

High Throughput Pharmacology of Na_v1.5 Channels on Nanion's SyncroPatch 384PE

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Summary

The Na_v1.5 channel, encoded by the SCN5A gene, is a voltage-gated sodium (NaV) channel found in skeletal muscle and heart¹. It is TTX insensitive with an IC₅₀ in the micromolar range¹. Na_v1.5 is responsible for the upstroke of the cardiac action potential in both ventricular and atrial myocytes² and is therefore critical for generation and propagation of the cardiac action potential in human heart. Block of this channel can lead to prolongation of the QRS interval of the electrocardiogram (ECG) and can have profound effects on the rate of cardiac depolarization and conduction velocity, thus causing potentially dangerous cardiac arrhythmias^{3,4}. Furthermore, effects of Na_v1.5 inactivation can modify cardiac repolarization⁴. Given the importance of this channel in maintaining cardiac function, it has become an important target in compound safety screening.

Local anaesthetics, such as lidocaine, have been shown to exhibit state- and use-dependence when acting on the cardiac sodium channel⁵. The IC₅₀ was shown to be approximately 30 times lower at depolarized holding potentials where inactivation was almost complete⁵. For this reason, it is important to test potency of compounds at different holding potentials.

Here we present high quality data with reliable pharmacology on hNa_v1.5 expressing HEK293 cells at a high throughput collected on the SyncroPatch 384PE. Current-voltage plots and concentration response curves for four NaV channel blockers are shown, including lidocaine at different holding potentials.

Results

For the evaluation of the performance of hNa_v1.5 (HEK293) cells, Seal Resistance, C_{slow} and the Series Resistance (R_{series}) were determined from one experiment (Fig. 1).

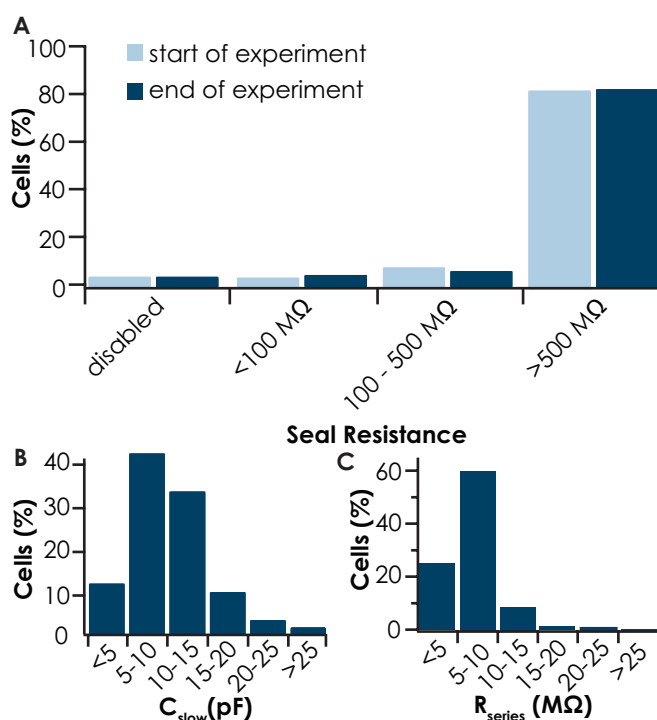


Figure 1: Statistics of hNa_v1.5 expressing HEK cells recorded on one NPC-384 chip on the SyncroPatch® 384PE

A Success rate (seal resistance) of individual HEK cells on the SyncroPatch 384PE. Shown is a bar graph of seal resistances at the start (light blue) and end of the experiment (dark blue). **B** Bar graph of C_{slow} and **C** R_{series} values for HEK cells expressing hNa_v1.5.

Application Note

Currents mediated by hNa_v1.5 could be reliably recorded on the SyncroPatch 384PE with a high success rate. Figure 2 shows the current-voltage relationship of hNa_v1.5 expressed in HEK293 cells recorded on the SyncroPatch 384PE. An average activation and inactivation IV plot is shown in Panels C and D, respectively. The V_{half} of activation was -51 mV and for inactivation was -84 mV in good agreement with the literature⁶.

