

High Throughput Pharmacology of $\text{Ca}_v1.2$ Channels on Nanion's SyncroPatch® 384PE

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Cells kindly provided by SB Drug Discovery.



Summary

The $\text{Ca}_v1.2$ channel is a voltage-gated calcium channel (VGCC) expressed in a variety of mammalian tissues and is essential for multiple processes including CNS function, cardiac and smooth muscle contraction and neuroendocrine regulation¹. VGCC are composed of 4 subunits, which include an α_1 subunit which forms the Ca^{2+} -selective pore, contains the voltage sensor and many of the binding sites for regulatory modulators and drugs² and accessory subunits $\alpha_2\delta$, β and γ which are involved in anchorage, trafficking and regulatory functions¹. The $\text{Ca}_v1.2$ channel contains the α_1C subunit, encoded by the CACNA1C gene on the human chromosome 12p13³. Mutations in the L-type Ca^{2+} channels have been associated with inherited arrhythmic disorders such as Timothy, Brugada and early repolarization syndromes⁴. In addition, in the light of the CiPA initiative, the L-type channel is likely to become an important target for cardiac safety testing^{5,6}.

Here we present high quality data with reliable pharmacology on $\text{Ca}_v1.2$ expressing HEK cells at a high throughput collected on the SyncroPatch® 384PE. Current-voltage plots and concentration response curves for the compounds nifedipine and verapamil are shown. The IC_{50} values for these compounds are within the expected range⁷⁻¹¹ and success rates of >75% for completed experiments were recorded. Importantly, $\text{Ca}_v1.2$ recorded on the SyncroPatch 384PE exhibited stable peak amplitude during the course of the experiment and displayed little or no run-down.

Results

For the evaluation of the performance of HEK $\text{Ca}_v1.2$ cells, Seal Resistance values were determined from one experiment (Fig. 1) at the start, after compound addition and at the end of the experiment. Experiments were performed in perforated patch mode using multi-hole (4 holes per well) chips and seal resistance values are calculated and displayed per hole.

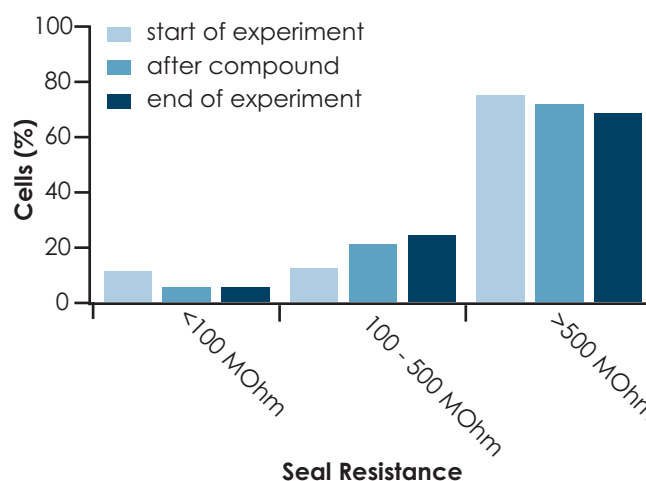


Figure 1: Statistics of $\text{Ca}_v1.2$ expressing HEK293 cells recorded on one NPC-384 chip on the SyncroPatch® 384PE. Success rate (seal resistance) of individual HEK cells on the SyncroPatch® 384. Shown is a bar graph of seal resistances at the start (light blue), after compound addition (mid-blue) and end of the experiment (dark blue). Multi-hole (4X) chips were used and the seal resistance values per hole are displayed.

Application Note

Currents mediated by $\text{Ca}_v1.2$ could be reliably recorded on the SyncroPatch® 384PE with a high success rate (>75% for completed experiments). Figure 2 shows a screenshot of the data acquisition and analysis software of the SyncroPatch® 384PE during an experiment recording the current-voltage relationship of $\text{Ca}_v1.2$ expressed in HEK cells. The current-voltage plot for an average of 279 cells is shown in Panel C. A Boltzmann equation reveals a $V_{0.5}$ of activation of -12 mV with a reversal potential of 53 mV in good agreement with the literature for L-type Ca^{2+} channels recorded from human atrium cells¹², rat neonatal ventricular cardiomyocytes⁷ and alpha-1C expressed in HEK cells¹³.

