SOPHION BIOSCIENCE

Application Report

hCa$_V$1.2 recordings on Qube 384 using step, train and CiPA protocols

A reproducible high-throughput assay with high success rates for biophysical and pharmacological studies of the Ca$_V$1.2 channel

Summary

The development of screening assays for the Ca$_V$1.2 channel has been challenging due to the tendency of Ca$_V$1.2 expressing cell lines to exhibit declining current levels (rundown) during the experiment[1,2]. Here we report a robust Ca$_V$1.2 assay yielding high success rates, low rundown and reliable pharmacology.

- Pharmacology and current-voltage relationship in accordance with literature values
- Success rates up to **94%**
- Stable currents with rundown as low as **(1.2 ± 0.9)%** per minute
- The CiPA protocol yields stable currents with rundown as low as **(1 ± 1)%** per minute

Introduction

The L-type voltage-gated calcium channel Ca$_V$1.2 is expressed in various mammalian tissues, including heart and smooth muscle[3-5], and its dysfunction has been implicated in a range of cardiovascular and neurological diseases[6,7]. Opening of the Ca$_V$1.2 channel results in an increase in the intracellular concentration of calcium ions (Ca^{2+}), affecting a variety of cellular processes including muscle contraction, hormone secretion and neuronal transmission[7-9], thus rendering the channel an important target in e.g. safety pharmacology screening.

Ca$_V$1.2 channels require a strong depolarization to activate, display relatively long-lasting activity and can be blocked by low micromolar concentrations of e.g. dihydropyridines, phenylalkylamines and benzothiazepines[8,9]. Following activation, the channel displays both Ca^{2+}- and voltage-dependent inactivation[10].
In this study, current traces from HEK-hCa$_V$1.2 cells were recorded on the high-throughput platform Qube 384 using both single-hole and multi-hole (10 patch holes) QChips. The current-voltage relationship and pharmacology of three different compounds were determined with high success rates and low rundown.

Results and discussion

The following experiments were carried out:

1. Recordings of Ca^{2+} currents
2. Current-voltage relationship of Ca$_V$1.2 channels
3. Pharmacology of Ca$_V$1.2 channels
4. Rundown analysis using multi-hole QChips
5. Recordings of Ca^{2+} currents and rundown analysis using a step-ramp (CiPA) protocol

1. Recordings of Ca^{2+} currents

Cells were clamped to -100 mV and Ca^{2+} currents were evoked by application of a depolarization step to +20 mV for 200 ms (Fig. 1).

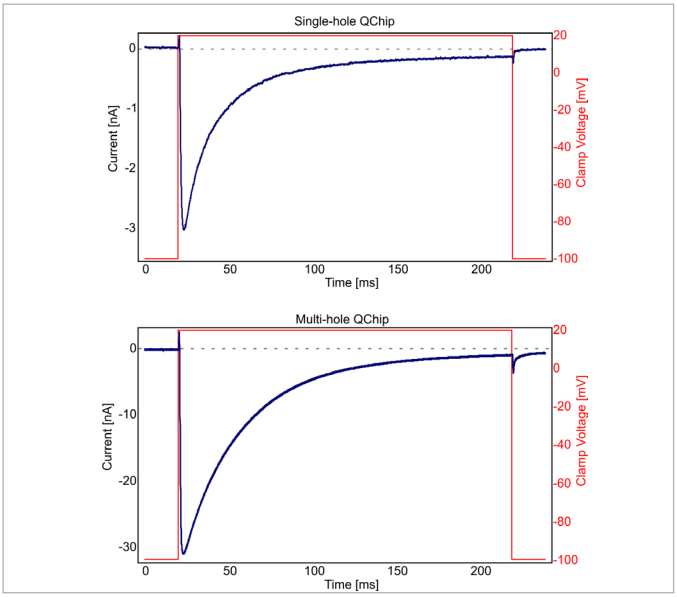

Fig. 1: Representative recordings of Ca^{2+} current (blue) on a single-hole (left) and multi-hole (right) QChip following a 200 ms depolarization from -100 mV to 20 mV (red) in Ca$_V$1.2 expressing cells.

The criteria for a successful recording were (average of first 10 sweeps):

Single-hole QChip:

I_{peak} < -100 pA

$R_{membrane}$ > 100 MΩ per cell

C_{total} > 4 pF per cell

Multi-hole QChip:

I_{peak} < -500 pA

$R_{membrane}$ > 100 MΩ per cell

C_{total} > 4 pF per cell

Where I_{peak} is the peak current, $R_{membrane}$ is the membrane resistance and C_{total} is the total capacitance.

