SOPHION BIOSCIENCE

Application Report

CHO-Na_V1.5 tested on QPatch

The biophysical characteristics were studied, and the values found correspond well to published literature values.

Summary

Here we demonstrate the functionality of CHO-Na_V1.5 when used on QPatch and the biophysical characteristics were investigated from high-resistance whole-cell recordings in IV- and dose-response experiments.

Introduction

The sodium ion channel Na_V1.5 is expressed as in integral membrane protein and contains a tetrodotoxin-resistant voltage-gated sodium channel subunit. The encoded protein is found primarily in cardiac muscle and is responsible for the initial upstroke of the action potential in an electrocardiogram. Mutations in the gene are associated with long QT syndrome type 3, Brugada syndrome, primary cardiac conduction disease and idiopathic ventricular fibrillation.

The aim of this report is to demonstrate the performance of BSYS cells on QPatch. Experiments were executed with CHO cells expressing the Na_V1.5 channel in order to show the capability of the QPatch to perform recordings on these voltage-gated ion channels.

Results

Figure 1 shows IV raw data from a single cell using the voltage protocol #1.

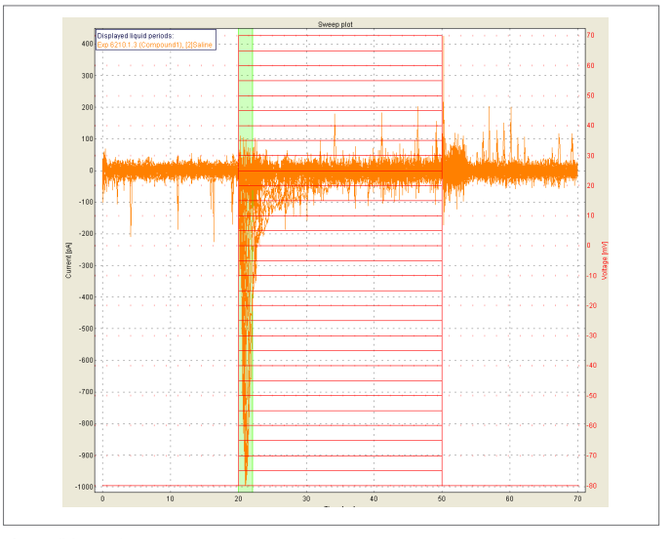


Fig. 1. IV raw data

The IV plot is represented in Figure 2.

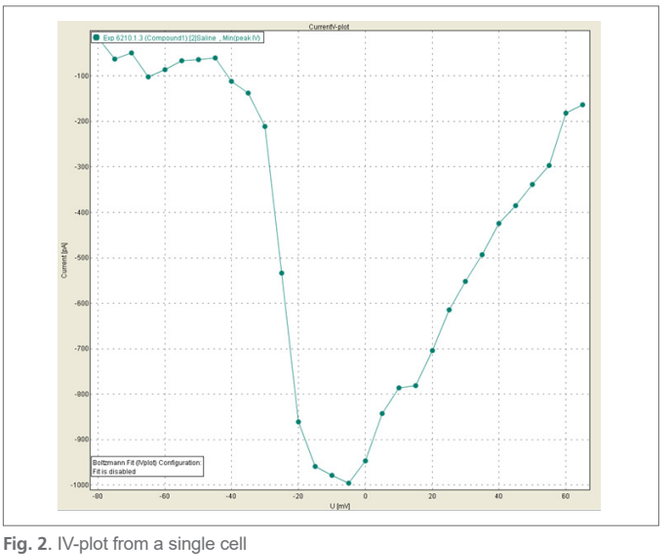


Fig. 2. IV-plot from a single cell

In the next section we provide a detailed concentration-response plot obtained by application of extracellular solutions with 4 increasing concentrations of [TTX]: 0.5 nM, 5 nM, 50 nM and 500 nM respectively. The current trace for a single cell is represented in Figure 3.

