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Report

Electrophysiological validation of $Na_v1.5$, $Ca_v1.2$ and hERG ion channel cell lines using the automated SyncroPatch platform

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Project Description

SyncroPatch automated electrophysiological assessment of three ion channel cell lines, $Na_v1.5$, hERG and $Ca_v1.2$.

Contents

1 Summary

We have assessed the performance of three cardiac ion channel cell lines ($Na_V1.5$, $Ca_V1.2$ and hERG) on SyncroPatch automated electrophysiology using two different protocols.

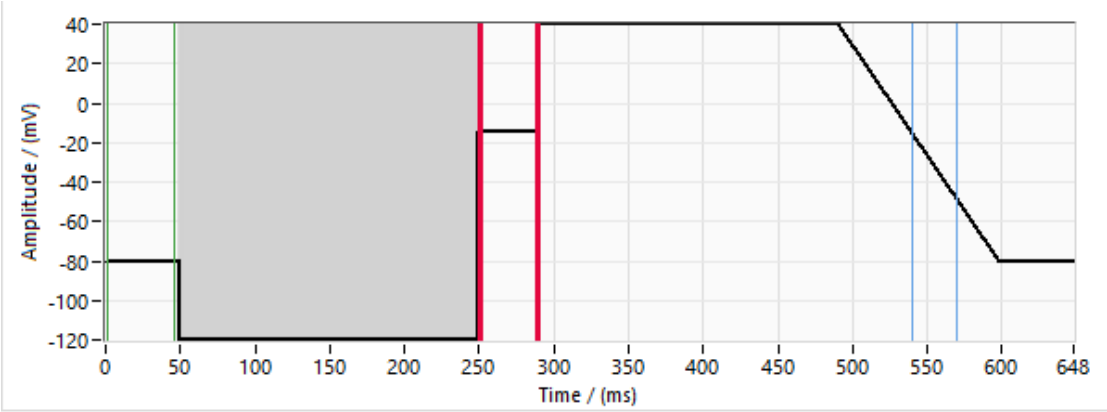
2 Methods

2.1 Assay summary

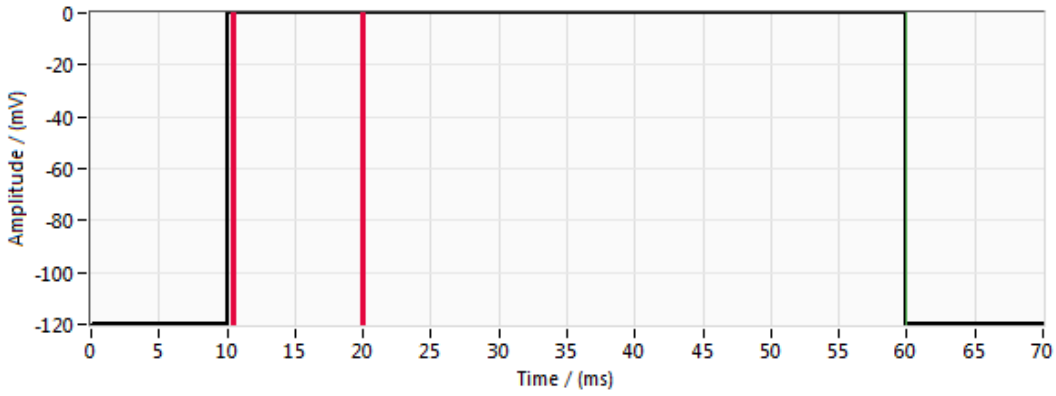
The automated electrophysiological assay was carried out on the SyncroPatch384 platform at room temperature. The series resistance and quality of seals were continuously monitored during the experiments. On the day of the experiment, the cell lines were each cultured according to standard operating procedures. Internal and external physiological solutions were freshly prepared prior to the assay.

2.2 Voltage protocols

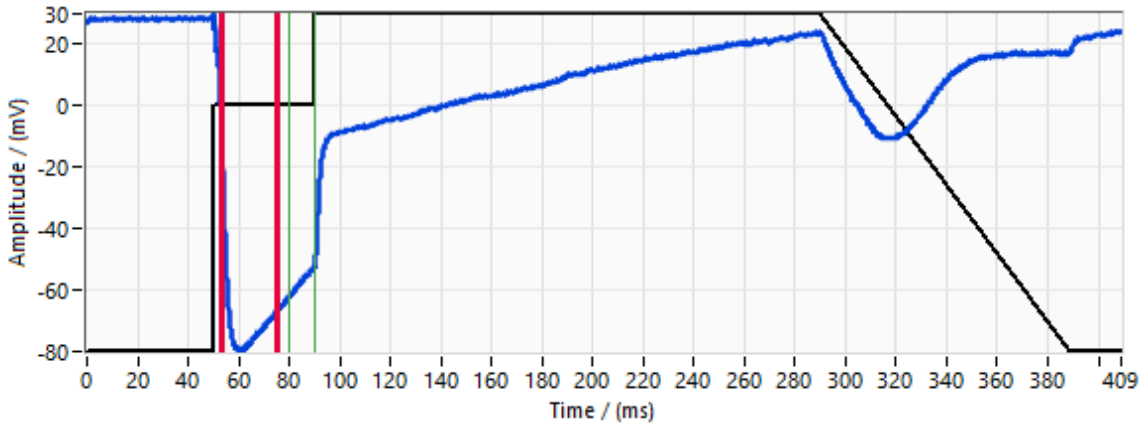
$Na_V1.5$ channel protocol 1: The protocol was repeated every 5 seconds, from a holding potential of -80 mV. The red lines indicate where the current was measured.



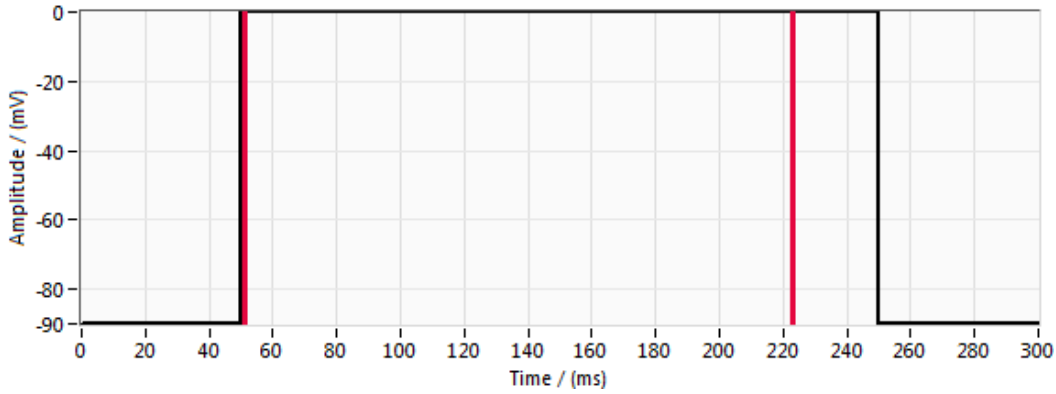
$Na_V1.5$ channel protocol 2: The protocol was repeated every 5 seconds, from a holding potential of -120 mV. The red lines indicate where the current was measured.