A plate-view of $Ca_V1.2$ Ca^{2+} currents recorded on a multi-hole QChip is shown in Figure 2. The success rates were up to 89% and 94%, for single-hole and multi-hole QChips, respectively. The mean success rates and peak currents were (85 ± 3)% and (-2.9 ± 0.4) nA for single-hole QChips (*N* = 3), and (82 ± 8)% and (-14 ± 2) nA for multi-hole QChips (*N* = 8). The success rates and peak currents started declining at cell passage numbers above 20.

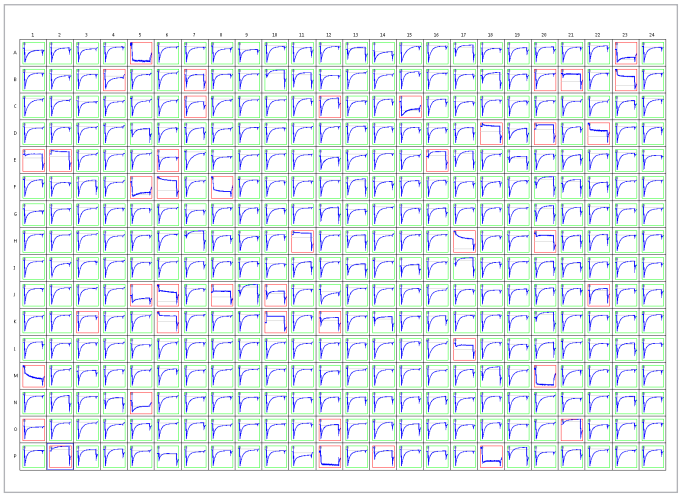

Fig. 2: Plate overview of $Ca_V1.2$ Ca^{2+} currents recorded on a multi-hole QChip. currents recorded on a multi-hole QChip.

2. Current-voltage relationship of $Ca_V1.2$ channels

The current-voltage relationship was quantified on single-hole and multi-hole QChips by applying a depolarization step protocol from -100 mV to +60 mV for 200 ms in increments of 10 mV. The time between sweeps was 15 s (Fig. 3).

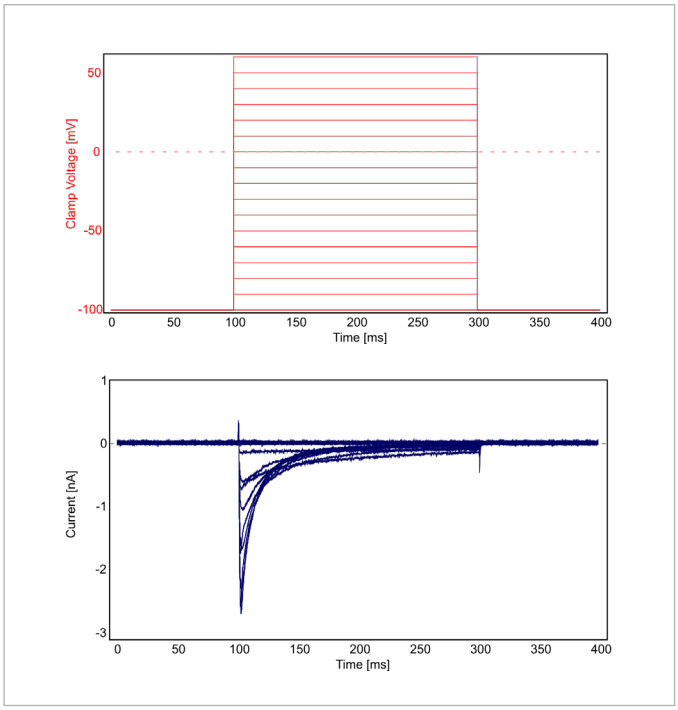

Fig. 3: Representative measurement of current-voltage relationship using a single-hole QChip. Top: Depolarization step protocol in 10 mV increments from -100 mV to +60 mV. Bottom: Ca^{2+} current response of a cell expressing $Ca_V1.2$ channels.

The peak current was extracted at each voltage step, normalized to the peak current at 10 mV and plotted as a function of voltage (Fig. 4).

