

Application Report:

CHO-Nav1.5

QPatch

On QPatch



The sodium ion channel Nav1.5 is expressed as an 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 μ l, 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.

Extracellular Ringer (in mM): 2 CaCl₂, 1 MgCl₂, 10 HEPES, 4 KCl, 145 NaCl, 0.1 CdCl₂, 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: Gating type
 Voltage gated
 Ligand gated

Description:

General | Filtering | Leak protocol | Online cursor | Rseries

Holding potential (Vhold): mV Run voltage protocol during idle periods

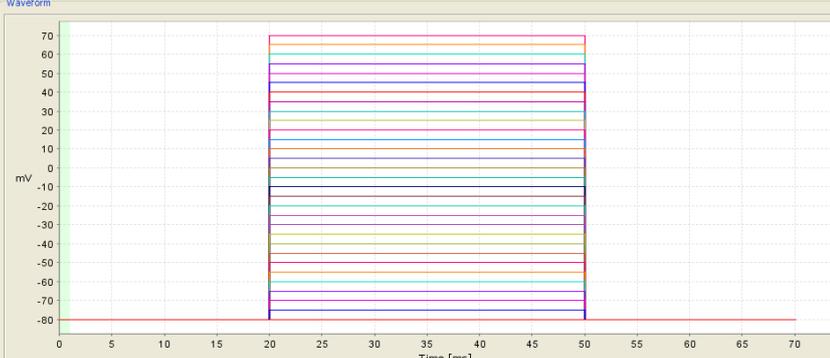
Minimum seal resistance: MΩ Enable C-slow and Rs measurement

Time between protocol start: ms Cslow and Rs in first VP run

Number of sweeps in protocol: Time between sweep start: ms

Time to first VP run: s Total protocol length (incl. leak): ms

Waveform



Start time [ms]	Duration [ms]	Start voltage [mV]	End voltage [mV]	Other	Data acquisition
0	20	-80	-80		<input checked="" type="checkbox"/>
20	30	-80	-80	-80 +5 mV	<input checked="" type="checkbox"/>
50	20	-80	-80		<input checked="" type="checkbox"/>

Voltage protocol #2 Pulse

Voltage protocol: HEK-Nav1.5 DR (B-sys) #1
 Description: 28/3-2008

Gating type:
 Voltage gated
 Ligand gated

General | Filtering | Leak protocol | Online cursor | Rseries

Holding potential (Vhold): -80 mV Run voltage protocol during idle periods
 Minimum seal resistance: 100 MΩ Enable C-slow and R_s measurement
 Time between protocol start: 13140 ms Cslow and R_s in first VP run
 Number of sweeps in protocol: 31 Time between sweep start: 360 ms
 Time to first VP run: 5.0 s Total protocol length (incl. leak): 11140 ms

Waveform

Start time [ms]	Duration [ms]	Start voltage [mV]	End voltage [mV]	Other	Data acquisition
0	20	-80	-80		<input checked="" type="checkbox"/>
20	30	0	0		<input checked="" type="checkbox"/>
50	20	-80	-80		<input checked="" type="checkbox"/>

Add
 Edit
 Group
 Delete
 Move Up
 Move Down

Voltage protocol #3 steady-state inactivation

Voltage protocol: Na ss-inactivation
 Description: with leak subtraction

Gating type:
 Voltage gated
 Ligand gated

General | Filtering | Leak protocol | Online cursor | Rseries

Holding potential (Vhold): -90 mV Run voltage protocol during idle periods
 Minimum seal resistance: 100 MΩ Enable membrane parameter measurement
 Time between protocol start: 150.0 s Membrane parameter measurement in first VP run
 Number of sweeps in protocol: 19 Time between sweep start: 7500 ms
 Time to first VP run: s Total protocol length (incl. leak): 142480 ms

Sweep

Start time [ms]	Duration [ms]	Start voltage [mV]	End voltage [mV]	Other	Data acquisition
0	10	-90	-90		<input checked="" type="checkbox"/>
10	1000	-90	-5 mV		<input checked="" type="checkbox"/>
1010	20	0	0		<input checked="" type="checkbox"/>
1030	200	-90	-90		<input checked="" type="checkbox"/>

