

Stable Recordings and High Throughput Pharmacology of Assay Ready Ca_v1.2 Cells on the SyncroPatch 384

The electrophysiology team at Nanion Technologies GmbH, Munich. Cells kindly provided by NMI TT Pharmaservices, Steinbeis-Innovationszentrum Zellkulturtechnik and acCELLerate.



Steinbeis-Innovationszentrum
Zellkulturtechnik



acCELLerate



Summary

The 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 alpha 1 subunit which forms the Ca²⁺-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 Ca_v1.2 channel contains the alpha-1C subunit, encoded by the CACNA1C gene on the human chromosome 12p13³. Mutations in the L-type Ca²⁺ channels have been associated with inherited arrhythmic disorders such as Timothy, Brugada and early repolarization syndromes⁴. In addition, the L-type Ca²⁺ channel is an important target for cardiac safety testing, especially in the light of the CiPA initiative^{5,6}.

Here we present high quality data with reliable pharmacology on Assay Ready Ca_v1.2 Cells at a high throughput collected on the SyncroPatch 384. Current-voltage plots and concentration response curves for the compounds nifedipine and verapamil are shown. The IC₅₀ values for these compounds are within the expected range⁷⁻¹¹ and success rates of >75% for completed experiments were recorded. Importantly, Ca_v1.2 recorded on the SyncroPatch 384 exhibited stable peak amplitude during the course of the experiment and displayed low rundown.

Results

CHO cells stably expressing Ca_v1.2 were provided as frozen stocks (Assay Ready Cells) that were thawed out and used without prior cultivation. Multi-hole chips were used on the SyncroPatch 384 (4 holes per well) and cells were captured with good success rates (94% of wells had $R_{\text{seal}} > 100 \text{ M}\Omega$). Rundown of current is a phenomenon common to VGCC channels¹² and can be a problem for electrophysiological measurements. Using the perforated patch mode and multi-hole chips we could reliably record Ca_v1.2-mediated currents for >10 mins with <10% rundown (Figure 1).

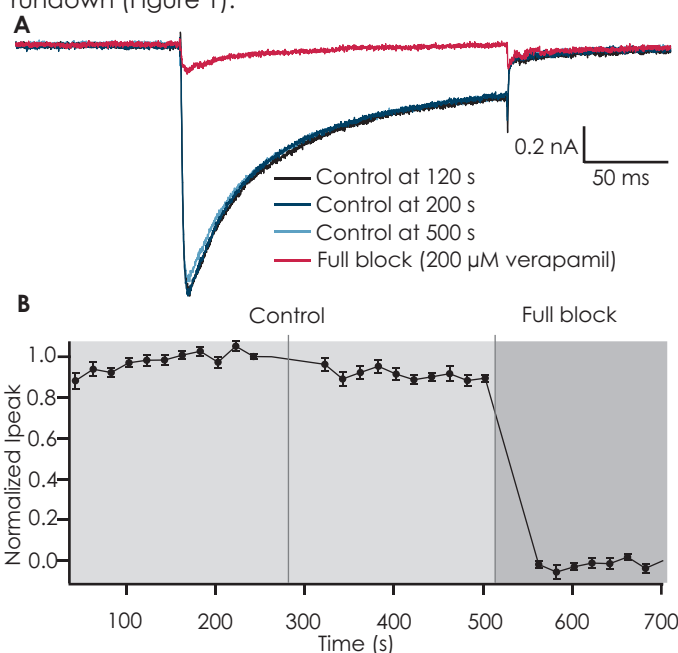
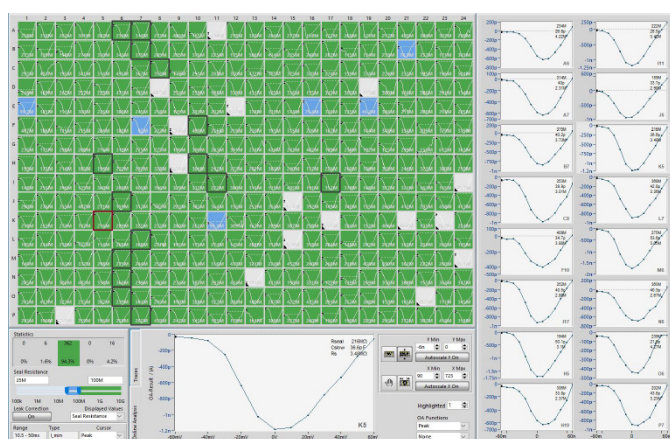


Figure 1: Ca_v1.2 expressed in CHO cells were reliably recorded on the SyncroPatch 384. **A** Traces from an example cell at 120 s, 200 s and 500 s overlay almost exactly. The current was blocked by 200 μM verapamil and the remaining current subtracted. **B** Timecourse of the experiment for an average of 26 wells. Rundown was low, <10% over 500 s.