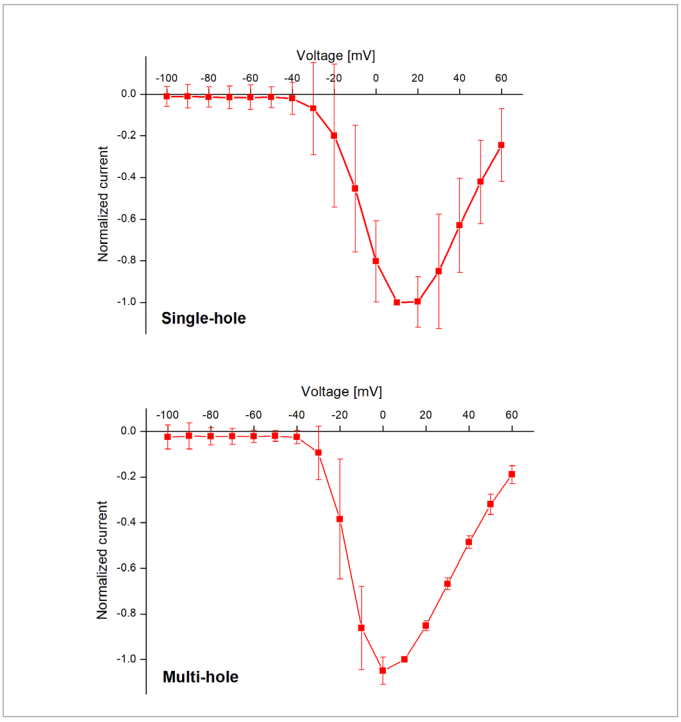

Fig. 4: Current-voltage plots of normalized current vs. voltage (mV) for a single-hole QChip (top) and a multi-hole QChip (bottom). The error bars are the SD.

The current-voltage relationship is in agreement with previous findings[2]. Note that the current-voltage plot recorded on multi-hole QChips is left-shifted due to a lack of R_S compensation.

3. Pharmacology of $Ca_V1.2$ channels

The $Ca_V1.2$ pharmacology was quantified on single-hole and multi-hole QChips. Cells were clamped to -100 mV and Ca^{2+} currents were evoked by application of a depolarization step to +20 mV for 200 ms (see Fig. 1). The experiment setup in the Sophion Viewpoint software is shown in Figure 5. After 10 initial depolarization steps (15 s between sweeps), 2 blocks of 10 depolarization trains (30 s between sweeps) were executed. Each train contained 5 pulses at 2.5 Hz (Fig. 5).

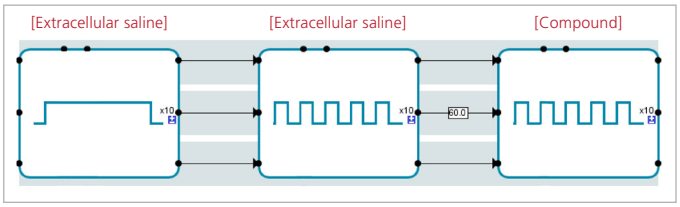

Fig. 5: Experiment setup in the Sophion Viewpoint software for $Ca_V1.2$ pharmacology experiments. Voltage protocols: block 1: 10 single depolarization steps (left), block 2 and 3: 10 train depolarizations (middle and right). The compound was applied in block 3 (right).

The saline baseline was established in the second block of the experiment and in the third block of the experiment the three compounds, diltiazem, nifedipine and verapamil, were applied in 7 concentrations (non-cumulative). The concentration range was a 3-fold dilution with the highest concentration being:

- Diltiazem 100 µM
- Nifedipine 3 µM
- Verapamil 50 µM

The peak current of the last train depolarization step in block 3 was normalized to the corresponding peak current in block 2 according to: $1- \left(\frac{I_{peak}(compound)}{I_{peak}(saline)} \right)$ and plotted as a function of concentration for the three compounds (Fig. 6 and Fig. 7).

The IC_{50} values were extracted by fitting the Hill equation to the data and listed in Table 1 together with literature values.