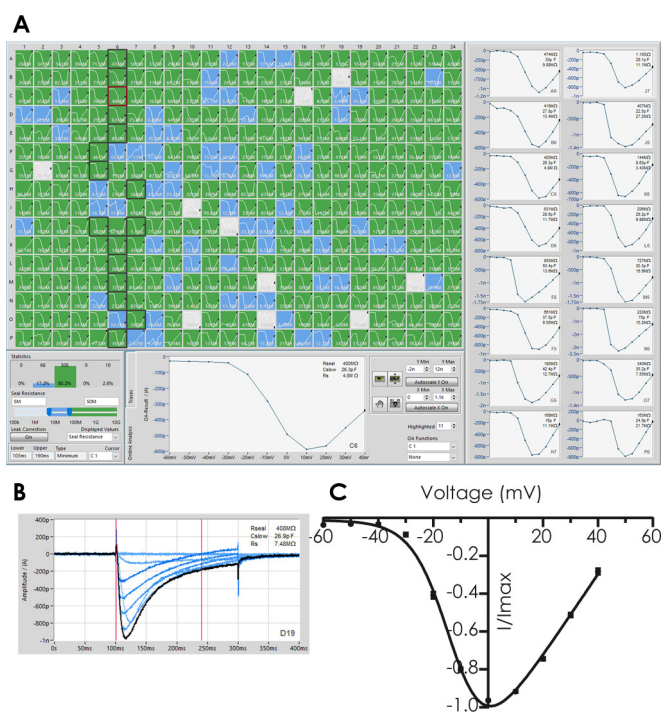


Figure 2: Typical recording of $\text{Ca}_v1.2$ expressed in HEK cells on the SyncroPatch® 384PE. **A** The screenshot shows the data acquisition and analysis software used on the SyncroPatch® 384PE. Three hundred and eighty-four small color-coded pictures shown in the upper left part from A1 to P24 display 384 recordings. Wells are color-coded based on seal resistance. One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. The online analysis values are shown for a current-voltage experiment. **B** The raw traces from an example cell elicited by depolarizing steps from -60 mV to 40 mV in 10 mV increments from a holding potential of -90 mV are shown. The black trace highlights the maximum current response elicited by a step to +10 mV. **C** The normalized current-voltage plot for an average of 279 cells. A Boltzmann equation fit revealed a $V_{0.5}$ of activation of -12 mV.

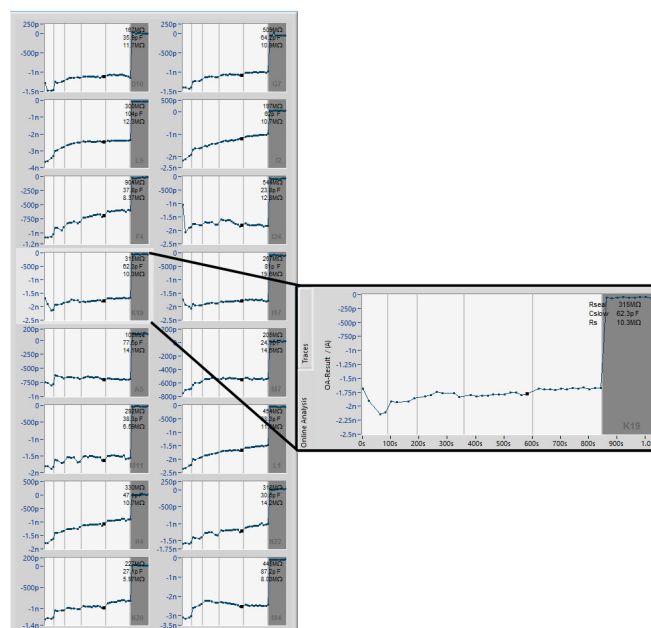


Figure 3: Recordings of $\text{Ca}_v1.2$ channels expressed in HEK cells on the SyncroPatch® 384PE are stable over time. Shown are online analysis values peak amplitude versus time for 16 example cells. A small amount of rundown is seen at the beginning of the recording but this stabilizes and recordings are stable for >15 mins. The inset shows an enlarged graph. At the end of the experiment, CdCl_2 was used to fully block the current.

Rundown of current is a phenomenon common to VGCC channels¹⁴ and can be a real problem for electrophysiological measurements. Using the perforated patch mode and multi-hole chips (4 holes per well) we could reliably record $\text{Ca}_v1.2$ -mediated currents for >20 mins (Figure 3).

