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Sophion Analyzer as a tool for biophysical and pharmacological characterization of eight Na_V subtypes evaluated in parallel on Qube

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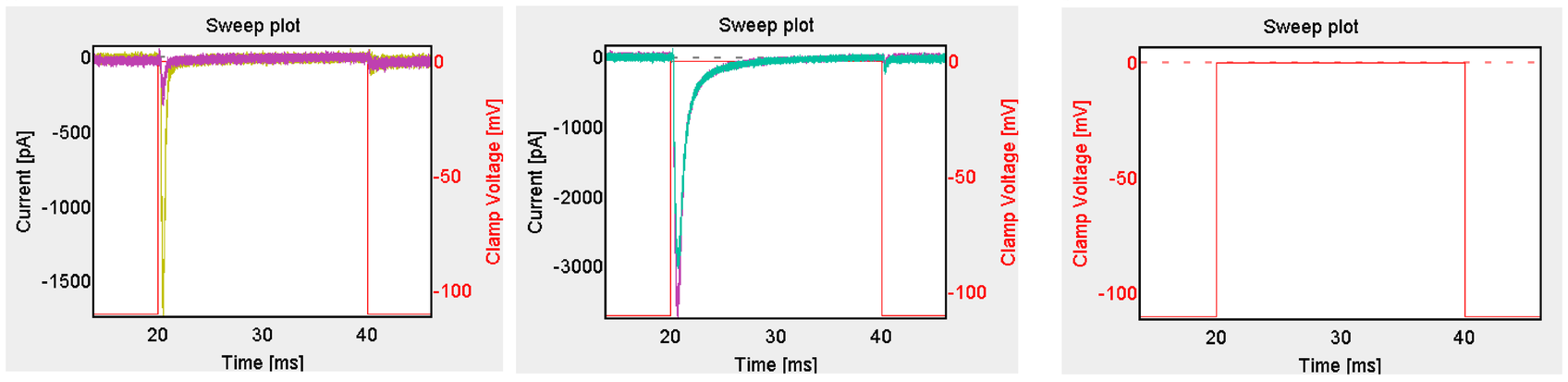
Introduction

Drug discovery on ion channels is a slow and complicated process and demands a high throughput system with high data quality, but also with a flexible design and easy-to-analyze data. Sophion Qube is a next-generation giga-seal automated patch clamp (APC) screening instrument, capable of testing thousands of compounds with a single click on a button. Data analysis is as important as data acquisition. In HTS the need for powerful analysis with efficient quality filtering is evident in order to handle the vast amount of data generated on an electrophysiological platform.

Qube is capable of testing up to 16 different cell lines or cell clones in one experiment. This can be utilized to test a different panel of cell lines or for selecting the best suited cell clone before embarking on a HTS campaign. The integrated analysis software, Sophion Analyzer, ensures analysis to keep track of all the results and whenever another QChip is assayed the analysis is done with the same set of user defined criteria. Here we demonstrate the power of automated analysis by exploring three types of experiments executed on eight different Nav channel subtypes; 1) TTX sensitivity, 2) IV-relationship for activation and inactivation and 3) pulse train suitable for screening for use dependent sodium channels blockers. For every run $Na_V1.1$, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7 and 1.8 were tested in parallel on a QChip. Recorded ion channel whole-cell currents were automatically analyzed for IV-relationships for activation and inactivation (V½ and Boltzmann fit) and concentration-dependent drug effects (Hill fit and IC50) were analyzed using the Sophion Analyzer. If preferred, data can by a click be exported and calculated by other programs as Spotfire, Genedata screener or implemented in in-house software.

For each subtype, the experiments identified the expected pharmacology for use- and state-dependent drugs as well as biophysical properties. The findings determined the differences between the different subtypes as expected and also that post experiment analysis can be performed with minimum of effort when using integrated, automated analysis software.