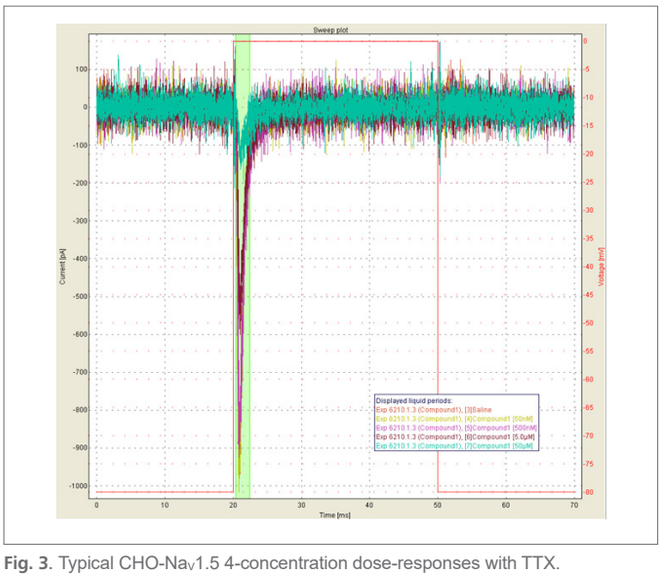
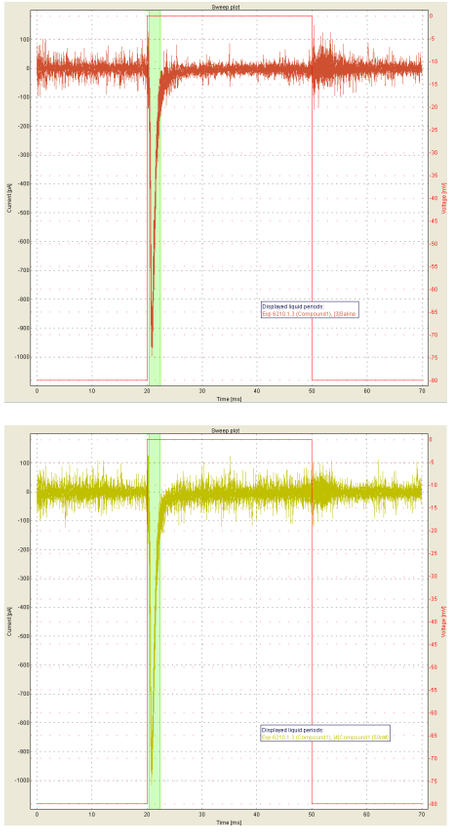


Fig. 3. Typical CHO-$Na_V1.5$ 4-concentration dose-responses with TTX.

The individual traces for each concentration are shown in figure 4.