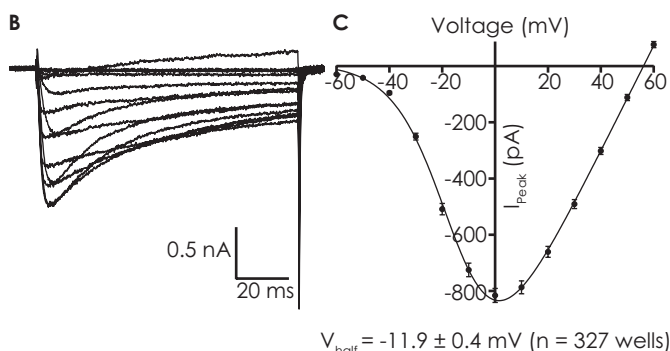
Application Note

Figure 2 shows a screenshot of the data acquisition and analysis software of the SyncroPatch 384 during an experiment recording the current-voltage relationship of $\text{Ca}_v1.2$ expressed in CHO cells. The current-voltage plot for an average of 327 wells is shown in Panel C. A Boltzmann equation reveals a $V_{0.5}$ of activation of -11.9 ± 0.4 mV with a reversal potential of 56.0 ± 0.5 mV in good agreement with the literature for L-type Ca^{2+} channels recorded from human atrium cells¹³, rat neonatal ventricular cardiomyocytes⁷, human ventricular cardiomyocytes¹⁴ and $\alpha\text{-1C}$ expressed in HEK cells¹⁵.

A



B



C

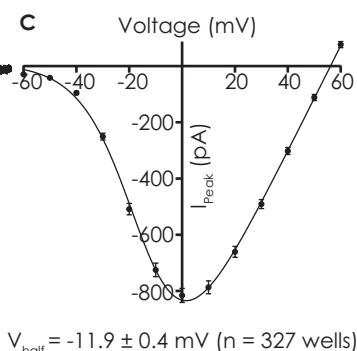


Figure 2: Typical recording of $\text{Ca}_v1.2$ expressed in CHO cells on the SyncroPatch 384. **A** The screenshot shows the data acquisition and analysis software used on the SyncroPatch 384. Three hundred and eighty-four small color-coded pictures shown in the upper left part from A1 to P24 display 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. **C** The normalized current-voltage plot for an average of 327 wells. A Boltzmann equation fit revealed a $V_{0.5}$ of activation of -11.9 ± 0.4 mV.

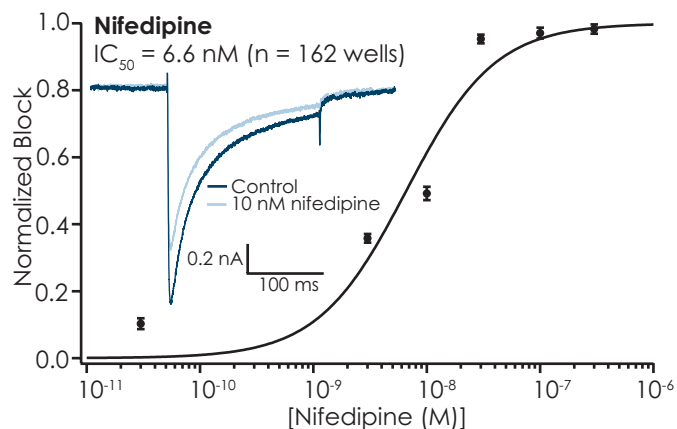


Figure 3: Average concentration response curve for nifedipine block of $\text{Ca}_v1.2$ recorded on the SyncroPatch 384. The cells received a single concentration of nifedipine and the concentration response curve constructed across multiple wells. Example traces from a single well are shown in the inset with block by 10 nM nifedipine. The IC_{50} was calculated using a Hill Equation and found to be 6.6 nM.