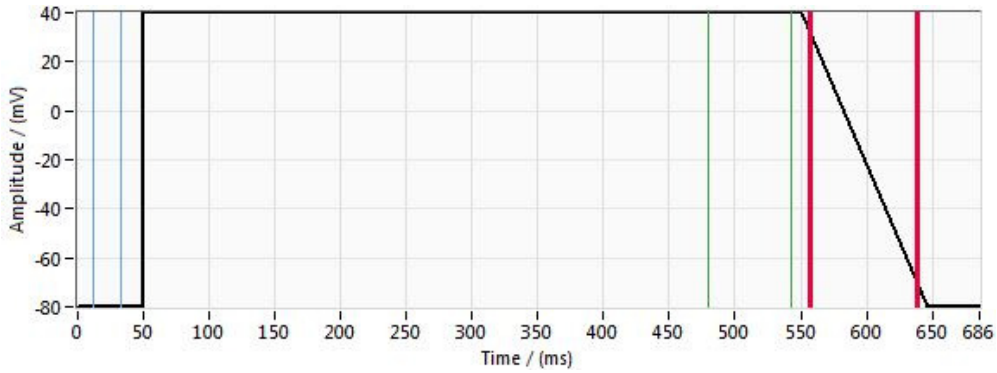
$Ca_V1.2$ channel protocol 1: This was repeated every 15 seconds, from a holding potential of -80 mV. The red lines indicate where the current was measured. The blue line indicates the currents.



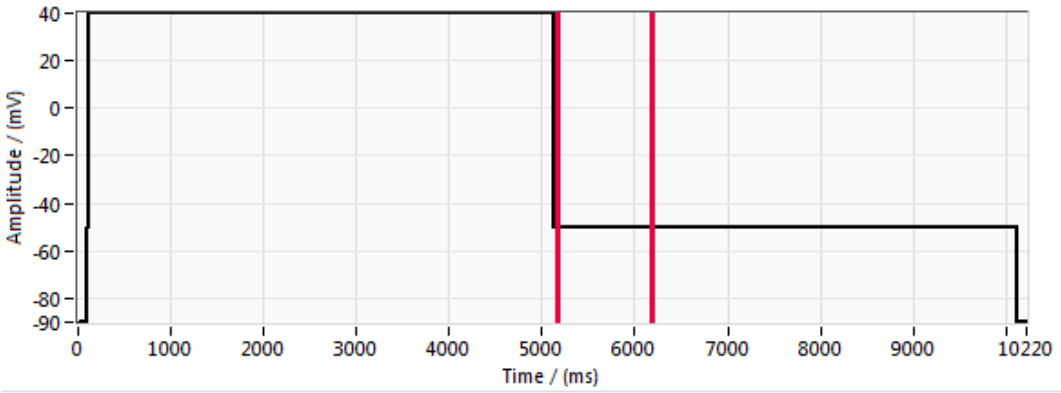
$Ca_V1.2$ channel protocol 2: The protocol was repeated every 20 seconds, from a holding potential of -90 mV. The red lines indicate where the current was measured.



hERG channel protocol 1: This protocol was repeated every 5 seconds, from a holding potential of -80 mV. The red lines indicate where the current was measured.



hERG channel protocol 2: The protocol was repeated every 12.5 seconds, from a holding potential of -90 mV. The red lines indicate where the current was measured.



2.3 Application protocol

The IC_{50} of each compound was calculated using a single concentration of antagonist per cell (at least 2 positive cells per concentration) and 6 concentrations per antagonist.

After recording for a minimum of 60 second control period, a single concentration of the antagonist was applied and allowed to reach steady-state, after which a saturating concentration of control antagonist was added.

2.4 Analysis

Data was analyzed using the Nanion Data Control384 software. The graphs have been plotted using the maximum inward or outward current values obtained during the patch-clamp recordings. Data were normalised using the maximum activation obtained during the pre-compound control as the bottom value (0.0) and maximum inhibition induced by the saturating blocker as the top value (1.0).

3 Results

3.1 $Na_V1.5$ channel

The IC_{50} value of the reference compound and average current are summarised in Table 1. Figures 1-4 show the superimposed current traces, the time-course plot and the IC_{50} graphs obtained for the positive (Propafenone) and negative (0.3% DMSO) controls.

Table 1: Summary of the IC_{50} values

Protocol	Propafenone IC_{50} (μM)	Cells (n)	DMSO IC_{50} (μM)	Cells (n)	Average current ±S.E.M (pA)
1	1.2	79	N.C	72	-4202± 176
2	1.4	65	N.C	41	-3581± 203

Table 1: Summary data: IC_{50} values and average current for $Na_V1.5$ cell line; N.C. not calculated due to lack of concentration-dependent block.