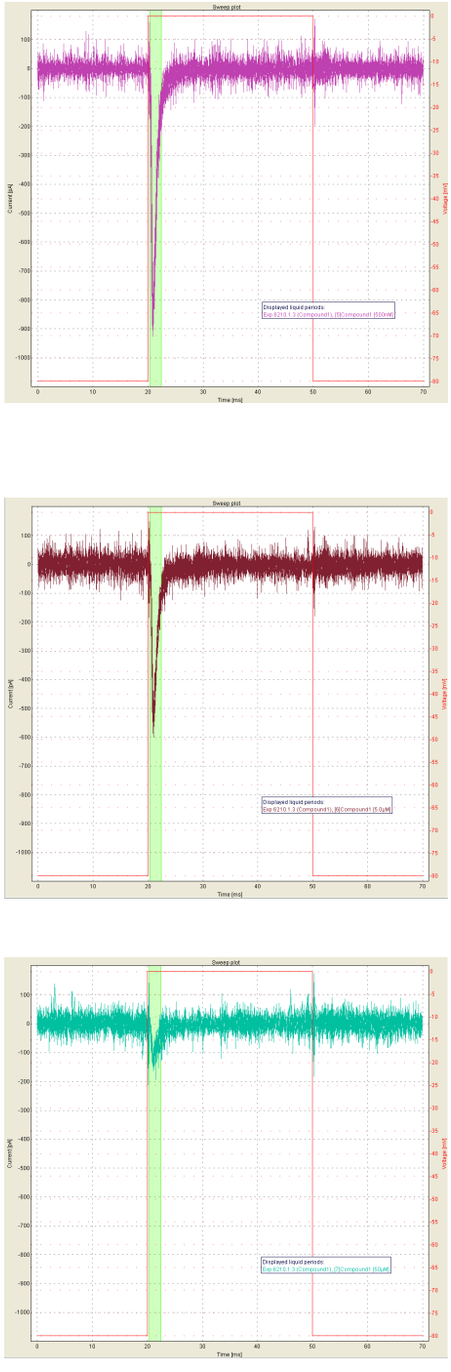


Fig. 4. Individual traces from each concentration of TTX on a single cell.

The corresponding Hill fits are depicted in Figure 5.

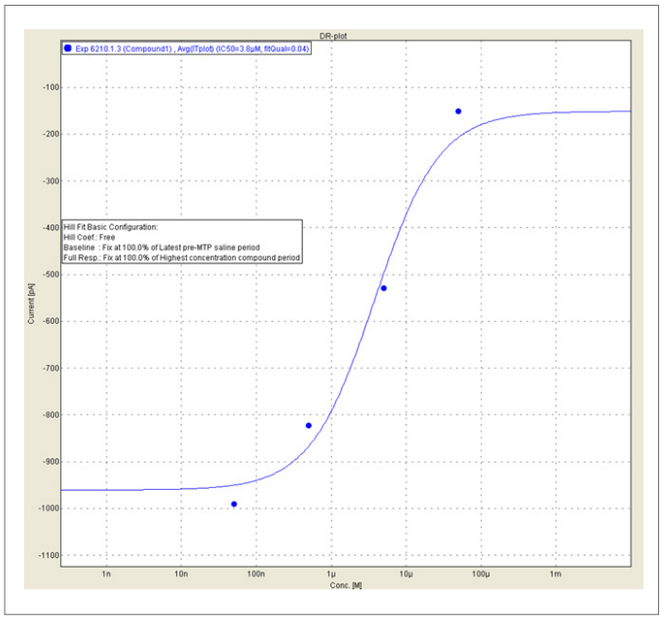


Fig. 5. Hill fit for 4-concentration dose-response.

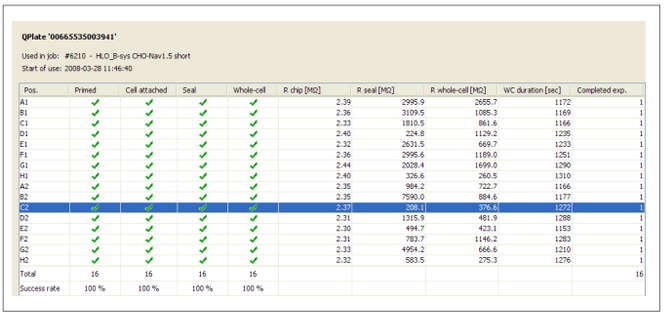
In average the IC_{50} value was 3.83±0.6 µM TTX (see Table 1). Literature value1 IC_{50}=2 µM

Table 1. Hill fit for 4-concentration dose-response.

Experiment	XC50(HillFit) [M]
Exp 6210.1.2 (Compound1)	1.76µ
Exp 6210.1.3 (Compound1)	3.75µ
Exp 6210.1.4 (Compound1)	476.85µ
Exp 6210.3.2 (Compound2)	3.08µ
Exp 6210.3.3 (Compound2)	1.43µ
Exp 6210.3.4 (Compound2)	2.16µ
Exp 6210.5.1 (Compound3)	5.71µ
Exp 6210.5.2 (Compound3)	1.69µ
Exp 6210.5.3 (Compound3)	5.36µ
Exp 6210.5.4 (Compound3)	6.40µ
Exp 6210.7.1 (Compound4)	4.36µ
Exp 6210.7.2 (Compound4)	6.92µ
Exp 6210.7.3 (Compound4)	6.70µ

Experimental statistics

In the first two experiments performed with CHO-Na_V1.5 cells the overall performance is shown from the QPlate statistics in Figure 6. Data shows that 100% of the experiments were completed. 56% of the cells had true giga-seals.