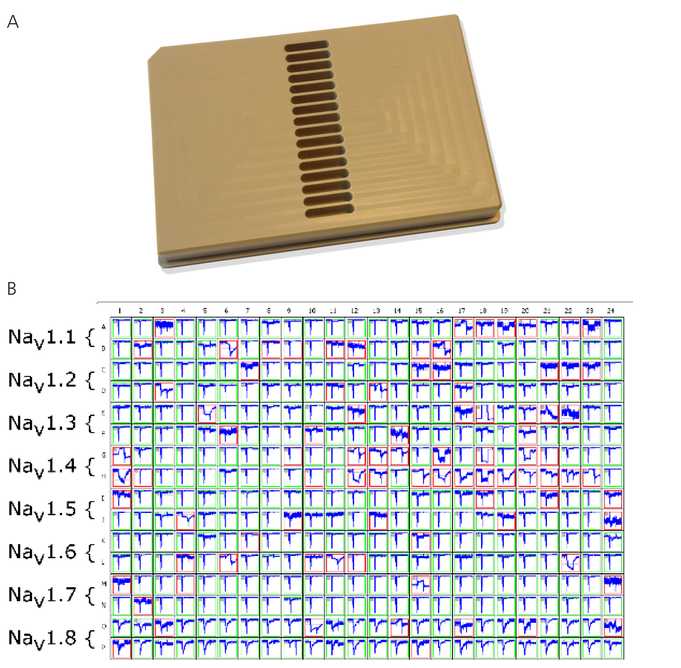


[Fig. 2]: Dose-response relationship in response to tetrodotoxin (TTX). A, representative whole-cell currents under control conditions and in the presence of 123 nM TTX for $Na_V1.1$ (left) and $Na_V1.5$ (right). B, currents were elicited by 20 ms depolarizations from -110 to 0 mV at 0.1 Hz. C, dose-response relationships for $Na_V1.4$ (blue), $Na_V1.5$ (grey) and $Na_V1.6$ (orange).

[Table 1]: Concentration dependent inhibition of sodium currents by TTX. Cells were stimulated with depolarizations from -110 to 0 mV at 0.1 Hz

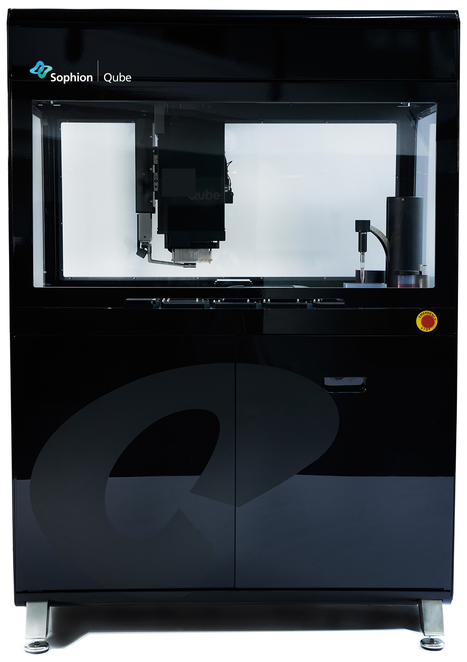
Channel isoform	IC_{50} ± SEM (nM)	n	Reference values*
$Na_V1.1$	22 ± 5	11 - 14	6
$Na_V1.2$	33 ± 4	11 - 15	12
$Na_V1.3$	12 ± 3	7 -14	4
$Na_V1.4$	44 ± 5	7 - 13	25
$Na_V1.5$	7800 ±	7 - 12	1 – 2 mM
$Na_V1.6$	9 ± 1	11 - 15	1 – 6 (rat/mouse)
$Na_V1.7$	93 ± 9	12 - 15	25
$Na_V1.8$	>10000	13 - 16	60 mM