Figure 1: Effect of Propafenone on the $Na_v1.5$ channel using Protocol 1

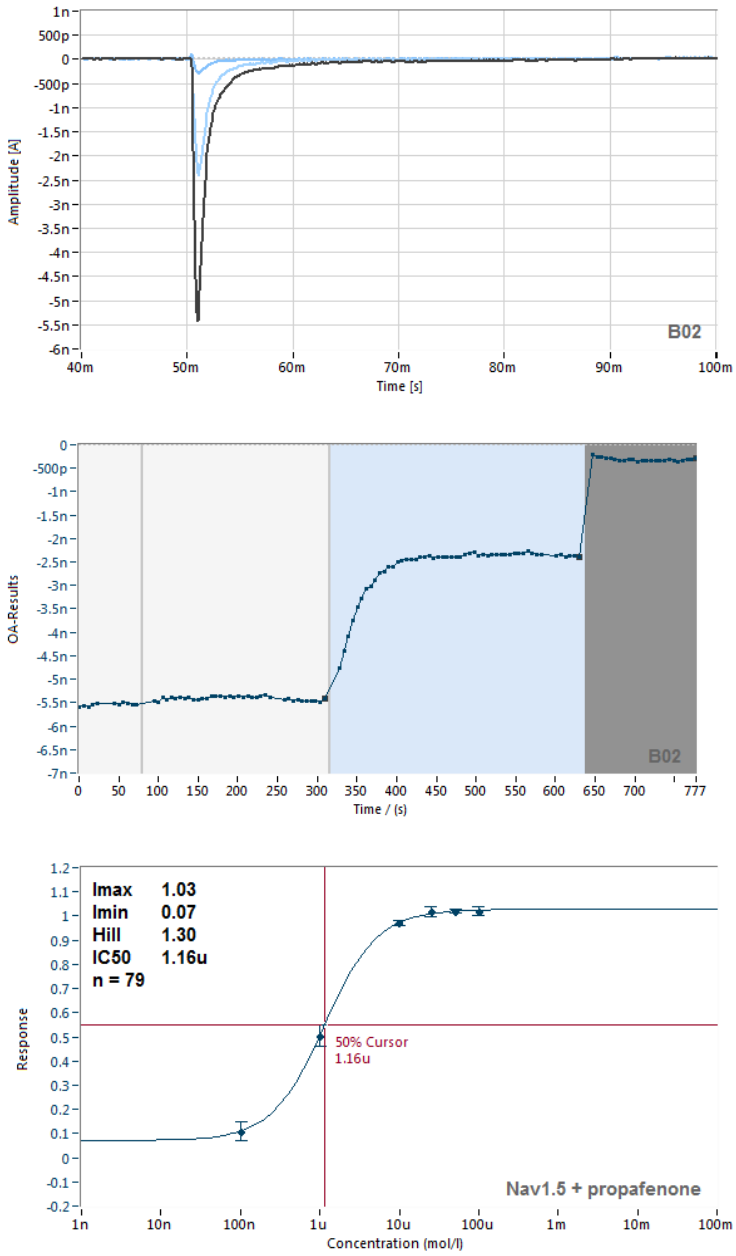


Figure 1: Top panel shows an example of superimposed currents traces during application of a single concentration of reference compound, the control current is shown in black. Middle panel shows the corresponding time-course of effect produced by the test compound (1 µM, blue area). The darker grey column shows maximum concentration of reference blocker. Bottom panel shows IC_{50} plot.

Figure 2: Effect of DMSO on the Nav1.5 channel using Protocol 1

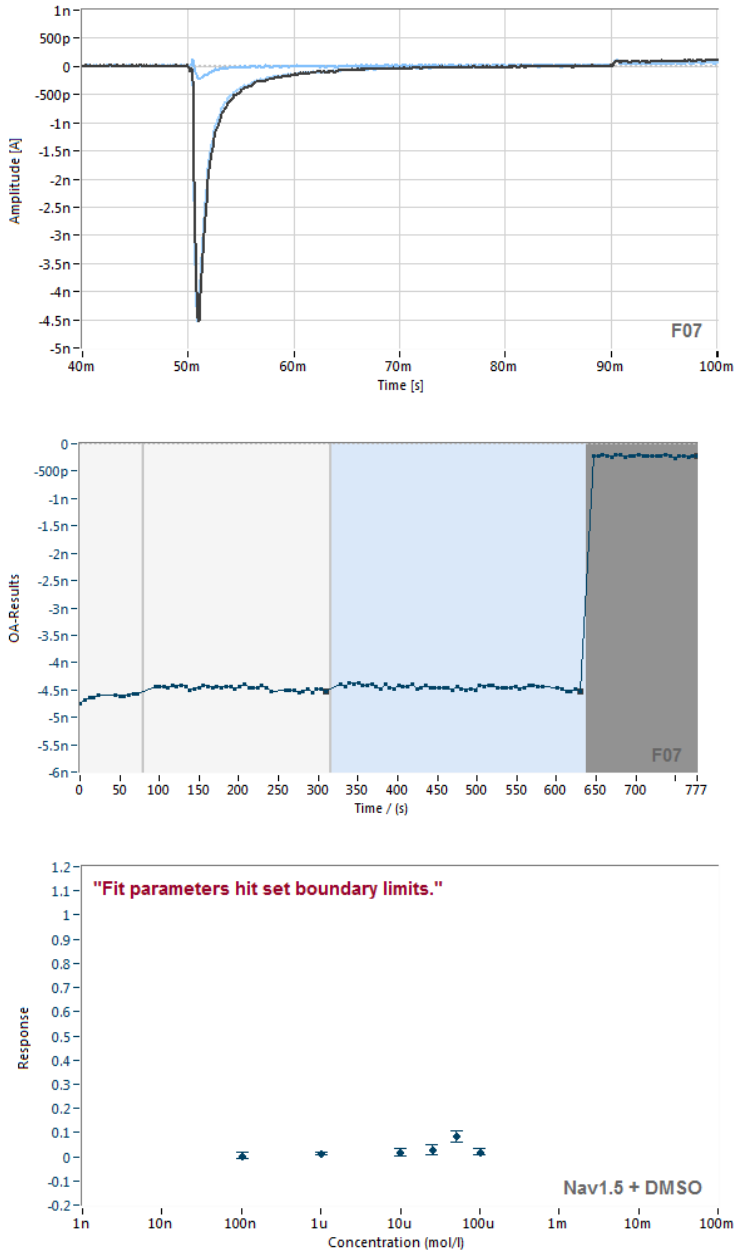


Figure 2: Top panel shows an example of superimposed currents traces during application of a single concentration of DMSO, the control current is shown in black. Middle panel shows the corresponding time-course of effect produced by DMSO (blue area). The darker grey column shows maximum concentration of reference blocker. Bottom panel shows IC_{50} plot.