QPlate '00665535003941'

Used in job: #6210 - HLO_B-sys CHO-Nav1.5 short

Start of use: 2008-03-28 11:46:40

Pos.	Primed	Cell attached	Seal	Whole-cell	R chip [MΩ]	R seal [MΩ]	R whole-cell [MΩ]	WC duration [sec]	Completed exp.
A1	✓	✓	✓	✓	2.39	2995.9	2655.7	1172	1
B1	✓	✓	✓	✓	2.36	3109.5	1085.3	1169	1
C1	✓	✓	✓	✓	2.33	1810.5	861.6	1166	1
D1	✓	✓	✓	✓	2.40	224.8	1129.2	1235	1
E1	✓	✓	✓	✓	2.32	2631.5	669.7	1233	1
F1	✓	✓	✓	✓	2.36	2995.6	1189.0	1251	1
G1	✓	✓	✓	✓	2.44	2028.4	1699.0	1290	1
H1	✓	✓	✓	✓	2.40	326.6	260.5	1310	1
A2	✓	✓	✓	✓	2.35	984.2	722.7	1166	1
B2	✓	✓	✓	✓	2.35	7590.0	884.6	1177	1
C2	✓	✓	✓	✓	2.37	508.1	376.6	1272	1
D2	✓	✓	✓	✓	2.31	1315.9	481.9	1288	1
E2	✓	✓	✓	✓	2.30	494.7	423.1	1153	1
F2	✓	✓	✓	✓	2.31	783.7	1146.2	1283	1
G2	✓	✓	✓	✓	2.33	4954.2	666.6	1210	1
H2	✓	✓	✓	✓	2.32	583.5	275.3	1276	1
Total	16	16	16	16					16
Success rate	100 %	100 %	100 %	100 %					

Fig. 6. QPlate statistics for the initial experiments, showing success rates for cell attachment to the QPlate orifice, seal quality, whole-cell success rates and number of completed experiments.

Conclusion

We have demonstrated the functionality of CHO-Na_V1.5 on the QPatch. Biophysical characteristics of the Na_V1.5 channels were studied from high resistance whole cell recordings in IV- and dose-response experiments and the values found on the QPatch correspond well to published literature values.

Methods

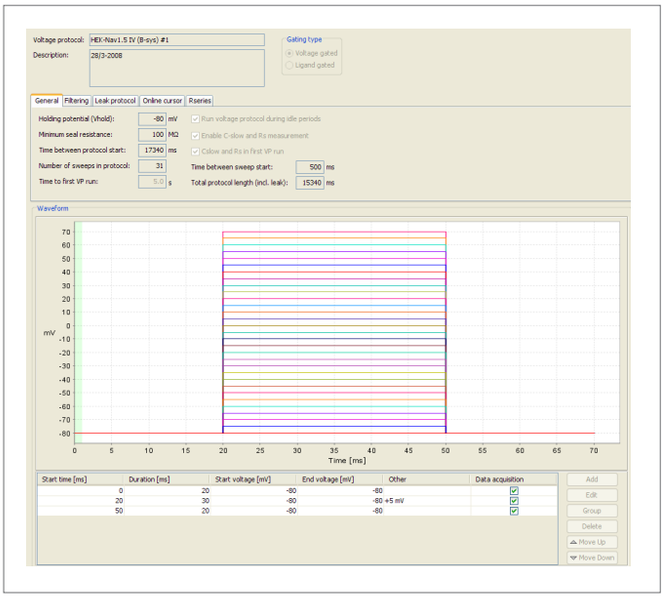
Cells

CHO-Na_V1.5 cells were grown and harvested according to the SOP's specified from BSYS and modified for use on QPatch by Sophion Bioscience.

