hCaV1.2 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 CaV1.2 channel

Summary
The development of screening assays for the CaV1.2 channel has been challenging due to the tendency of CaV1.2 expressing cell lines to exhibit declining current levels (rundown) during the experiment1,2. Here we report a robust CaV1.2 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

Results and discussion
The following experiments were carried out:
1. Recordings of Ca2+ currents
2. Current-voltage relationship of CaV1.2 channels
3. Pharmacology of CaV1.2 channels
4. Rundown analysis using multi-hole QChips
5. Recordings of Ca2+ currents and rundown analysis using a step-ramp (CiPA) protocol

1. Recordings of Ca2+ currents
Cells were clamped to -100 mV and Ca2+ currents were evoked by application of a depolarization step to +20 mV for 200 ms (Fig. 1).

Introduction
The L-type voltage-gated calcium channel CaV1.2 is expressed in various mammalian tissues, including heart and smooth muscle3-5, and its dysfunction has been implicated in a range of cardiovascular and neurological diseases6-7. Opening of the CaV1.2 channel results in an increase in the intracellular concentration of calcium ions (Ca2+), affecting a variety of cellular processes including muscle contraction, hormone secretion and neuronal transmission7-9, thus rendering the channel an important target in e.g. safety pharmacology screening.

CaV1.2 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 benzothiazepines8,9. Following activation, the channel displays both Ca2+- and voltage-dependent inactivation10. In this study, current traces from HEK-hCaV1.2 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.

Fig. 1: Representative recordings of Ca2+ 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 CaV1.2 expressing cells.
The criteria for a successful recording were (average of first 10 sweeps):

**Single-hole QChip:**
- $I_{\text{peak}} < -100 \text{ pA}$
- $R_{\text{membrane}} > 100 \text{ M}\Omega \text{ per cell}$
- $C_{\text{total}} > 4 \text{ pF per cell}$

**Multi-hole QChip:**
- $I_{\text{peak}} < -500 \text{ pA}$
- $R_{\text{membrane}} > 100 \text{ M}\Omega \text{ per cell}$
- $C_{\text{total}} > 4 \text{ pF per cell}$

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

A plate-view of Ca$_{\text{V}1.2}$ 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.

**2. Current-voltage relationship of Ca$_{\text{V}1.2}$ 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).

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).
The current-voltage relationship is in agreement with previous findings². Note that the current-voltage plot recorded on multi-hole QChips is left-shifted due to a lack of Rᵢ compensation.

3. Pharmacology of Caᵥ1.2 channels

The Caᵥ1.2 pharmacology was quantified on single-hole and multi-hole QChips. Cells were clamped to -100 mV and Ca²⁺ 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).

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 - \( \frac{I_{\text{peak}}(\text{compound})}{I_{\text{peak}}(\text{saline})} \) and plotted as a function of concentration for the three compounds (Fig. 6 and Fig. 7).

The IC₅₀ values were extracted by fitting the Hill equation to the data and listed in Table 1 together with literature values.
The IC$_{50}$ values were in good agreement with IC$_{50}$ values measured on other Ca$_{v}1.2$ 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$_{v}1.2$ 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).

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

\[
\text{Rundown (\% per min): } \left( \frac{I_{\text{peak,1}} - I_{\text{peak,50}}}{I_{\text{peak,1}}} \right) \cdot 100\%
\]

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 1$^{st}$ and 25$^{th}$ 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.

### 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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (µM) Single-hole</th>
<th>N$_{\text{QChip}}$</th>
<th>IC$_{50}$ (µM) Multi-hole</th>
<th>N$_{\text{QChip}}$</th>
<th>IC$_{50}$ (µM) Literature values$^{11}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diltiazem</td>
<td>11 ± 2</td>
<td>3</td>
<td>24 ± 9</td>
<td>3</td>
<td>5.6 ± 0.2</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>0.097 ± 0.01</td>
<td>3</td>
<td>0.16 ± 0.08</td>
<td>3</td>
<td>0.056 ± 0.002</td>
</tr>
<tr>
<td>Verapamil</td>
<td>6 ± 1</td>
<td>3</td>
<td>8 ± 2</td>
<td>3</td>
<td>2.69 ± 0.09</td>
</tr>
</tbody>
</table>
5. Recordings of Ca\textsuperscript{2+} currents and rundown analysis using a step-ramp (CiPA) protocol

Here we report the response of Ca\textsubscript{1.2} cells to a step-ramp voltage protocol, which is similar to the voltage protocol proposed in the Comprehensive in \textit{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\textsubscript{1.2} – into account. For the Ca\textsubscript{1.2} CiPA voltage protocol, cells were clamped to -80 mV and Ca\textsuperscript{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).
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\(_{1.2}\) 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\(_{1.2}\) experiments for screening as well as characterization.

Material and methods

Experiments in this study were performed on HEK-hCa\(_{1.2}\) cells, kindly provided by SB Drug Discovery. The cell line is stably transfected with the Ca\(_{1.2}\) channel and cultured in 30 µM verapamil according to SB Drug Discovery guidelines. HEK-hCa\(_{1.2}\) cells were harvested according to Sophion standard procedures.

References:

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