Add
 Edit
 Group
 Delete
 Move Up
 Move Down

Export... Import... Delete Copy... Apply Cancel

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

The screenshot shows the QPatch software interface. At the top, the 'Voltage protocol' is set to 'HEK-Nav1.5 IV (B-sys)' and the 'Description' is '27/3-2008'. The 'Gating type' is set to 'Voltage gated'. Below this, there are tabs for 'General', 'Filtering', 'Leak protocol', 'Online cursor', and 'Rseries'. The 'Filtering' tab is active, showing the following settings: 'Sampling frequency' is 10000 Hz, 'Filter type' is Bessel, 'Filter order' is 8, and 'Cut-off frequency' is 3000 Hz.

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.

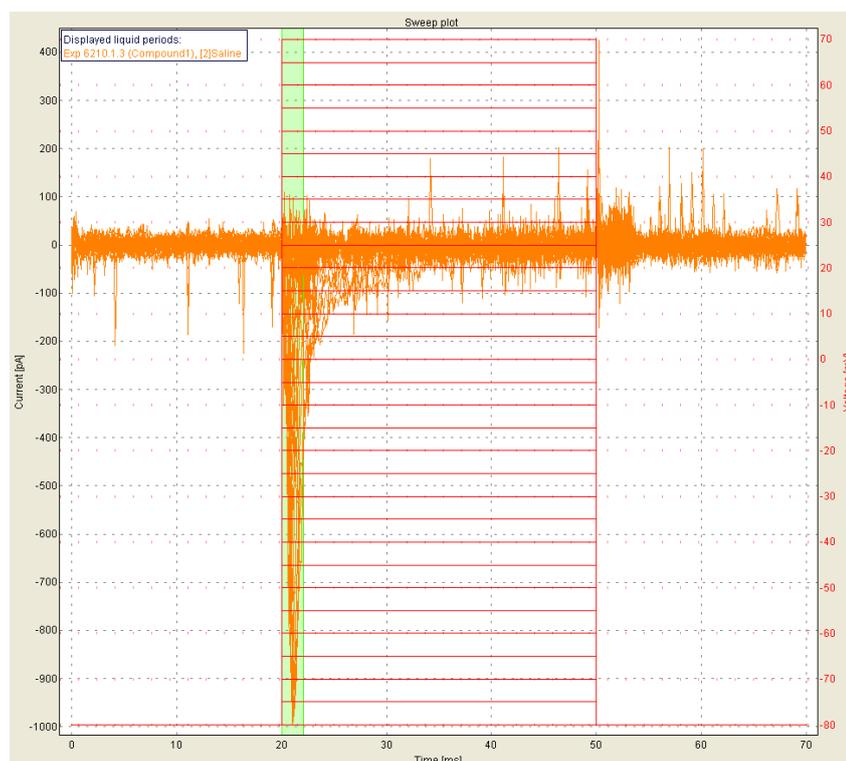


Figure 2 IV raw data

The IV plot is represented in Figure 3

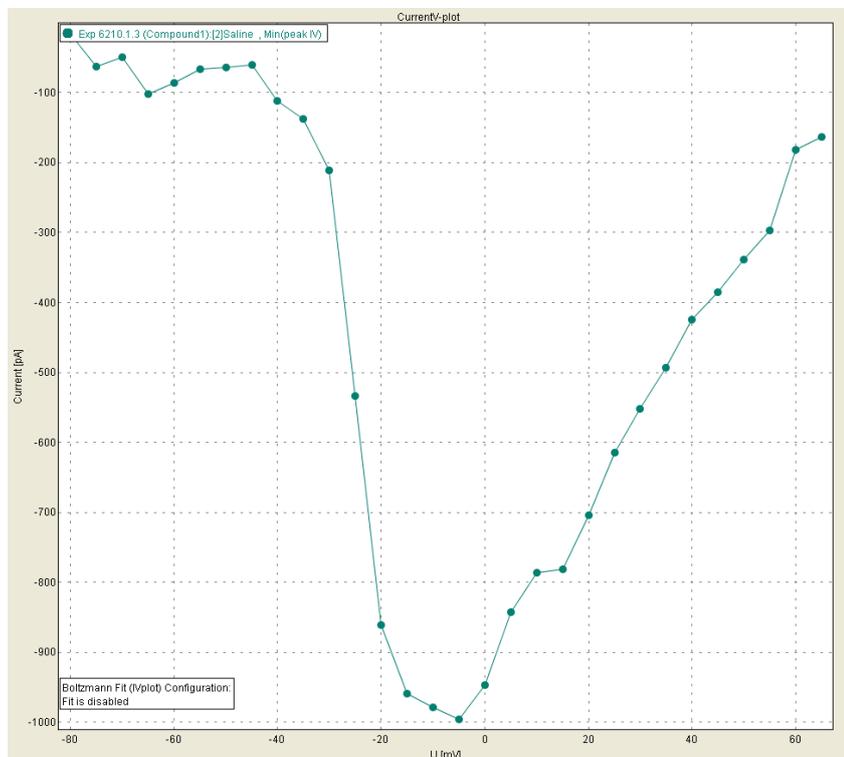


Figure 3 IV-plot from a single cell

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.

