Application Report:

CHO-Nav1.5

QPatch

On QPatch

The sodium ion channel Nav1.5 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.

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Aim

The aim of this report is to demonstrate the performance of BSYS cells on the QPatch HT and QPatch HTX. Experiments were executed with CHO cells expressing the Nav1.5 channel in order to show the capability of the QPatch to perform recordings on these voltage-gated ion channels.

Materials and Methods

Cells

CHO-Nav1.5 cells were grown and harvested according to the SOP's specified from B-sys and modified for use on QPatch by Sophion Bioscience.

Cell handling on the QPatch HT

CHO-Nav1.5 cells were harvested and placed in the cell-containing facility on the QPatch, the QStirrer. Here, the cells were kept stirred in serum-free medium for up to 4 hours. When an experiment is started on the QPatch, the pipettes pick up 1.5 ml cells from the QStirrer, and transfer them to the centrifuge unit on the platform, the QFuge. The cell pellet is washed twice by the QPatch and resuspended in a user-defined volume of extracellular Ringer's solution ranging from 200-500 µl, depending on cell density.

Ringer solutions

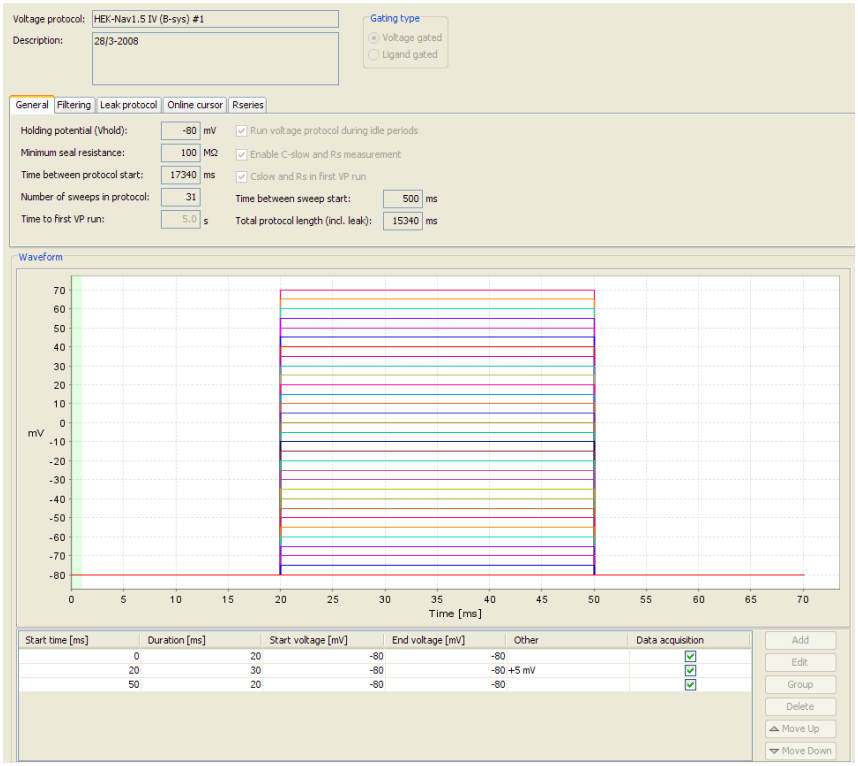
Intracellular Ringer (in mM): 135 CsF, 1mM/5mM CsOH, 10 HEPES, 10 NaCl. The pH value was set to 7.3 and the osmolarity was adjusted to 320 mOsm with sucrose before use.

Extracelluar Ringer (in mM): 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 4 KCl, 145 NaCl, 0.1 $CdCl_2$, 20 TEA-Cl, 10 Glucose. The pH value was set 7.3, respectively, and the osmolarity was adjusted to 320 mOsm with sucrose before use.