$\text{Ca}_v1.2$ currents were blocked by nifedipine and verapamil in a concentration-dependent manner. The average concentration response curves are shown in Figure 4 and the IC_{50} values summarized in Table 1. The estimated IC_{50} values agree well with those found in the literature⁷⁻¹¹.

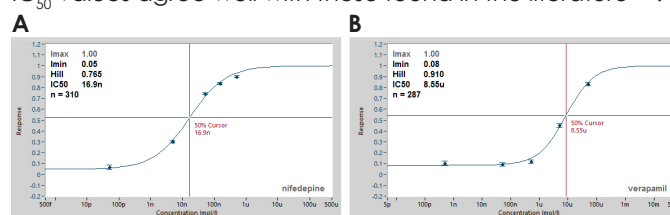


Figure 4: Average concentration response curves for 2 different calcium channel blockers on the SyncroPatch® 384PE. The concentration response curves were constructed across the whole plate as shown in Figure 5. The SyncroPatch® 384PE analysis software (DataControl® 384) was used to calculate the average concentration response curves, normalized to maximum block and fitted with a standard Hill-equation. A summary of the IC_{50} values and success rates is shown in Table 1.

Application Note

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

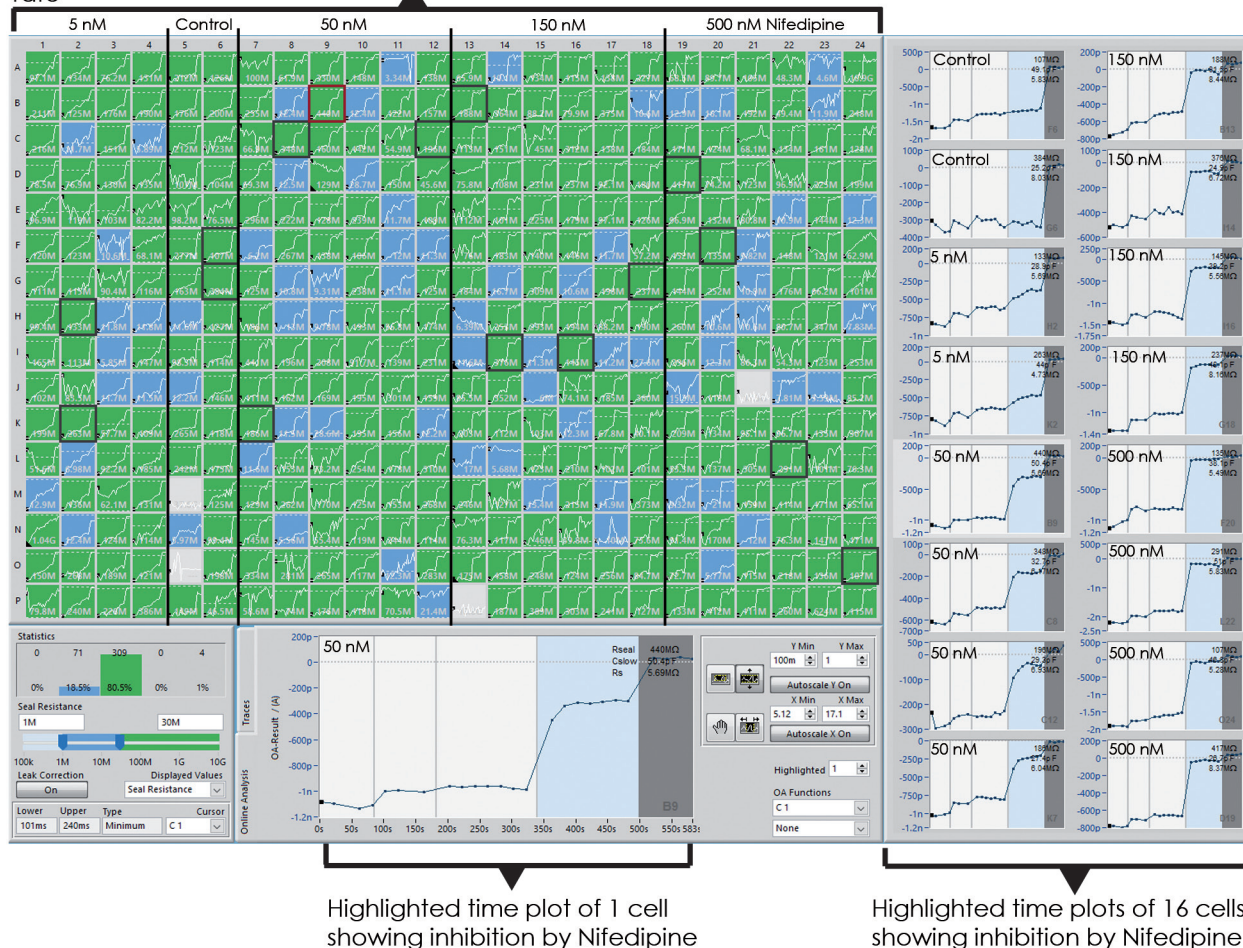


Figure 5: Graphical user interface of the screening and data analysis software used on the SyncroPatch® 384PE. Screenshot of depiction of online analysis data of $Ca_v1.2$ expressing HEK cells as recorded on one NPC-384 patch clamp chip (multi-hole, 4X). A single compound (in this case nifedipine) was applied to individual wells at varying concentrations (including control wells, 0.3% DMSO) across the plate. 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 ($R_{\text{memb}} > 30 \text{ M}\Omega$), blue ($R_{\text{memb}} = 1 - 30 \text{ M}\Omega$), light blue or grey ($R_{\text{memb}} < 1 \text{ M}\Omega$ or wells disabled). One highlighted experiment is displayed at the bottom, 16 selected experiments are displayed on the right. Graphs show current amplitudes of $Ca_v1.2$ channels from the test pulse to 0 mV during application of reference solution (white region), inhibition by different concentrations of nifedipine (blue region; concentrations indicated) and full block by $200 \mu\text{M}$ CdCl₂.

Exp end and chip unload

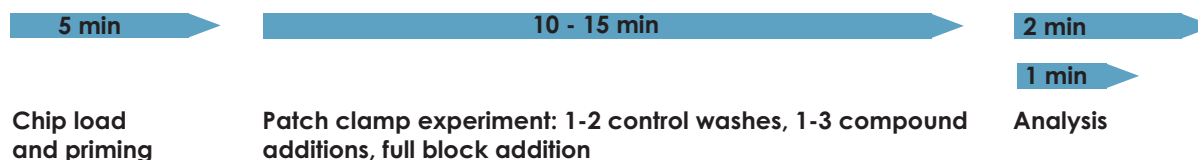


Figure 6: Timeline of an experiment on the SyncroPatch® 384PE. The completion of 1 experiment on the SyncroPatch® 384 patch clamp chip (384 wells) for a single point concentration response curve on $Ca_v1.2$ -mediated currents took approximately 17 - 22 min.