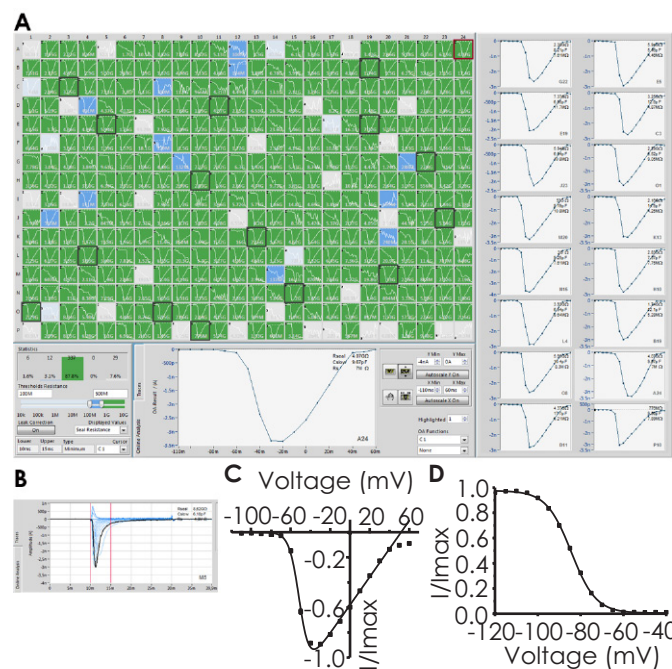


Figure 2: Na_v1.5 expressed in HEK293 cells recorded on the SyncroPatch 384PE. **A** The screenshot shows the data acquisition and analysis software used on the SyncroPatch 384PE. Wells are color-coded based on seal resistance (green: R_{seal} > 500 MΩ, blue: R_{seal} = 100 - 500 MΩ, light blue or grey: R_{seal} < 100 MΩ or disabled). Shown is the online analysis where peak current is plotted against voltage to show the current-voltage relationship **B** Na_v1.5-mediated current responses to a voltage step protocol from -110 mV to 60 mV increasing in 10 mV increments. **C** Average current-voltage plot of 337 cells. The points were fit with a Boltzmann equation and the V_{half} of activation was -51 mV. **D** Average inactivation plot for 271 cells. The points were fit with a Boltzmann equation and the V_{half} of inactivation was -84 mV.

Pharmacology of the hNa_v1.5 channel could be performed on the SyncroPatch 384PE with success rates of up to 76% for completed experiments. Figure 3 shows the concentration response curves for four Na_v channel blockers on Na_v1.5-mediated currents. In these experiments holding potential was -120 mV. The IC₅₀ values are summarised in Table 1.

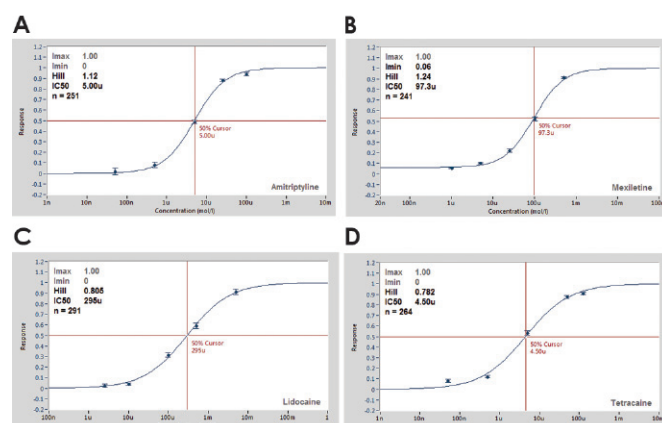


Figure 3: Average concentration response curves for 4 different Na_v1.5 blockers on the SyncroPatch 384PE. The concentration response curves were constructed across the whole plate (single concentration per cell). The SyncroPatch 384PE analysis software (DataControl 384) was used to calculate the average concentration response curves, normalized to maximum block and fitted with a standard Hill-equation. A summary of the IC₅₀ values and success rates is shown in Table 1.