$\text{Ca}_v1.2$ currents were blocked by nifedipine and verapamil in a concentration-dependent manner. Each well received one concentration of either nifedipine (0-300 nM) or verapamil (0-100 μM) and the concentration response curves calculated across multiple wells. The average concentration response curve for nifedipine is shown in Figure 3. The IC_{50} was 6.6 nM in good agreement with the range found in the literature⁷⁻¹⁰. The average concentration response curve for verapamil is shown in Figure 4. The IC_{50} was 1.61 μM in good agreement with the range found in the literature⁹⁻¹¹. The IC_{50} values and success rates are summarized in Table 1.

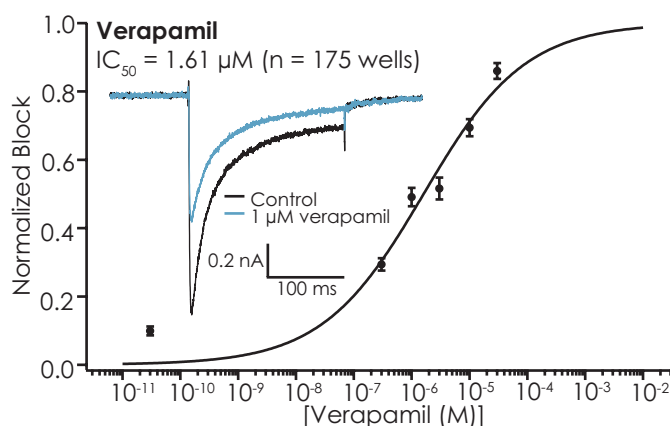


Figure 4: Average concentration response curve for verapamil block of $\text{Ca}_v1.2$ recorded on the SyncroPatch 384. The cells received a single concentration of verapamil and the concentration response curve constructed across multiple wells. Example traces from a single well are shown in the inset with block by 1 μM verapamil. The IC_{50} was calculated using a Hill Equation and found to be 1.61 μM .