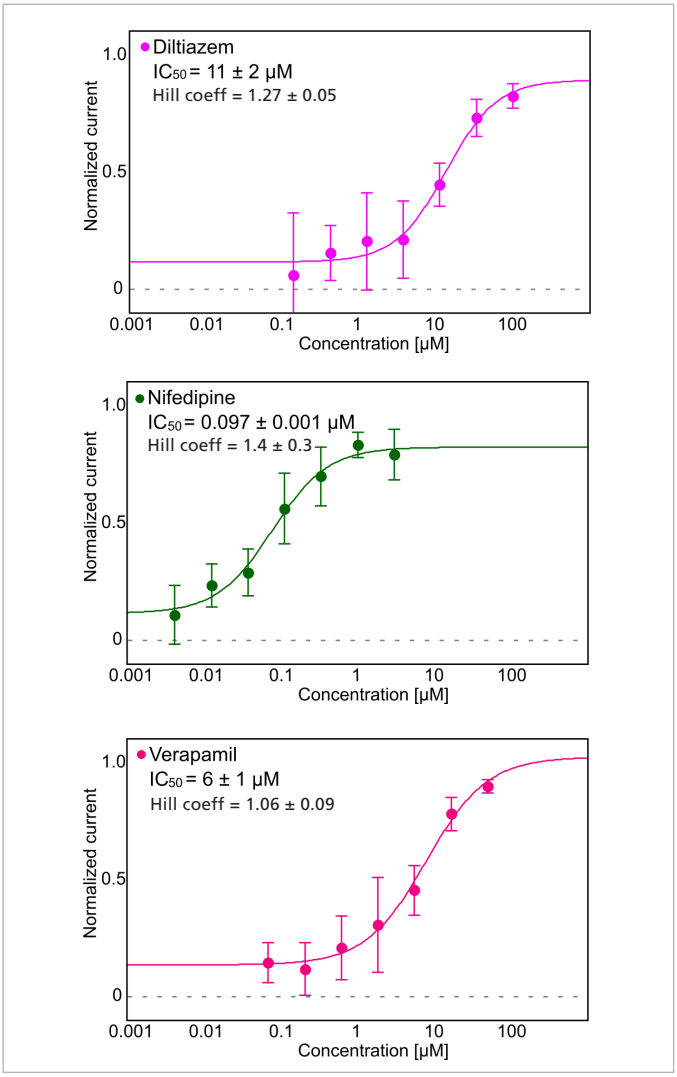

Fig. 6: Dose-response relationships measured using **single-hole QChips** for diltiazem (left), nifedipine (middle) and verapamil (right). The IC_{50} values and Hill coefficients were estimated by fitting the Hill equation to the data and the reported values are the average ± SD of three QChips with a total of 48 experiments per compound.

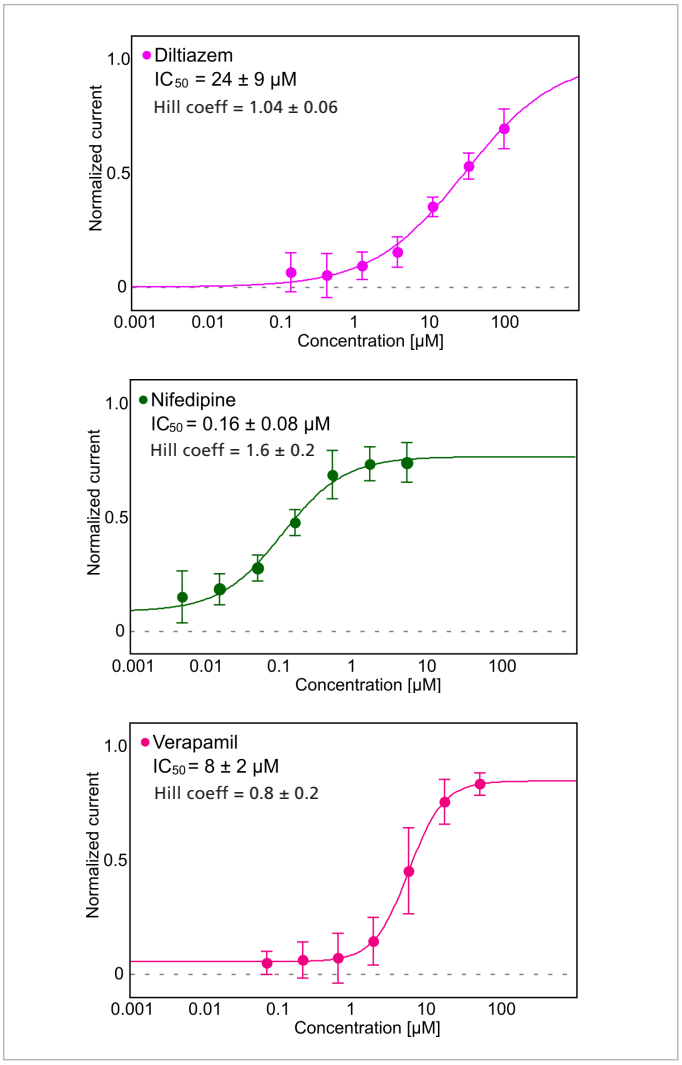

Fig. 7: Dose-response relationships measured using **multi-hole QChips** for diltiazem (left), nifedipine (middle) and verapamil (right). The IC_{50} values and Hill coefficients were estimated by fitting the Hill equation to the data and the reported values are the average ± SD of three QChips with a total of 48 experiments per compound.