Figure 3: Effect of Propafenone on the $Na_v1.5$ channel using Protocol 2

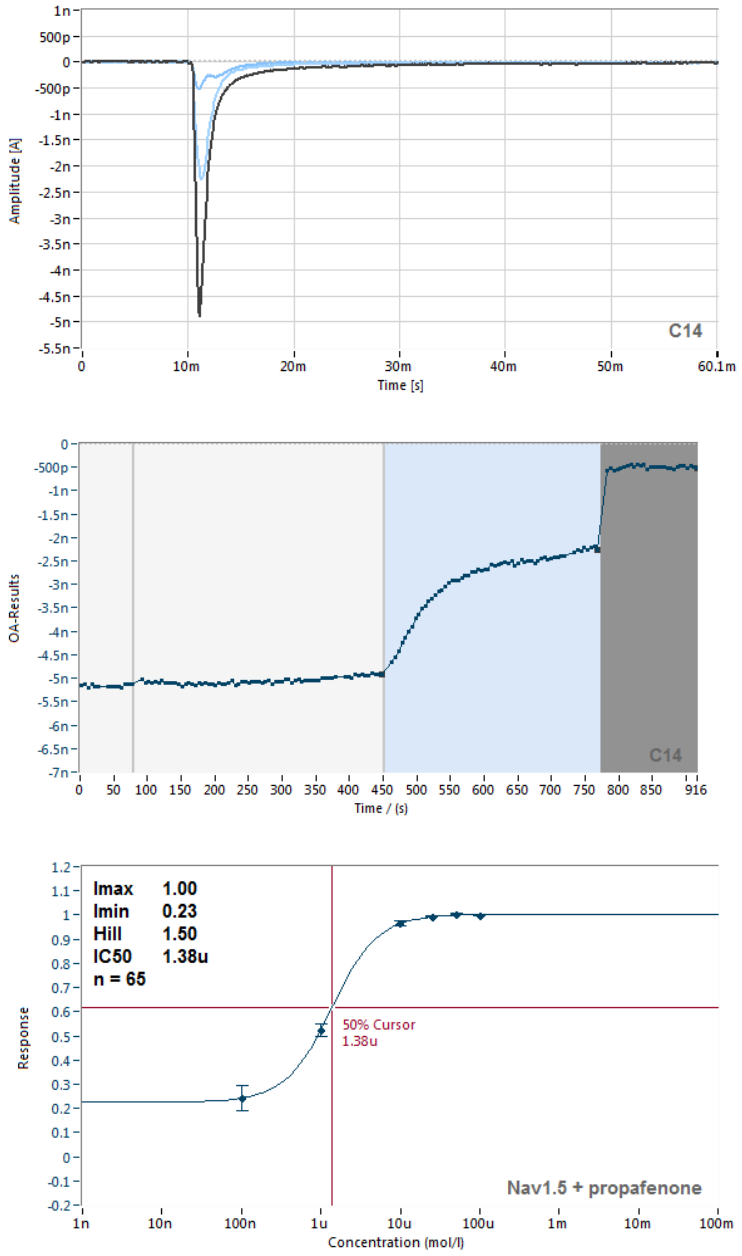


Figure 3: Top panel shows an example of superimposed currents traces during application of a single concentration of reference compound, the control current is shown in black. Middle panel shows the corresponding time-course of effect produced by the test compound (1 µM, blue area). The darker grey column shows maximum concentration of reference blocker. Bottom panel shows IC_{50} plot.

Figure 4: Effect of DMSO on the Nav1.5 channel using Protocol 2

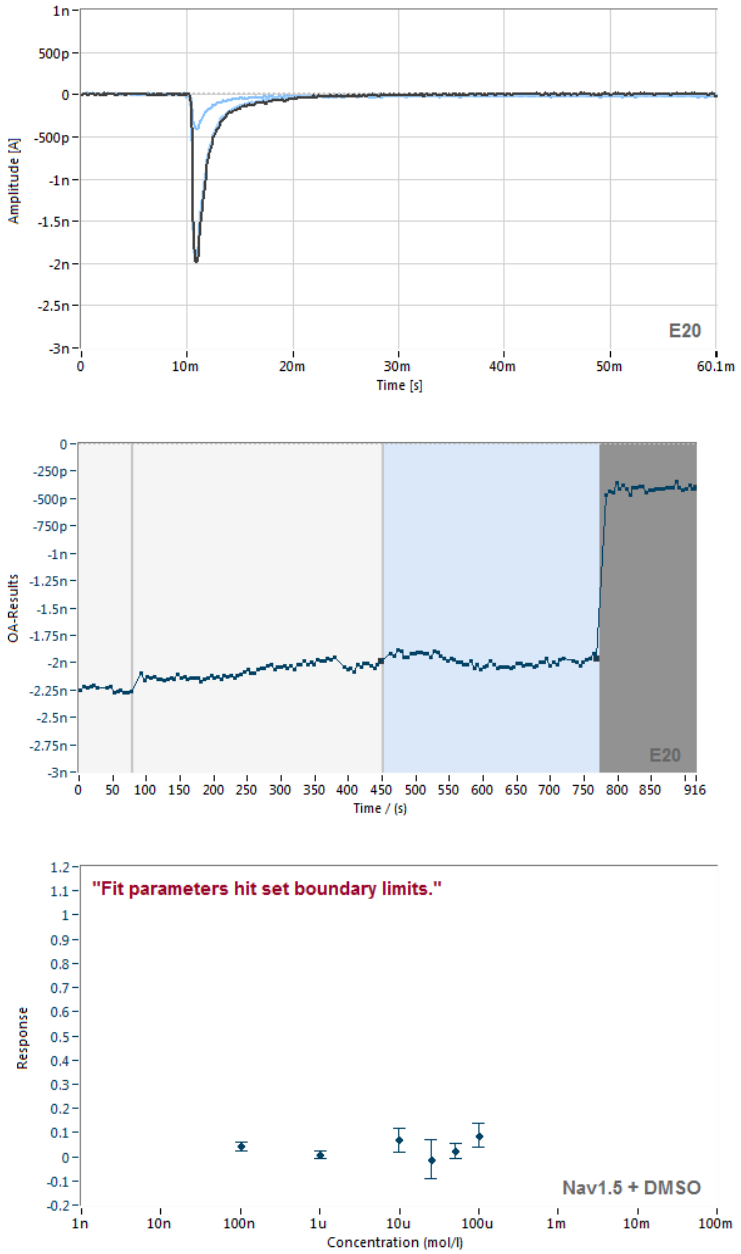


Figure 4: Top panel shows an example of superimposed currents traces during application of a single concentration of DMSO, the control current is shown in black. Middle panel shows the corresponding time-course of effect produced by DMSO (blue area). The darker grey column shows maximum concentration of reference blocker. Bottom panel shows IC_{50} plot.

3.2 $Ca_V1.2$ channel

The IC_{50} value of the reference compound and average current are summarised in Table 2. Figures 5-8 show the superimposed current traces, the time-course plot and the IC_{50} graphs obtained for the positive (Nifedipine) and negative (0.3% DMSO) controls.

Table 2: Summary of the IC_{50} values

Protocol	Nifedipine IC_{50} (μM)	Cells (n)	DMSO IC_{50} (μM)	Cells (n)	Average current ±S.E.M (pA)
1	0.016	39	N.C	44	-2003 ±101
2	0.037	35	N.C	25	-2019 ±170

Table 2: Summary data: IC_{50} values and average current amplitude for $Ca_V1.2$ cell line; N.C. not calculated due to lack of concentration-dependent block.