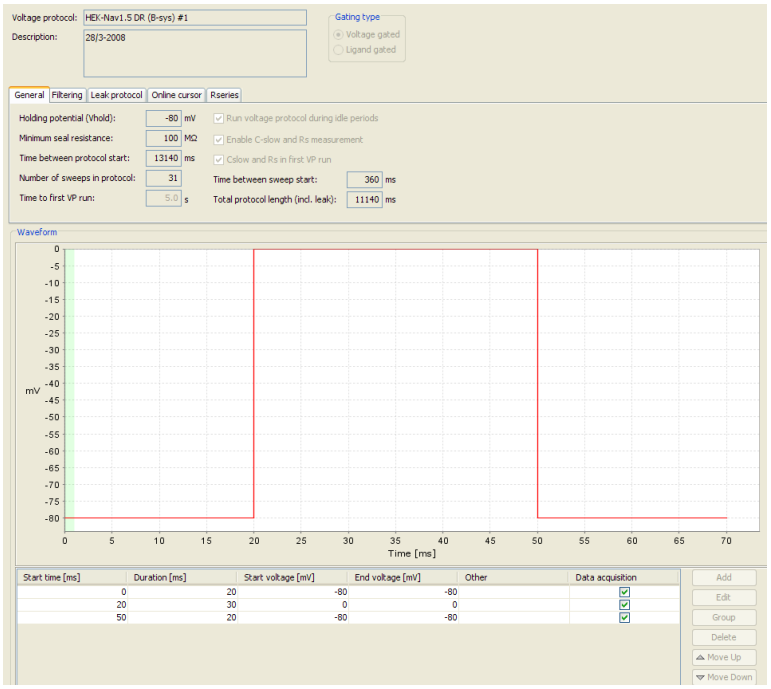
Voltage protocols

For experiments with CHO-Nav1.5 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.

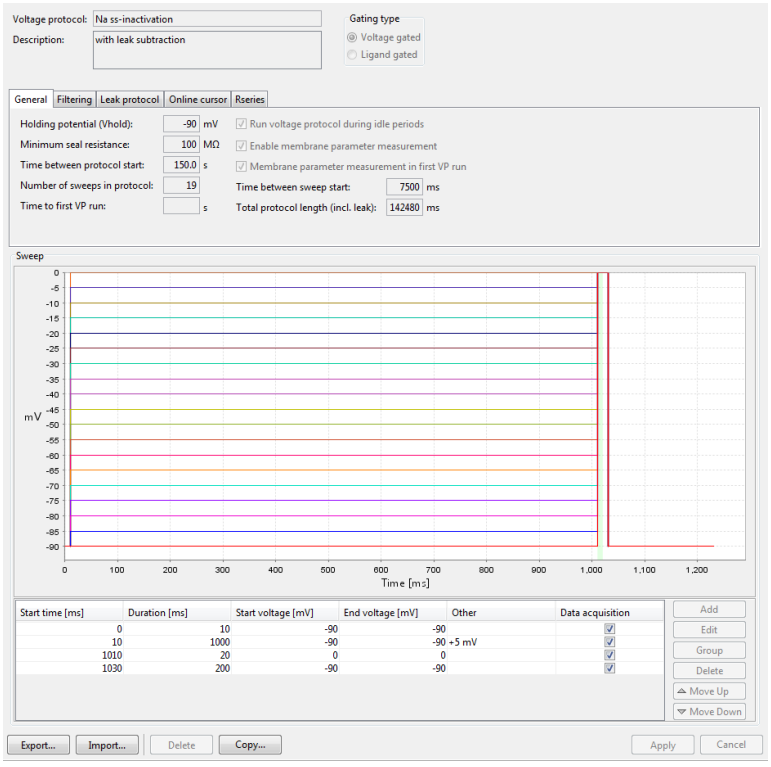
Voltage protocol #1 IV



Voltage protocol #2 Pulse



Voltage protocol #3 steady-state inactivation



Data were sampled at a frequency of 10 kHz (see Figure 1). Rseries compensation was 100%.