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Application Note

Compound	IC ₅₀	Success rate (%)	Literature range
Nifedipine	17 nM (310)	81	9 - 220 nM ⁷⁻¹⁰
Verapamil	8.6 μM (287)	75	0.17 - 110 μM ⁹⁻¹¹

Table 1: IC₅₀ values for nifedipine and verapamil on Ca_v1.2-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. IC₅₀ values recorded on the SyncroPatch® 384PE agree well with the literature values⁷⁻¹¹.

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 versus 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 (vehicle; 0.3% DMSO) solution and blue is nifedipine.

References

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Methods

Cells

HEK293 cells expressing Ca_v1.2 provided by SB Drug Discovery, Catalog Ref: SB-HEK-Cav1.2.

In conclusion, Ca_v1.2 expressed in HEK cells can be recorded on the SyncroPatch® 384PE with high success rates for completed experiments (typically >75%). The timeline of each experiment was about 17 - 22 minutes (start – end) and included wash with vehicle followed by incubation in 1 - 3 concentrations of blocker (or control solution) and full-block at the end of the experiment. Importantly, Ca_v1.2 displayed little or no run-down and current-voltage relationship recorded on the SyncroPatch® 384PE is in good agreement with the literature^{7,12,13}. The IC₅₀'s calculated using the SyncroPatch® 384PE's analysis software, DataControl® 384, of two calcium channel blockers were in good agreement with the literature⁷⁻¹¹.

The SyncroPatch® 384PE is a high throughput and highly reliable automated patch clamp device for recording Ca_v1.2 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.

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol.

Electrophysiology

Perforated patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch® 384PE and multi-hole chips (4X). The current-voltage relationship was elicited by depolarizing steps from -60 mV to 40 mV for 200 ms in 10 mV increments from a holding potential of -90 mV. A voltage step protocol from -90 mV (holding potential) to 0 mV for 200 ms was applied to the cells every 20 s for pharmacology experiments.