Application Note

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

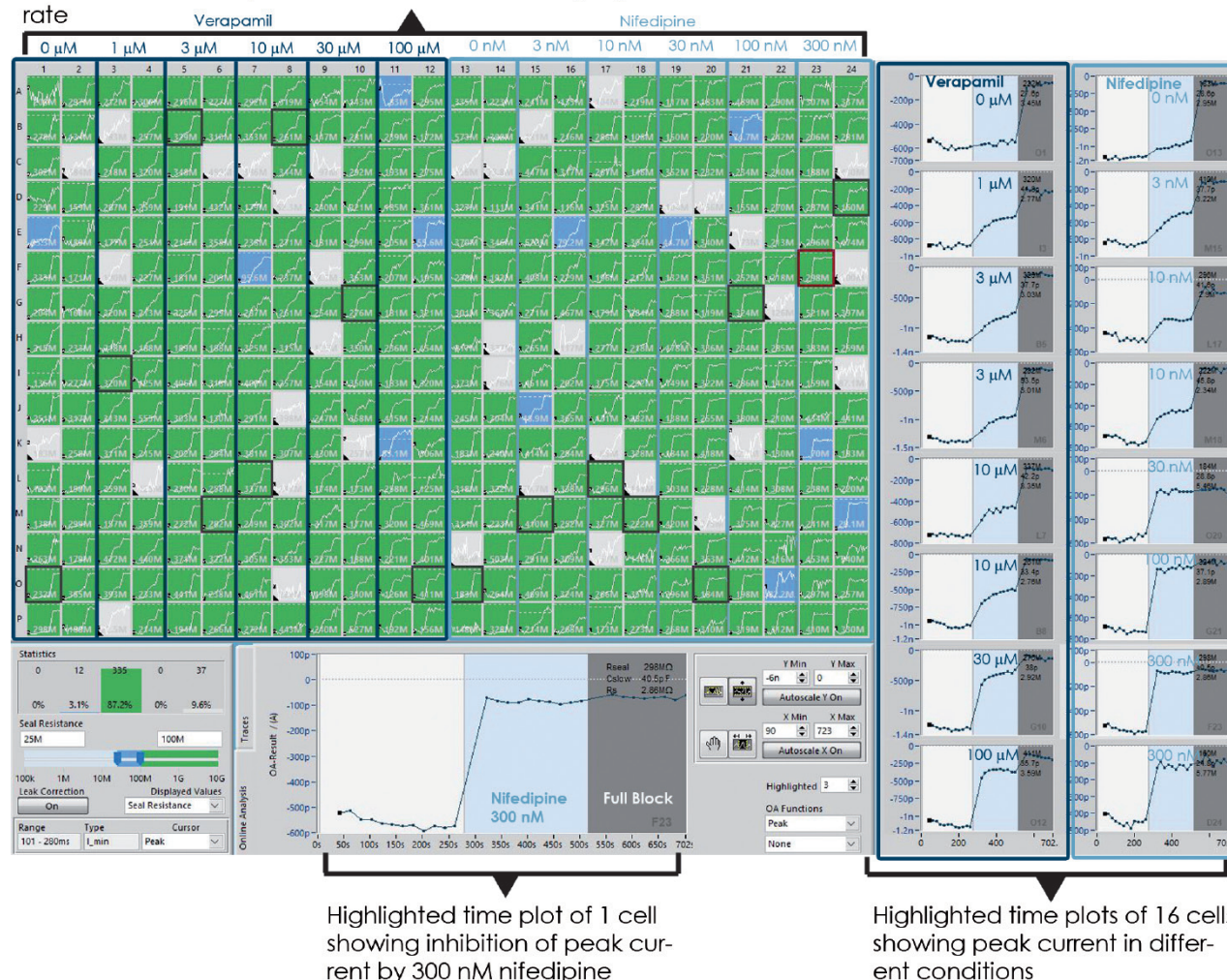


Figure 5: Graphical user interface of the screening and data analysis software used on the SyncroPatch 384. Screenshot of depiction of online analysis data of $Ca_v1.2$ expressing CHO cells as recorded on one NPC-384 patch clamp chip (multi-hole, 4X). A single concentration of compound (in this case either verapamil or nifedipine) was applied to individual wells. 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 ($R_{\text{seal}} > 100 \text{ M}\Omega$), blue ($R_{\text{seal}} = 25 - 100 \text{ M}\Omega$), light blue or grey ($R_{\text{seal}} < 25 \text{ M}\Omega$ 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 or verapamil (blue region; concentrations indicated) and full block by 200 μM verapamil (dark grey).

Exp end and chip unload

2 min

1 min

Analysis

5 min

Chip load and priming

~12 min

Patch clamp experiment: 1 control wash, 1 compound addition, full block addition

Figure 6: Timeline of an experiment on the SyncroPatch 384. 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 19 min.

Application Note

Compound	IC ₅₀	Success rate (%)	Literature range
Nifedipine	6.6 nM (162)	84	9 - 220 nM ⁷⁻¹⁰
Verapamil	1.7 μ M (175)	91	0.17 - 110 μ M ⁹⁻¹¹

Table 1: IC₅₀ values for nifedipine and verapamil on Ca_v1.2-mediated currents recorded on the SyncroPatch 384i. Shown are IC₅₀ values (number of wells shown in brackets), success rate for completed experiments and the expected literature IC₅₀ values. IC₅₀ values recorded on the SyncroPatch 384 agree well with the literature values⁷⁻¹¹.

Figure 5 shows a screenshot of the SyncroPatch 384 software during the experiment where half of the plate (192 wells) received nifedipine and the other half received verapamil. 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.

References

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Methods

Cells

This recombinant CHO-Ca_v1.2 cell line was developed by Steinbeis-Innovationszentrum Zellkultur und NMI TT using their proprietary IGAMI® expression system for the development of stable cell lines. This technology uses special stabilizing and expression enhancing sequences,

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, blue is verapamil or nifedipine and grey is full block (200 μ M verapamil).

In conclusion, Ca_v1.2 Assay Ready Cells can be recorded on the SyncroPatch 384 with high success rates for completed experiments (typically >84%). The timeline of each experiment was about 19 minutes (start – end) and included wash with control solution followed by incubation in a single concentration of blocker and full-block at the end of the experiment. Importantly, Ca_v1.2 displayed little rundown (<10% rundown over 10 mins) and current-voltage relationship recorded on the SyncroPatch 384 is in good agreement with the literature^{7,14,15}. The IC₅₀s calculated using the SyncroPatch 384's analysis software, DataControl 384, of two calcium channel blockers were in good agreement with the literature⁷⁻¹¹.

which prevent gene silencing of neighboring transgenes and therefore allow a stable co-expression. The combination of the IGAMI technology with an inducible gene expression of Ca_v1.2 protein subunits led to the generation of Assay Ready Ca_v1.2 Cells which can be used instantly after thawing without prior cultivation. The standardized production and freezing process of Assay Ready Cells significantly increases the reproducibility of experiments since all variations occurring from cell culture can be excluded.

Electrophysiology

Cells were thawed out and used directly for experiments. Perforated patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch 384 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. The current remaining after full-block application was subtracted from current in control and drug conditions.