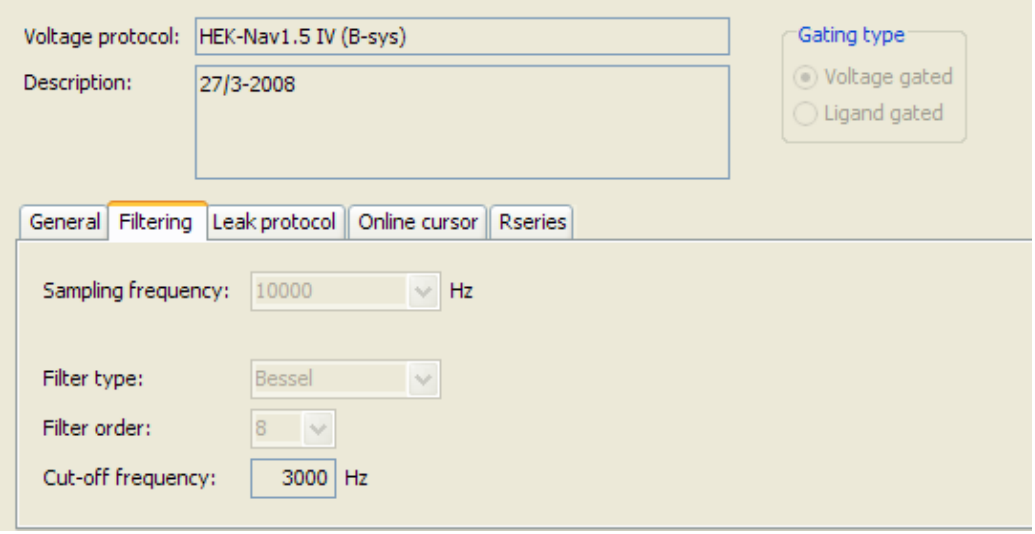


Figure 1 Sampling frequency settings for CHO-Nav1.5 experiments

Results

Figure 2 shows IV raw data from a single cell using the voltage protocol #1.