Table 1: Potency of the three tested compounds. IC_{50} values (average ± SD) for the listed compounds, quantified on single-hole and multi-hole QChips, together with literature values.

Compound	IC_{50} (µM) Single-hole	N_{QChip}	IC_{50} (µM) Multi-hole	N_{QChip}	IC_{50} (µM) Literature values[11]
Diltiazem	11 ± 2	3	24 ± 9	3	5.6 ± 0.2
Nifedipine	0.097 ± 0.01	3	0.16 ± 0.08	3	0.056 ± 0.002
Verapamil	6 ± 1	3	8 ± 2	3	2.69 ± 0.09

The IC_{50} values were in good agreement with IC_{50} values measured on other $Ca_V1.2$ cell lines in-house (11 µM ± 3 µM, 0.052 µM ± n/a µM, 5 µM ± 2 µM for diltiazem, nifedipine and verapamil, respectively).

4. Rundown analysis using multi-hole QChips

A concern in $Ca_V1.2$ assays is the rundown of the current under continuous stimulation. Here, the rundown was quantified using multi-hole QChips. Cells were clamped to -100 mV and Ca^{2+} currents were evoked by application of a depolarization step to +20 mV for 200 ms (see Fig.1). The experiment setup in the Sophion Viewpoint software is shown in Figure 8. After 10 initial depolarization steps (block 1, 15 s between sweeps), an additional 50 sweeps of single depolarization steps were applied (block 2, 15 s between sweeps), followed by 25 depolarization trains, each containing 5 pulses at 2.5 Hz (block 3, 30 s between sweeps).

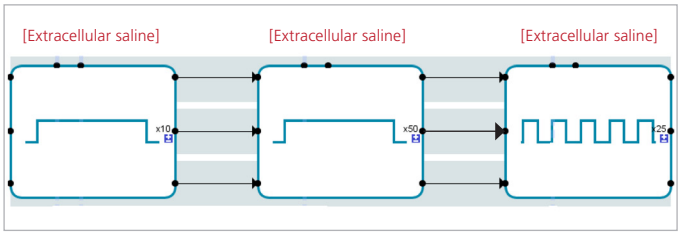

Fig. 8: Experiment setup for a $Ca_V1.2$ rundown experiment in the Sophion Viewpoint software. Voltage protocols: block 1: 10 single depolarization steps (left), block 2: 50 depolarization steps (middle), and block 3: 25 train depolarizations.

The rundown was quantified for the second experiment block (see Fig. 8) by comparing the peak currents evoked by the 1st and 50th depolarization (Fig. 8):

$$\text{Rundown (\% per min):} \quad \frac{I_{peak_1} - I_{peak_{50}}}{I_{peak_1} \cdot \Delta t} \cdot 100\%$$

where Δt is the time between sweep 1 and 50 in minutes. The mean rundown was (1.2 ± 0.9)% per min.

The rundown was also quantified for the third experiment block (see Fig. 8), by comparing the 1st and 25th train depolarization (see Fig. 9). For the first depolarization within the train the mean rundown was (2.0 ± 0.5)% per min. For the last depolarization within the train the mean rundown was (2.5 ± 0.5)% per min.