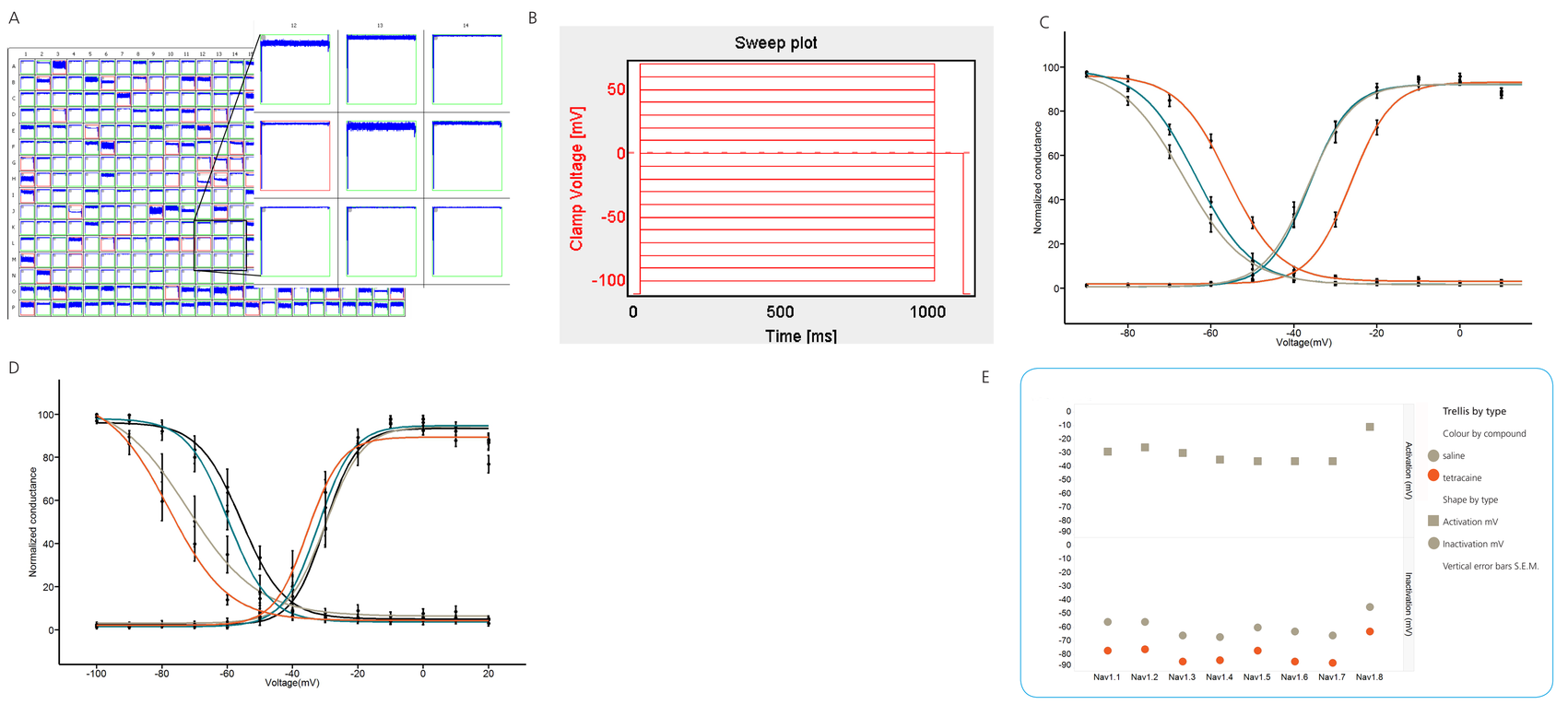
*Values from Catteral W.A. et al. Pharmacol Rev. 57:397-409, 2005 and references therein.