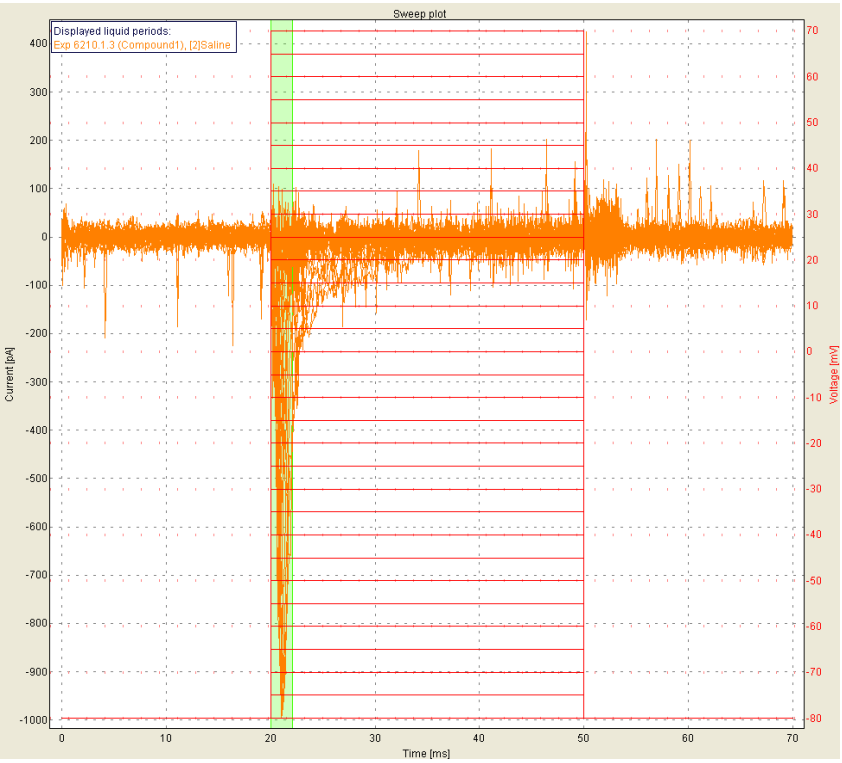


Figure 2 IV raw data

The IV plot is represented in Figure 3

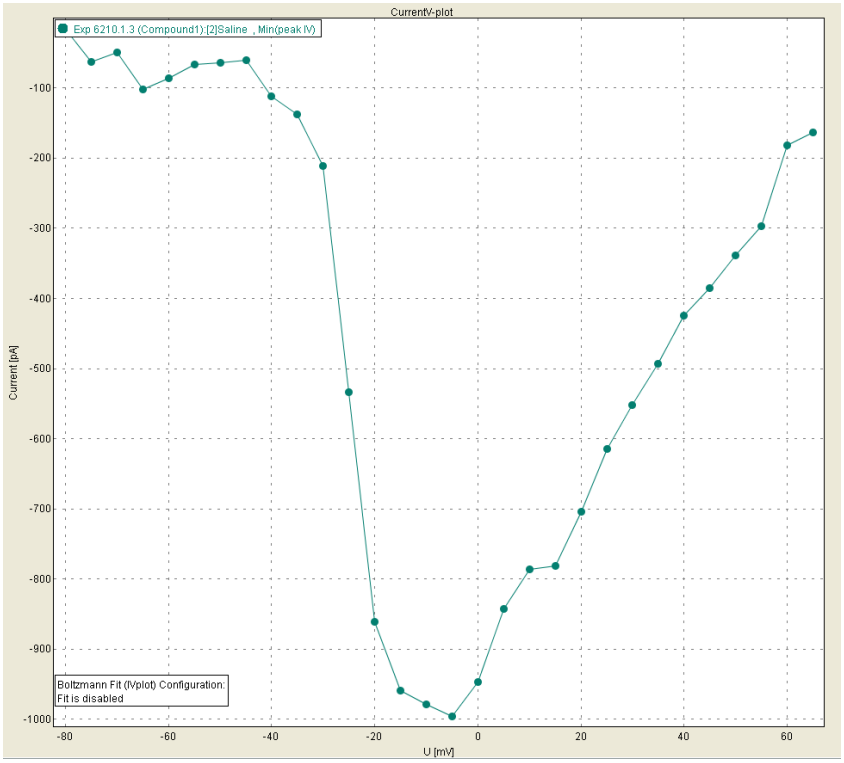


Figure 3 IV-plot from a single cell

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 4.