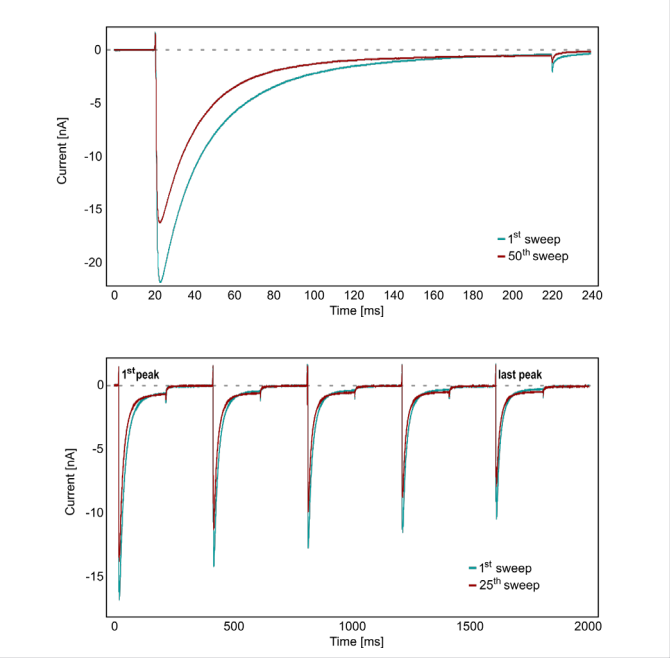

Fig. 9: Example current traces used for quantifying rundown. Top 1st (green) and 50th (red) depolarization in experiment block 2. Bottom: 1st (green) and 25th (red) depolarization train in experiment block 3. We quantified rundown for the first and last peak of the train.

5. Recordings of Ca^{2+} currents and rundown analysis using a step-ramp (CiPA) protocol

Here we report the response of $Ca_V1.2$ cells to a step-ramp voltage protocol, which is similar to the voltage protocol proposed in the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative. The objective of the CiPA initiative is to initiate a new paradigm for safety assessment, taking several ion channels – including $Ca_V1.2$ – into account. For the $Ca_V1.2$ CiPA voltage protocol, cells were clamped to -80 mV and Ca^{2+} currents were evoked by application of a depolarization step to 0 mV for 40 ms, followed by an additional depolarization step to +20 mV for 200 ms and a 100 ms ramp to -80 mV (Fig. 10). In this study, a total of 100 sweeps (15 or 30 s between sweeps) of the CiPA protocol stimulations (block 2 and 3) were flanked by five 200 ms standard depolarization pulses from -100 mV to 20 mV (block 1 and 4), as indicated in the Sophion Viewpoint software (Fig. 11).

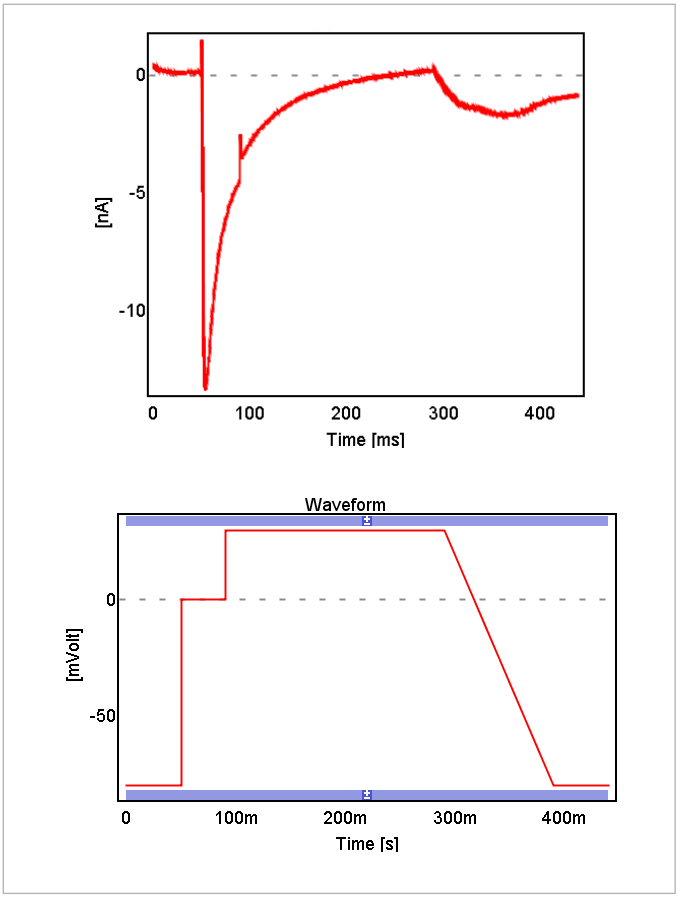

Fig. 10: Original recording on a multi-hole QChip of a $Ca_V1.2$ Ca^{2+} current (top) following a depolarization according to the CiPA protocol (bottom).