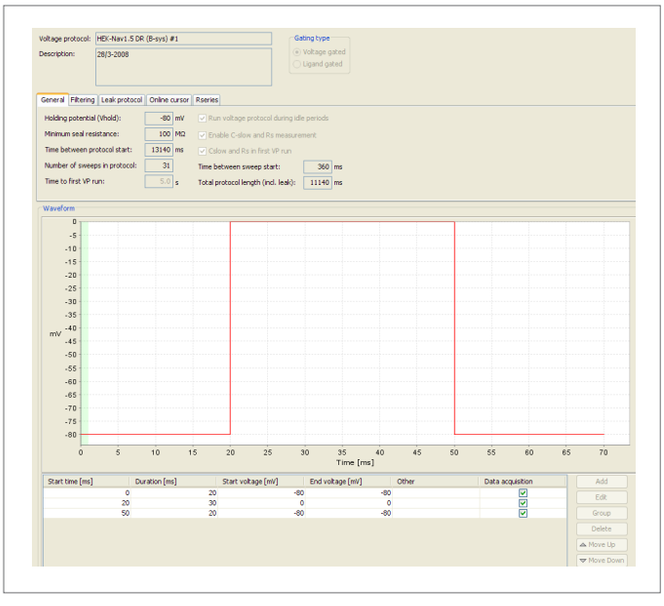
Voltage protocols

For experiments with CHO-Na_V1.5 the following protocols were used. 1) IV step protocol 2) simple depolarization pulse with a holding potential of -80 mV 3) steady-state inactivation protocol and 4) a paired pulse protocol.

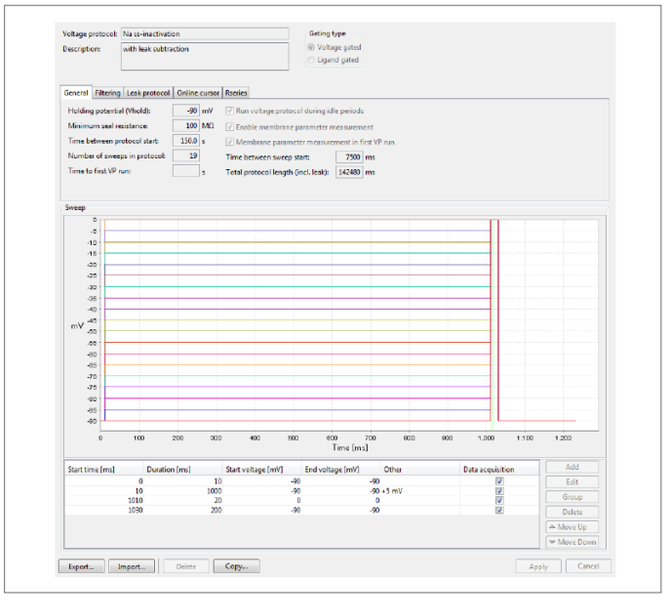
Voltage protocol #1 IV



Voltage protocol #2 pulse



Voltage protocol #3 steady-state activation



Data were sampled at a frequency of 10kHz (see figure 7). Rseries compensation was 100%.

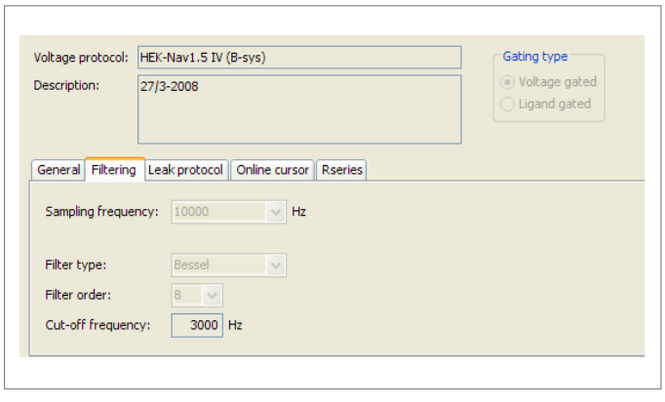


Fig. 7. Sampling frequency settings for CHO-$Na_V1.5$ experiments.

References

1. M. P. Korsgaard, P Christophersen, P. K. Ahring, S-P. Olesen. Identification of a novel voltage-gated Na^+ channel $rNa_V1.5a$ in the rat hippocampel progenitor stem cell line HiB5. 2001. Pflügers Arch – Eur J Physiol 443:18-30