Figure 5: Effect of Nifedipine on the $Ca_V1.2$ channel using Protocol 1

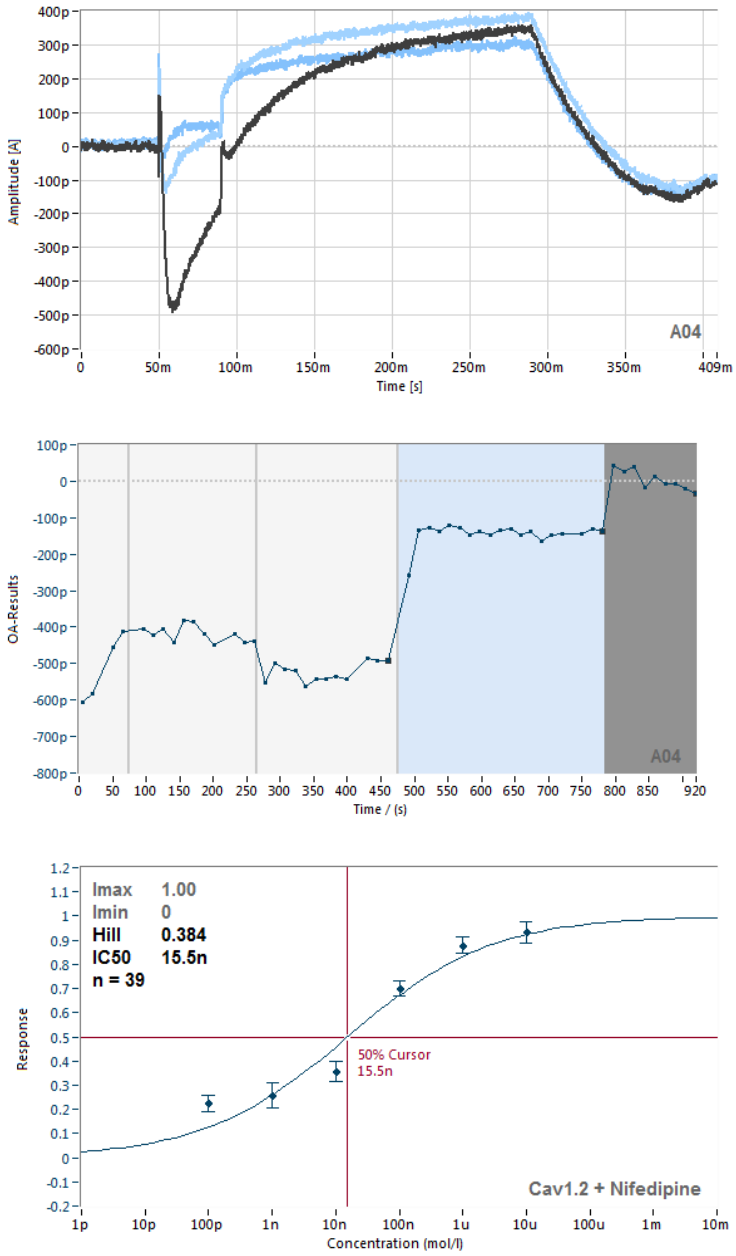


Figure 5: Top panel shows an example of superimposed currents traces during application of a single concentration of reference compound, the control current is shown in black. Middle panel shows the corresponding time-course of effect produced by the test compound (100 nM, blue area). The darker grey column shows maximum concentration of reference blocker. Bottom panel shows IC_{50} plot.

Figure 6: Effect of DMSO on the $Ca_v1.2$ channel using Protocol 1

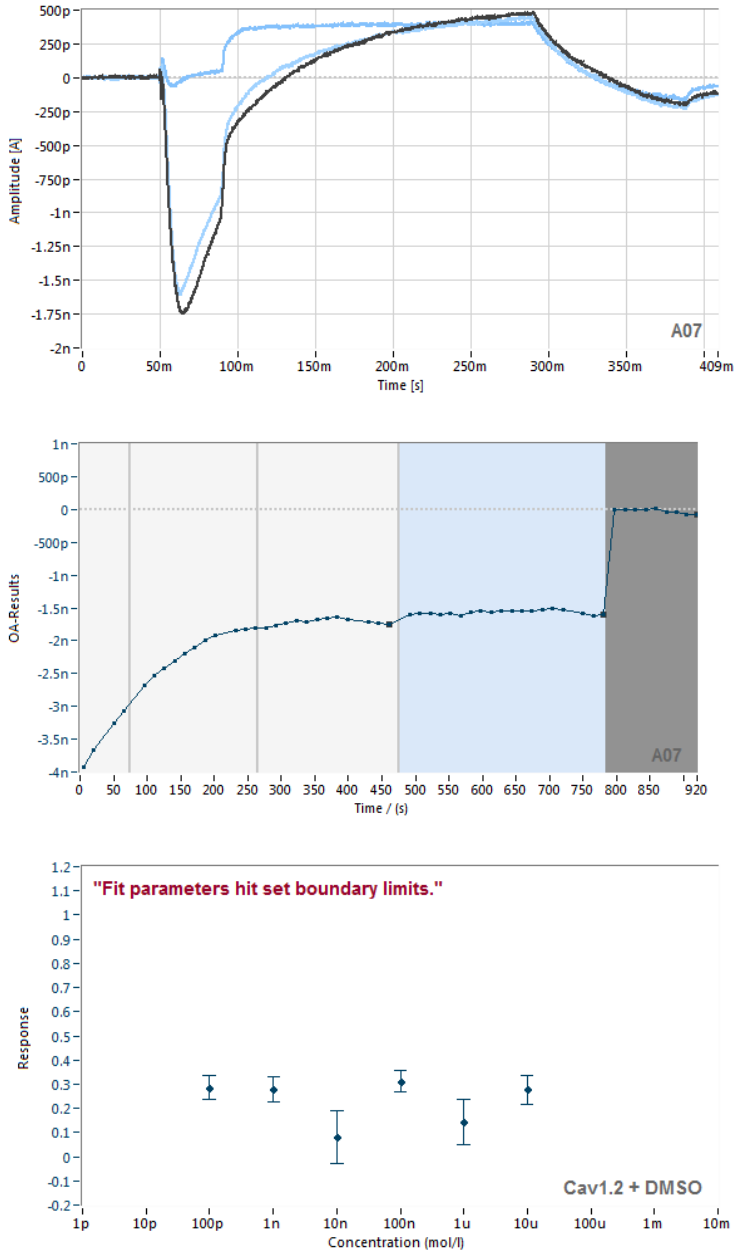


Figure 6: Top panel shows an example of superimposed currents traces during application of a single concentration of DMSO, the control current is shown in black. Middle panel shows the corresponding time-course of effect produced by DMSO (blue area). The darker grey column shows maximum concentration of reference blocker. Bottom panel shows IC_{50} plot.