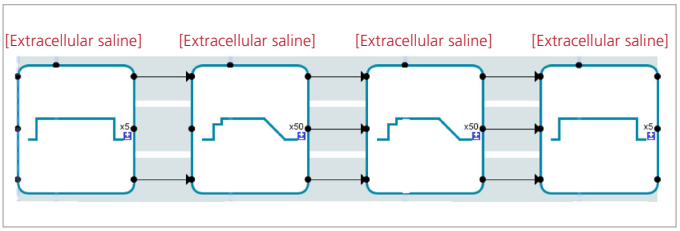

Fig. 11: CiPA experiment setup for a $Ca_V1.2$ rundown measurements in the Sophion Viewpoint software. Voltage protocols: block 1 and 4: 5 single depolarization steps (15 s between sweeps), block 2: 50 CiPA depolarization steps (30 s between sweeps), block 3: 50 CiPA depolarization steps (15 s between sweeps).

According to the previously defined success criteria, the CiPA protocol execution had a 67% success rate and rundown of (1.0 ± 0.7)% per min (30 s in between sweeps) and (1 ± 1)% per min (15 s in between sweeps).

Conclusion

$Ca_V1.2$ Ca^{2+} currents were recorded on the high-throughput platform Qube 384 on both single-hole and multi-hole QChips. The reported current-voltage relationship and pharmacology experiments were in accordance with literature values and highly reproducible. The current levels were stable over time with an average rundown of **(1.2 ± 0.9)%** per min and high success rates were achieved (up to **89%** and **94%** for single-hole and multi-hole QChips, respectively) with cells at passage numbers below 20. In addition, the CiPA protocol exhibited stable currents albeit with slightly lower success rates. Conclusively, Qube is an ideal platform for running $Ca_V1.2$ experiments for screening as well as characterization.

Material and methods

Experiments in this study were performed on HEK-h$Ca_V1.2$ cells, kindly provided by SB Drug Discovery. The cell line is stably transfected with the $Ca_V1.2$ channel and cultured in 30 µM verapamil according to SB Drug Discovery guidelines. HEK-h$Ca_V1.2$ cells were harvested according to Sophion standard procedures.

Author:
Melanie Schupp, application scientist, Sophion Bioscience A/S
Kadla R Rosholm, application scientist, Sophion Bioscience A/S

References:

1. Balazubramanian, B et al. Optimization of $Ca_V1.2$ screening with an automated planar patch clamp platform, J Phamacol Tox Met 2009;**59**:62-72
2. Kuryshev YA et al. Evaluating State Dependence and Subtype Selectivity of Calcium Channel Modulators in Automated Electrophysiology Assays. Assay Drug Dev Technol 2014;**12**(2): 110–119
3. Sinnegger-Brauns MJ et al. Isoform-specific regulation of mood behavior and pancreatic β cell and cardiovascular function by L-type Ca^{2+} channels. J Clin Invest 2004;**113**:1430-9
4. Moosmang S et al. Dominant role of smooth muscle L-type calcium channel Cav1.2 for blood pressure regulation. EMBO J 2003;**22**:6027-34
5. Zhang J et al. Role of $Ca_V1.2$ L-type Ca^{2+} channels in vascular tone: effects of nifedipine and Mg^{2+}. Am J Physiol Heart Circ Physiol 2007;**292**:H415-25
6. Zamponi, GW Targeting voltage-gated calcium channels in neurological and psychiatric diseases. Nat Rev Drug Discovery 2007;**15**:19-34
7. Liao P & Soong TW $Ca_V1.2$ channelopathies: from arrhythmias to autism, bipolar disorder, and immunodeficiency. Eur J Physiol 2016;**460**:353-359
8. Hofman F et al. L-type $Ca_V1.2$ calcium channels: from in vitro findings to in vivo function. Physiol Rev 2014;**94**:303-326
9. Reuter H Calcium channel modulation by neurotransmitters, enzymes and drugs. Nature 1983;**301**:569–574
10. Hennessey JA et al. A CACNA1C variant associated with reduced voltage-dependent inactivation, increased $Ca_V1.2$ channel window current, and arrhythmogenesis. PLOS One 2014;**9**,e106982
11. Elkins CR et al. Variability in high-throughput ion-channel screening data and consequences for cardiac safety assessment J Phamacol Tox Met 2013;**68**:112-122

Sophion Bioscience A/S, Baltorpvej 154, 2750 Ballerup, Denmark
Phone: +45 4460 8800, E-mail: info@sophion.com

sophion.com