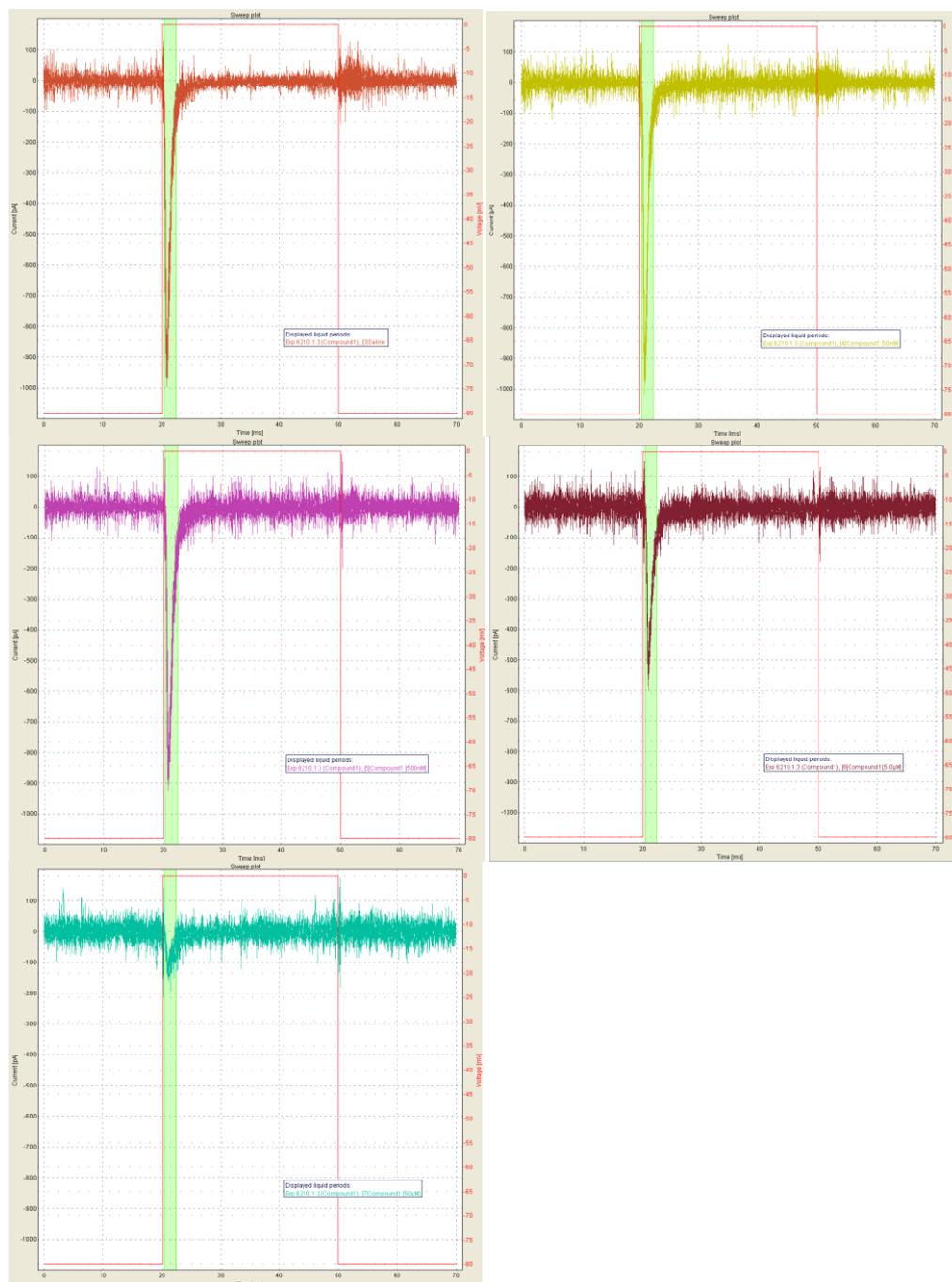


Figure 5 Individual traces from each concentration of TTX on a single cell.

The corresponding Hill fits are depicted in Figure 6.

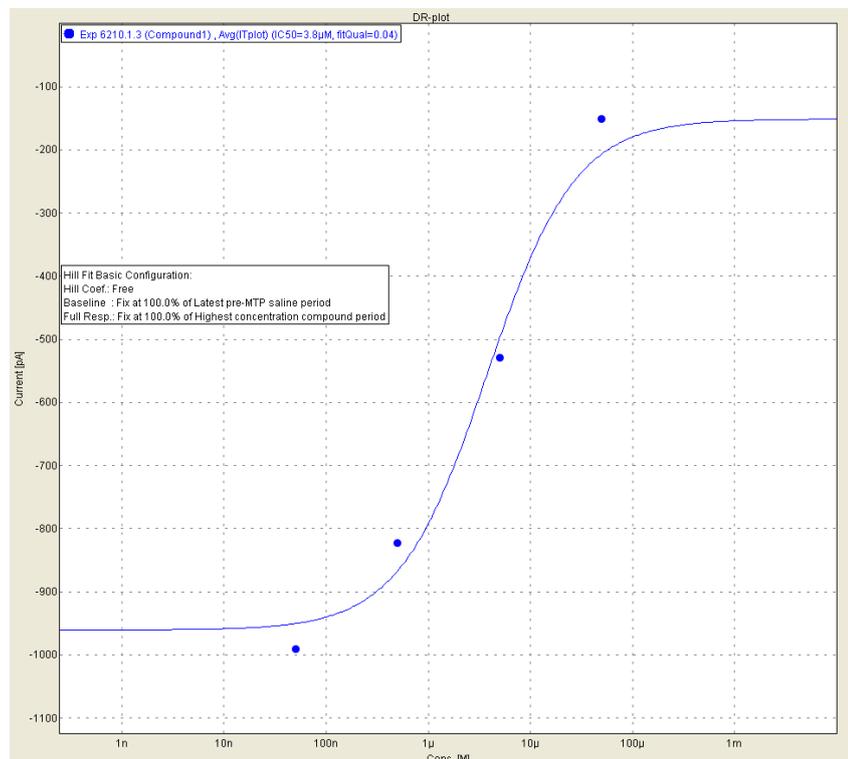


Figure 6 Hill fit for 4-concentration dose-response

In average the IC_{50} value was $3.83 \pm 0.6 \mu\text{M}$ TTX (see Table 1). Literature value¹ $IC_{50} = 2 \mu\text{M}$

Experiment	IC_{50} (HillFit) [M]
Exp 6210.1.2 (Compound1)	1.76 μ
Exp 6210.1.3 (Compound1)	3.75 μ
Exp 6210.1.4 (Compound1)	476.85n
Exp 6210.3.2 (Compound2)	3.08 μ
Exp 6210.3.3 (Compound2)	1.43 μ
Exp 6210.3.4 (Compound2)	2.16 μ
Exp 6210.5.1 (Compound3)	5.71 μ
Exp 6210.5.2 (Compound3)	1.69 μ
Exp 6210.5.3 (Compound3)	5.36 μ
Exp 6210.5.4 (Compound3)	6.40 μ
Exp 6210.7.1 (Compound4)	4.36 μ
Exp 6210.7.2 (Compound4)	6.92 μ
Exp 6210.7.3 (Compound4)	6.70 μ

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 hippocampal 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.