Figure 7: Effect of Nifedipine on the $Ca_V1.2$ channel using Protocol 2

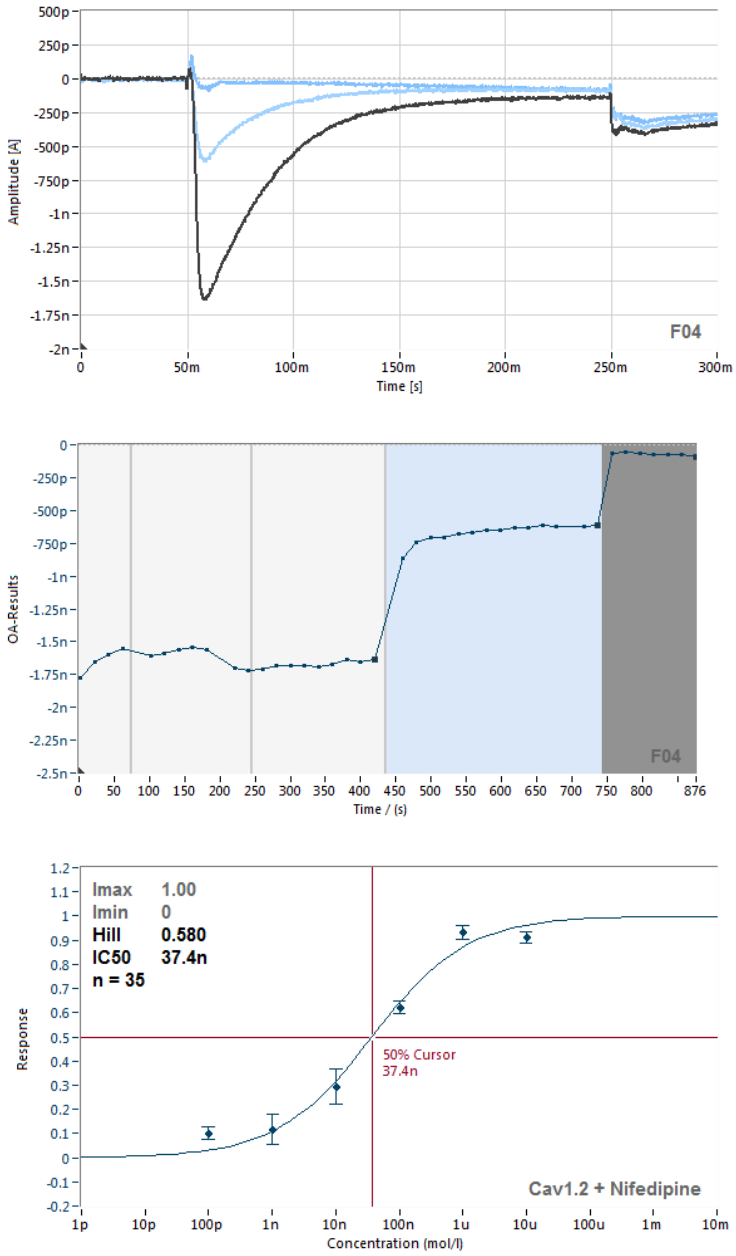


Figure 7: Top panel shows an example of superimposed currents traces during application of a single concentration of reference compound, the control current is shown in black. Middle panel shows the corresponding time-course of effect produced by the test compound (100 nM, blue area). The darker grey column shows maximum concentration of reference blocker. Bottom panel shows IC_{50} plot.

Figure 8: Effect of DMSO on the Cav1.2 channel using Protocol 2

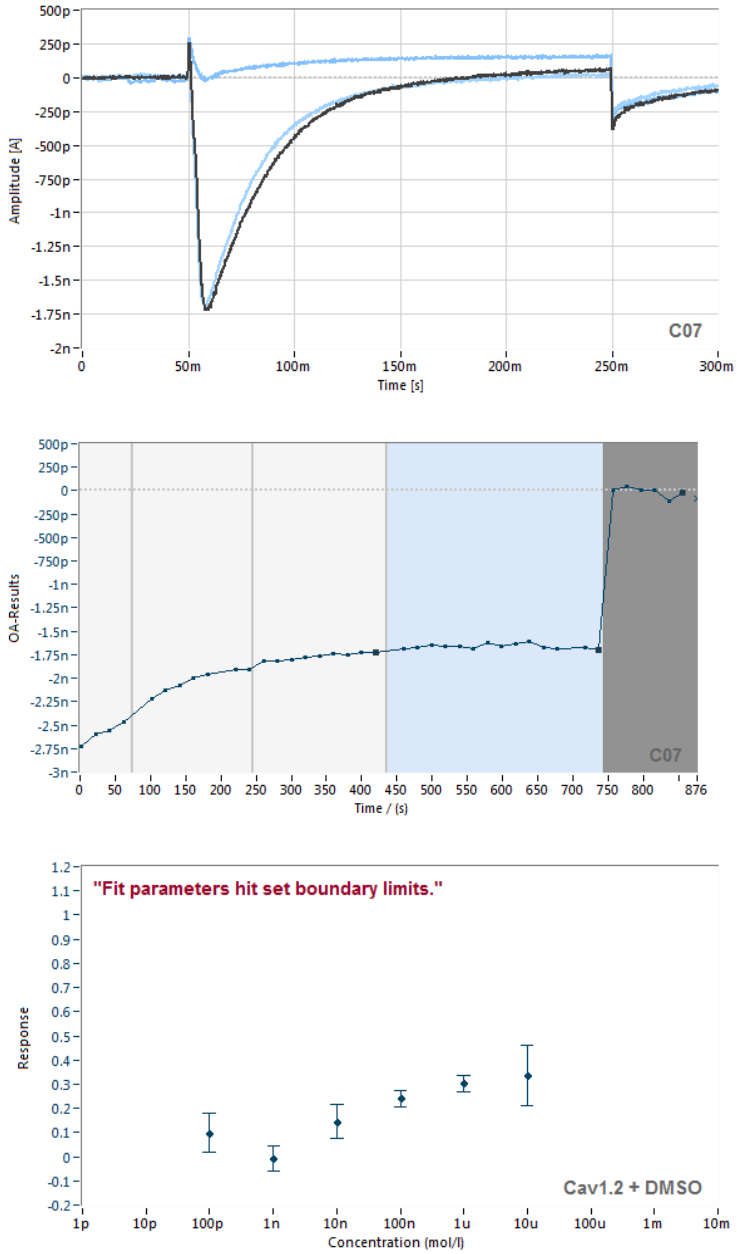


Figure 8: Top panel shows an example of superimposed currents traces during application of a single concentration of DMSO, the control current is shown in black. Middle panel shows the corresponding time-course of effect produced by DMSO (blue area). The darker grey column shows maximum concentration of reference blocker. Bottom panel shows IC_{50} plot.

3.3 hERG channel

The IC_{50} value of the reference compound, average current and % positive cells are summarised in Table 3. Figures 9-12 show the superimposed current traces, the time-course plot and the IC_{50} graphs obtained for the positive (Cisapride) and negative (0.3% DMSO) controls.

Table 3: Summary of the IC_{50} values

Protocol	Cisapride IC_{50} (µM)	Cells (n)	DMSO IC_{50} (µM)	Cells (n)	Average current ±S.E.M (pA)
1	0.193	47	N.C	43	697±477
2	0.029	40	N.C	50	752±38

Table 3: Summary data: IC_{50} values and average current amplitude for hERG cell line; N.C. not calculated due to lack of concentration-dependent block.