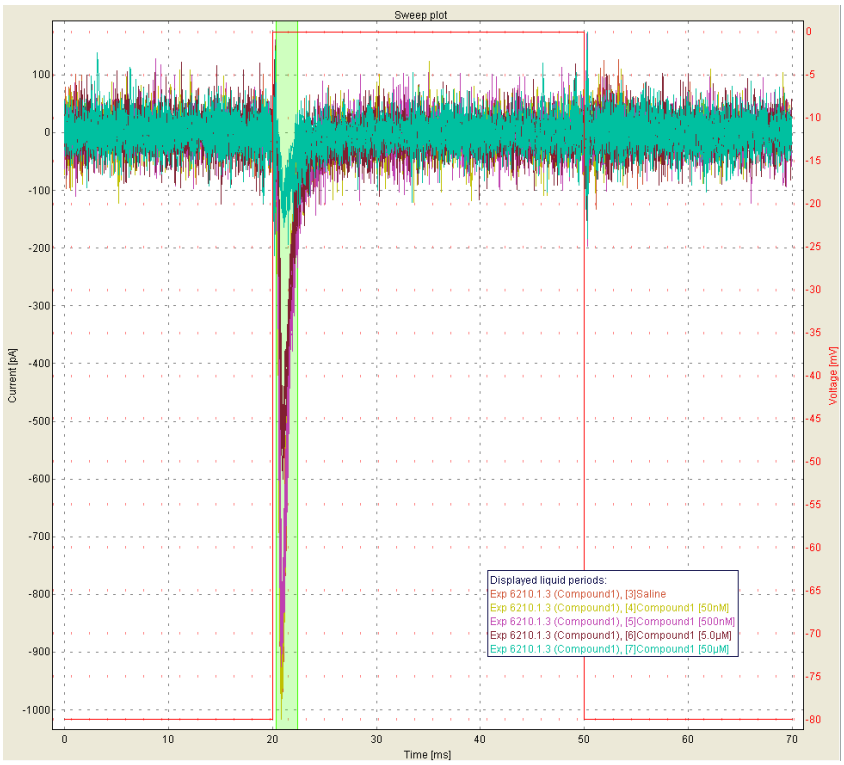


Figure 4 Typical CHO-Nav1.5 4-concentration dose-responses with TTX.

The individual traces for each concentration is shown in Figure 5.

