mV. For the inactivation curves voltages were stepped from holding of -120 mV for 1 s to voltages between -80 mV and -35 mV followed by 10 ms at 0 mV. Pharmacology experiments used a single voltage step protocol to 0 mV for 20 ms from a holding potential as indicated in the text, repeated every 5 s.

Nanion Technologies GmbH
Erzgiessereistr. 4
80335 Munich, Germany

phone +49 89 218997972
fax +49 89 218997960
www.nanion.de • info@nanion.de