Figure 9: Effect of Cisapride on the hERG channel using Protocol 1

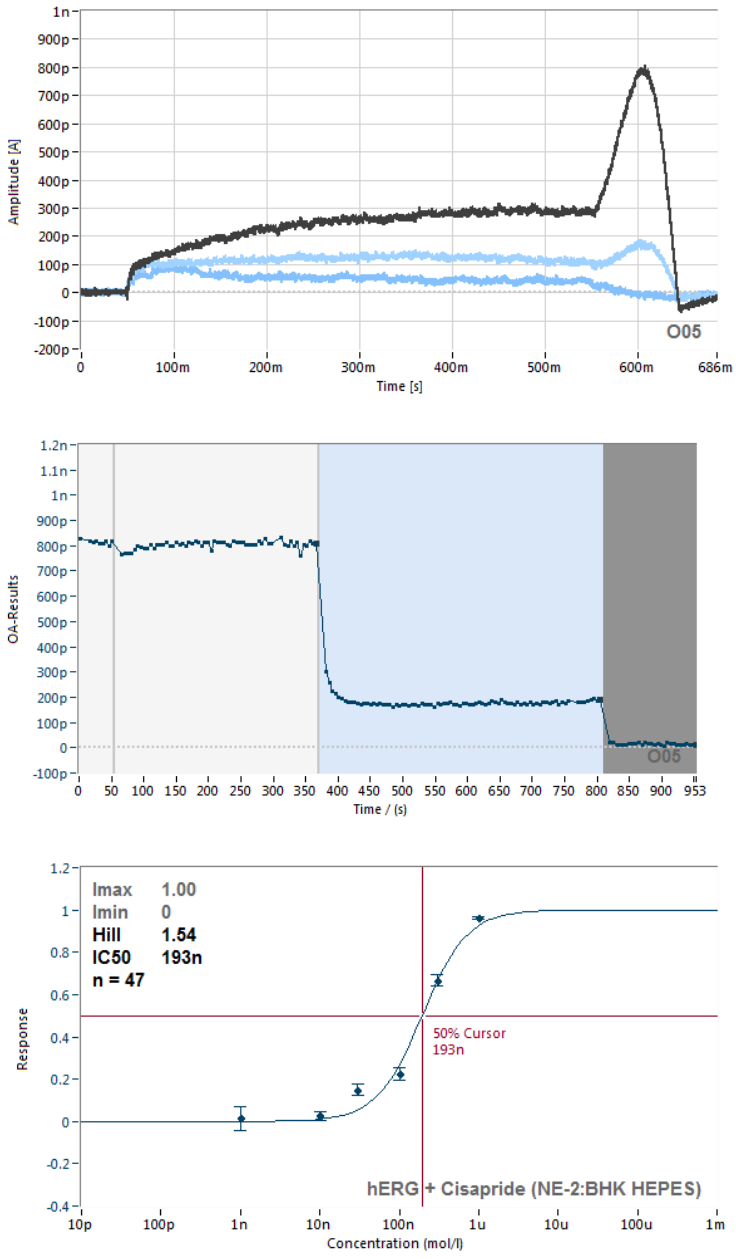


Figure 9: Top panel shows an example of superimposed currents traces during application of a single concentration of reference compound, the control current is shown in black. Middle panel shows the corresponding time-course of effect produced by the test compound (300 nM, blue area). The darker grey column shows maximum concentration of reference blocker. Bottom panel shows IC_{50} plot.

Figure 10: Effect of DMSO on the hERG channel using Protocol 2

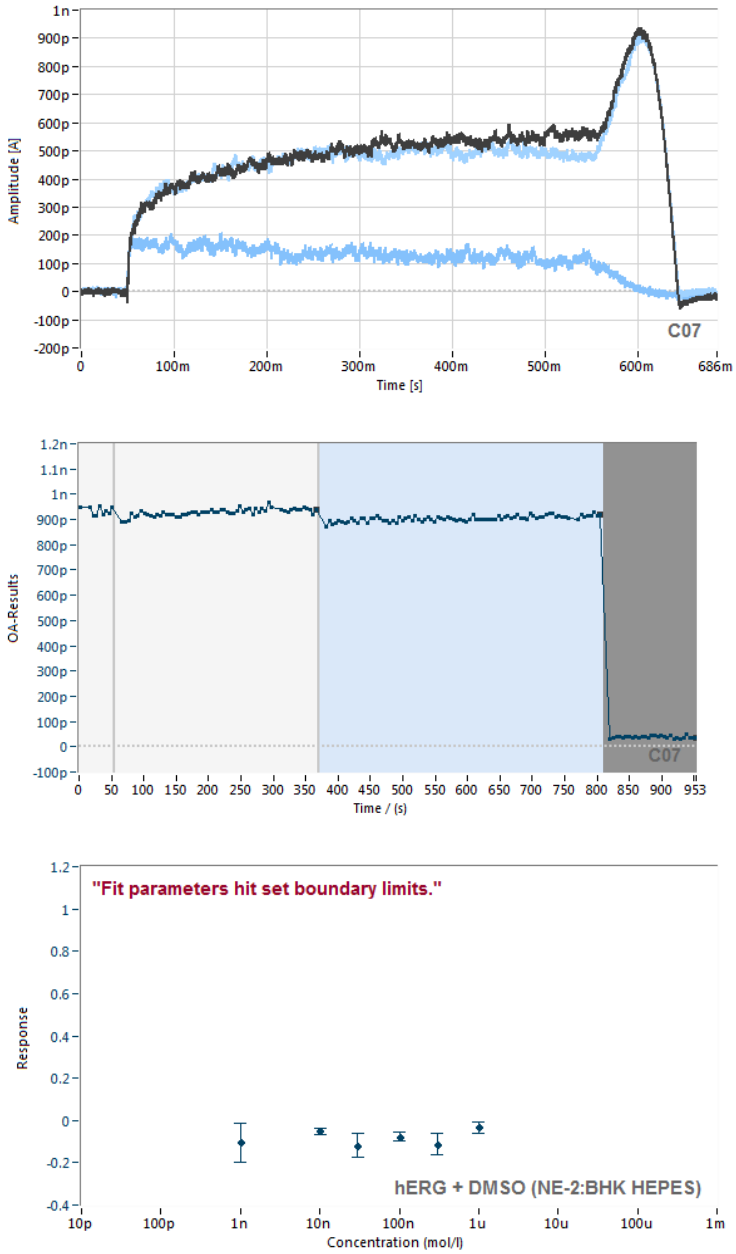


Figure 10: Top panel shows an example of superimposed currents traces during application of a single concentration of DMSO, the control current is shown in black. Middle panel shows the corresponding time-course of effect produced by DMSO (blue area). The darker grey column shows maximum concentration of reference blocker. Bottom panel shows IC_{50} plot.