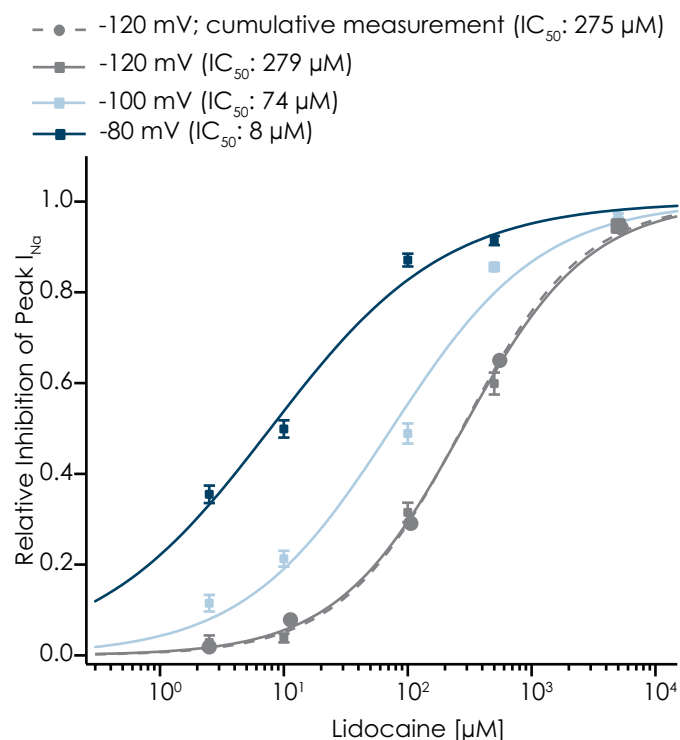


Figure 4: Effect of holding potential on potency of lidocaine block of Na_v1.5. The concentration response curves were constructed at different holding potentials (as indicated) either using a single concentration of compound per cell (solid lines) or cumulative concentration response curves (dashed line). The IC₅₀ for lidocaine was shifted by a factor of 35 when holding potential was changed from -120 mV to -80 mV.

Application Note

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

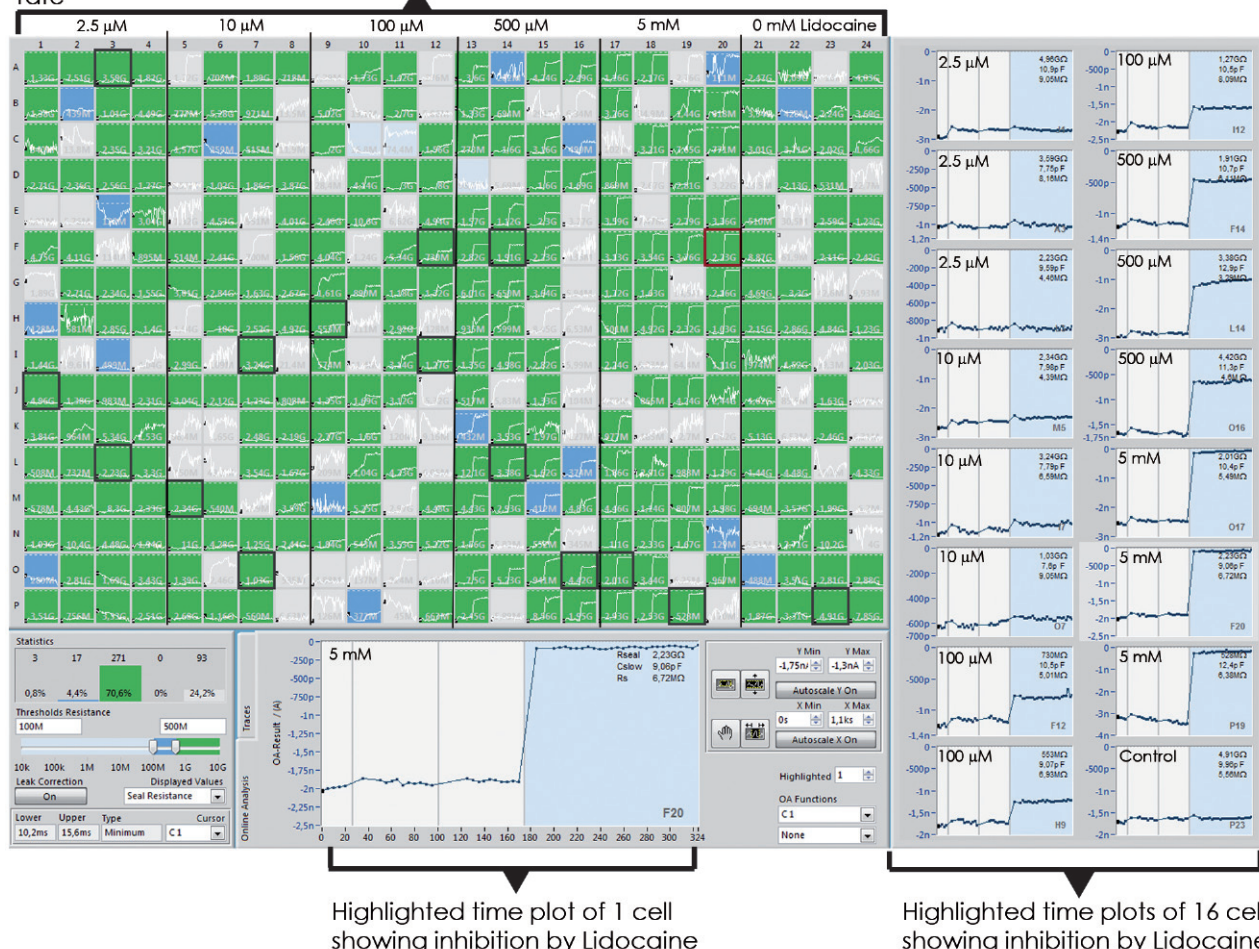


Figure 5: Graphical user interface of the screening and data analysis software used on the SyncroPatch 384PE. Screenshot of depiction of online analysis data of $\text{Na}_v1.5$ expressing HEK293 cells as recorded on one NPC-384 patch clamp chip. Three hundred and eighty-four small color-coded pictures as seen in the upper left part display 384 recordings. Depending on the seal resistance, pictures are green ($R_{\text{memb}} > 500 \text{ M}\Omega$), blue ($R_{\text{memb}} = 100 - 500 \text{ M}\Omega$), light blue or grey ($R_{\text{memb}} < 100 \text{ M}\Omega$ or cells disabled). One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. Graphs show current amplitudes of $\text{Na}_v1.5$ channels during the voltage step to -10 mV during application of control solution (white region) and inhibition by lidocaine (blue region).