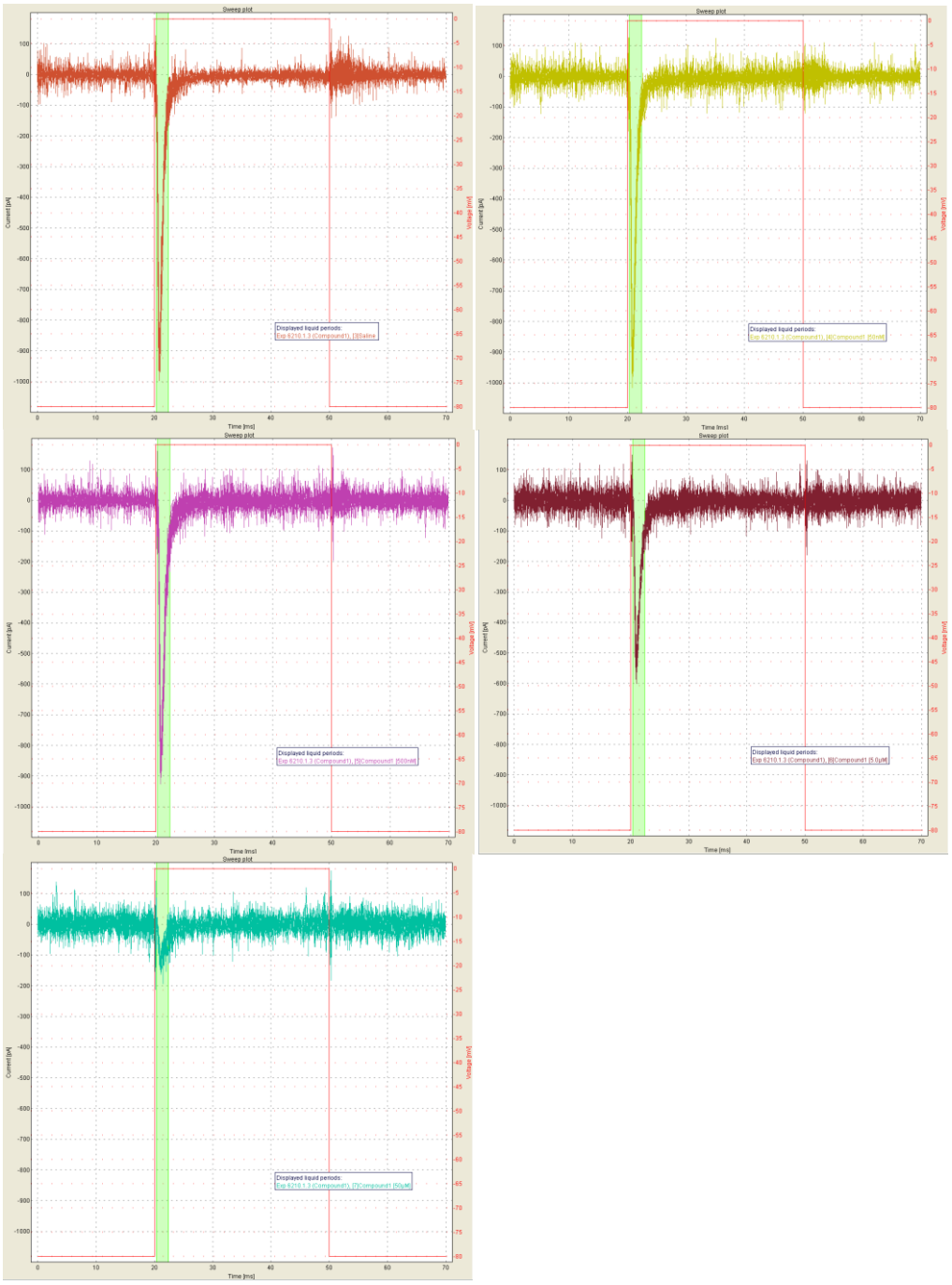


Figure 5 Individual traces from each concentration of TTX on a single cell.

The corresponding Hill fits are depicted in Figure 6.

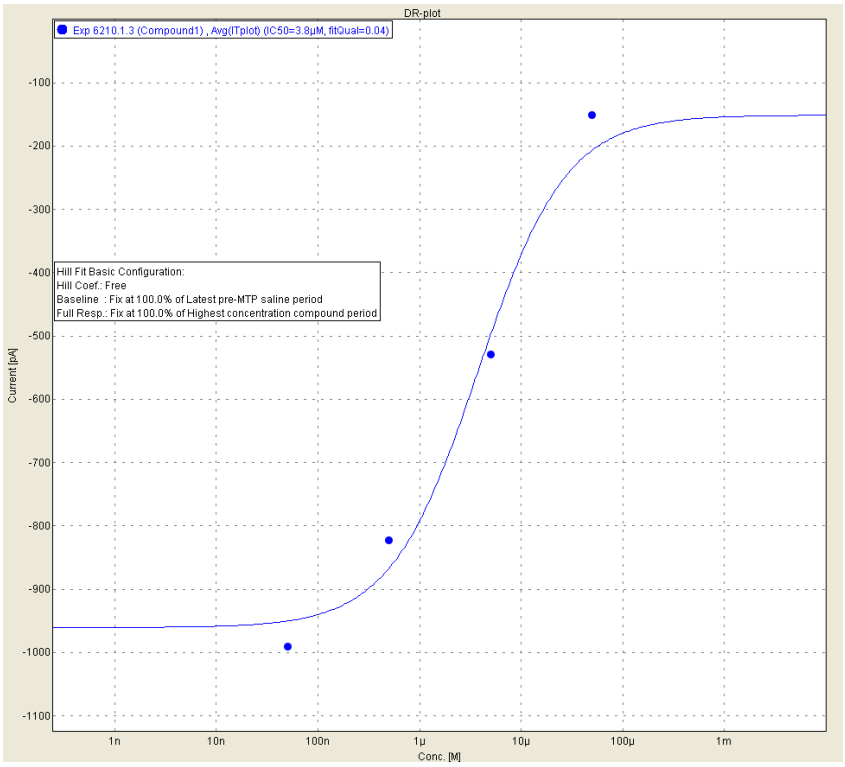


Figure 6 Hill fit for 4-concentration dose-response

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

Experiment	XC50(HillFit) [M]
Exp 6210.1.2 (Compound1)	1.76µ
Exp 6210.1.3 (Compound1)	3.75µ
Exp 6210.1.4 (Compound1)	476.85n
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

Table 1 Individual values for the 4-concentration TTX dose-response are shown in the top table.

Experimental statistics

In the first two experiments performed with CHO-Nav1.5 cells the overall performance is shown from the QPlate statistics in Figure 7. 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	208.1	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 %					

Figure 7 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.

References

Identification of a novel voltage-gated Na^+ channel rNav1.5a in the rat hippocampel progenitor stem cell line HiB5 (2001). M. P. Korsgaard, P Christophersen, P. K. Ahring, S-P. Olesen. Pflügers Arch – Eur J Physiol **443**:18-30

Conclusion

We have demonstrated the functionality of CHO-Nav1.5 on the QPatch. Biophysical characteristics of the Nav1.5 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.