Figure 11: Effect of Cisapride on the hERG channel using Protocol 2

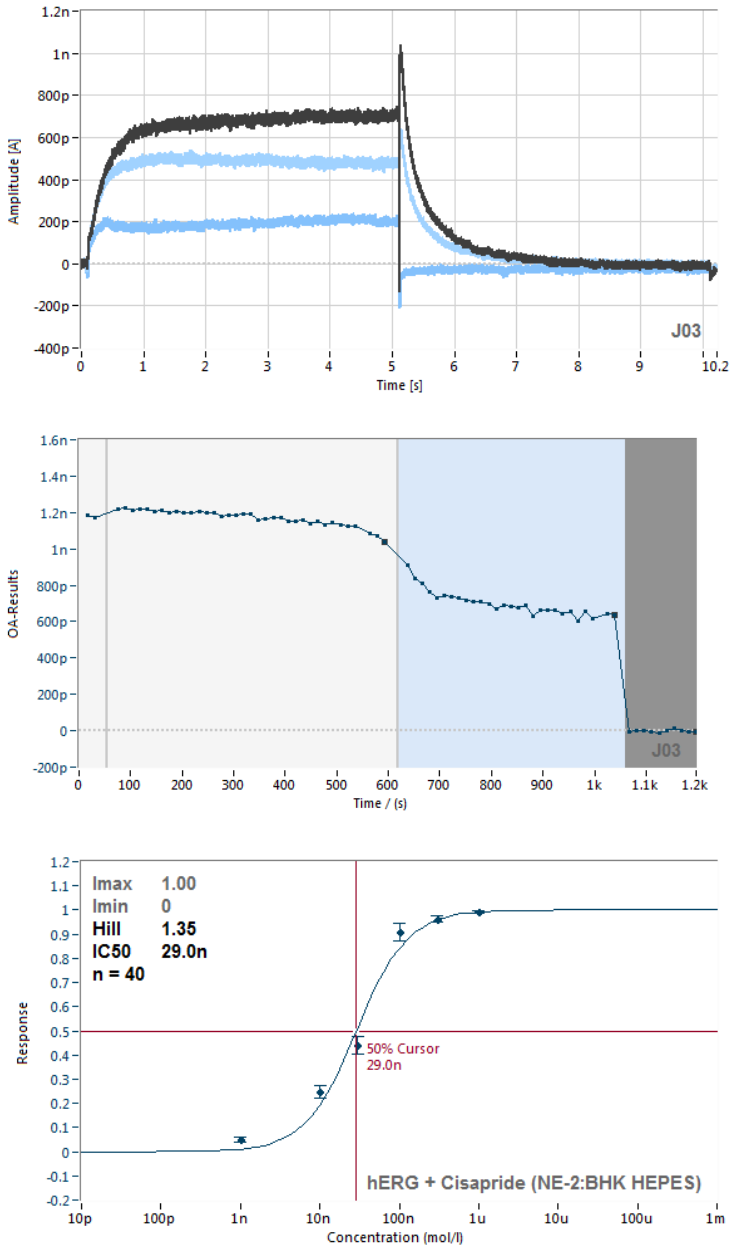


Figure 11: Top panel shows an example of superimposed currents traces during application of a single concentration of reference compound, the control current is shown in black. Middle panel shows the corresponding time-course of effect produced by the test compound (30 nM, blue area). The darker grey column shows maximum concentration of reference blocker. Bottom panel shows IC_{50} plot.

Figure 12: Effect of DMSO on the hERG channel using Protocol 2

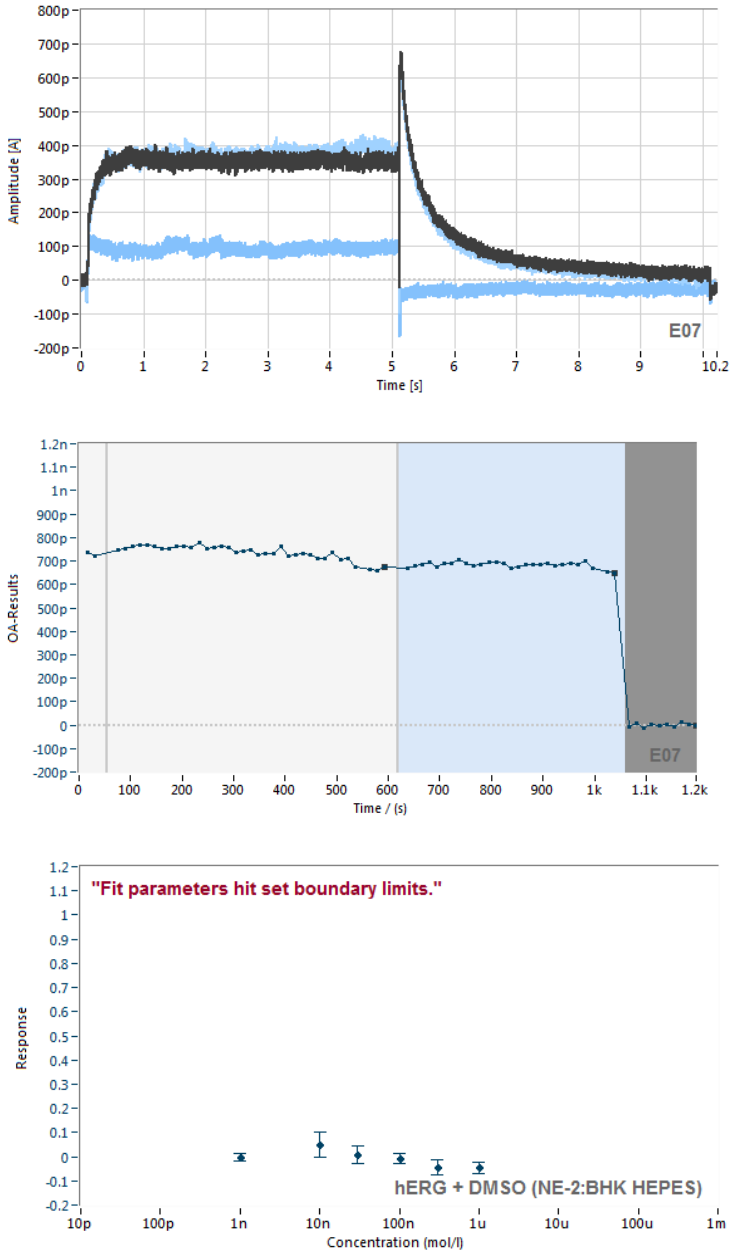


Figure 12: Top panel shows an example of superimposed currents traces during application of a single concentration of DMSO, the control current is shown in black. Middle panel shows the corresponding time-course of effect produced by DMSO (blue area). The darker grey column shows maximum concentration of reference blocker. Bottom panel shows IC_{50} plot.

4 Conclusions

All cells used in these experiments display functional $Na_v1.5$, $Ca_v1.2$ or hERG channels which were inhibited by a saturating concentration of antagonist, therefore validating the assay. Our negative control, DMSO (up to 0.3% final), showed a good level of sustained current amplitude for all three channels.

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Compliance to Standard Operating Procedures and Guidelines

Where appropriate, processes described in SB Drug Discovery standard operating procedures have been applied to this study. These documents are stored on file within SB Drug Discovery. However, due to the nature of the molecules and processes under investigation, some of the work represents novel research requiring adaptation of existing methodologies and may not be covered by these standard operating procedures. SB Drug Discovery is not a GLP accredited company and no claims of GLP are made regarding work carried out in its laboratories.

Storage of Raw Data

All raw data is stored at SB Drug Discovery. Electronic data generated from laboratory equipment together with spreadsheets, statistical files and documents generated as part of the study are stored electronically in restricted access folders to individual clients. Similarly, copies of any email communications relevant to the work are held in restricted accounts. An electronic version of the report and a hard copy version, signed and dated by the author are held on file at SB Drug Discovery. Sensitive files (electronic), when required, are deleted using software capable of removing all traces of the file. Sensitive documents, when required, are shredded.