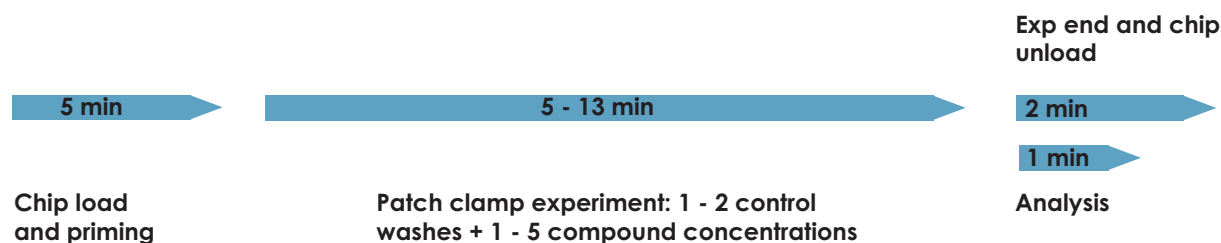


Figure 6: Timeline of an experiment on the SyncroPatch 384PE. The completion of 1 experiment on the SyncroPatch 384 patch clamp chip (384 wells) for a single point or cumulative concentration response curve on $\text{Na}_v1.5$ -mediated currents took approximately 12-20 min.

Application Note

Compound	IC ₅₀ (μM)	Success rate (%)	Literature value (μM)
Amitriptyline	5.0 (251)	65	1.6 ⁷
Mexiletine	97.3 (241)	63	49 ⁸
Lidocaine	295 (291)	76	353 ⁵
Tetracaine	4.5 (264)	69	9.3 ⁹

Table 2: IC₅₀ values for amitriptyline, mexiletine, lidocaine and tetracaine on Na_v1.5-mediated currents recorded on the SyncroPatch 384PE. Shown are IC₅₀ values (number of cells shown in brackets), success rate for completed experiments and the expected literature IC₅₀ values.

To investigate whether the potency of lidocaine on Na_v1.5 was influenced by holding potential, concentration response curves were constructed at different holding potentials (Fig. 4). As expected⁵, the IC₅₀ for lidocaine was shifted by a factor of >30 using a holding potential of -80 mV vs. -120 mV. The IC₅₀ was not changed when the experiments were performed as cumulative (dashed line) or single point concentration response curves (solid line) at -120 mV.

Figure 5 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 and blue is lidocaine.

In conclusion, hNa_v1.5 expressed in HEK293 cells can be recorded on the SyncroPatch 384PE with high success rates for completed experiments (>70%). The IC₅₀'s of four NaV channel blockers were in good agreement the literature⁵⁻⁹ and the IC₅₀ for lidocaine was influenced by holding potential⁵.

The SyncroPatch 384PE is a high throughput and highly reliable automated patch clamp device for recording Na_v1.5 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

1. Gellens, M.E., *et al.*, 1992. PNAS. 89: 554-558
2. Nerbonne, J.M. & Kass, R.S. 2005. Physiol. Rev. 85: 1205-1253
3. Bowes, J., *et al.*, 2012. Nat. Rev. Drug. Disc. 11: 909-922
4. Erdemli, G., *et al.*, 2012. Front. Pharmacol. doi: 10.3389/fphar.2012.00006
5. Bean, B.P., *et al.*, 1983. J.Gen. Physiol. 81: 613 - 642
6. Sheets, M.F. & Hanck, D.A. 1999. J. Physiol. 514.2: 425-436
7. Harmer, A.R., *et al.*, 2011. Br. J. Pharmacol. 164: 260-273
8. Wang, D.W., *et al.*, 1997. J. Clin. Invest. 99 (7): 1714-1720
9. Finkel, A., *et al.*, 2006. J. Biomol. Screen. 11(5): 488-496

Methods

Cells

HEK293 cells expressing hNa_v1.5.

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. A voltage step protocol from a holding potential of -120 mV (unless otherwise indicated) to -10 mV was applied to the cells every 5 s for pharmacology experiments. Peak amplitude at -10 mV was used for analysis.