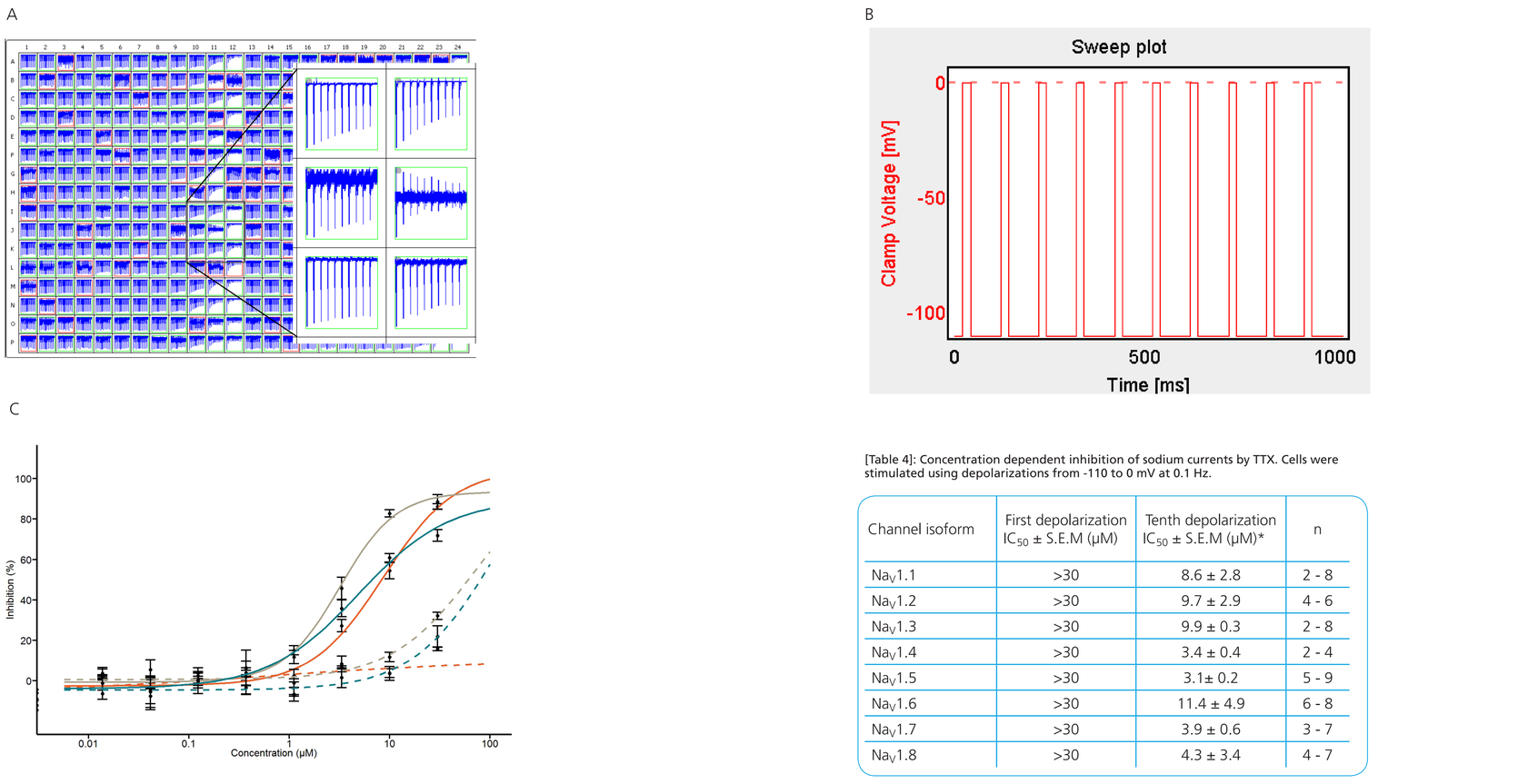
[Fig. 1]: Experiment layout. A, cell clone Cell Transfer Plate (ccCTP) for the Qube instrument. The ccCTP makes it possible to run up to 16 different cell lines or clones on a single QChip. In the experiments shown here cells expressing the sodium channel isoforms $Na_V1.1$ to $Na_V1.8$ were added to the individual wells of the ccCTP and subsequently transferred to the QChip. B, whole-cell currents from a typical experiment. Currents were elicited by a depolarizing pulse from a holding potential of -110 to 0 mV.

Sophion Qube





[Fig. 3]: Voltage-dependent activation and steady-state inactivation for $Na_V1.X$ isoforms. A, typical currents in response to a depolarizing pulse from -110 to 0 mV and B, voltage protocol. C, conductance-voltage and steady-state inactivation for $Na_V1.2$ (blue), $Na_V1.6$ (orange) and $Na_V1.7$ (grey). D, activation and steady-state in-activation for $Na_V1.7$ under control conditions (black) and in the presence of 10 µM of amitriptyline (blue), bupivacaine (orange) and tetracaine (grey) respectively. E, Half maximal activation (top panel, ■) and inactivation (bottom panel,●) without (grey) and with (orange) 10 µM tetracaine for the respective channel isoform. Data for (E) exported from Qube software and analyzed using TIBCO Spotfire v7.7.



[Table 4]: Concentration dependent inhibition of sodium currents by TTX. Cells were stimulated using depolarizations from -110 to 0 mV at 0.1 Hz.

Channel isoform	First depolarization IC_{50} ± S.E.M (µM)	Tenth depolarization IC_{50} ± S.E.M (µM)*	n
$Na_V1.1$	>30	8.6 ± 2.8	2 - 8
$Na_V1.2$	>30	9.7 ± 2.9	4 - 6
$Na_V1.3$	>30	9.9 ± 0.3	2 - 8
$Na_V1.4$	>30	3.4 ± 0.4	2 - 4
$Na_V1.5$	>30	3.1± 0.2	5 - 9
$Na_V1.6$	>30	11.4 ± 4.9	6 - 8
$Na_V1.7$	>30	3.9 ± 0.6	3 - 7
$Na_V1.8$	>30	4.3 ± 3.4	4 - 7

[Fig. 4]: Dose-response relationship in response to tetracaine. A, whole-cell currents in the presence of increasing concentrations of tetracaine. Inserts show details of currents from $Na_V1.5$ and $Na_V1.6$ expressing cells and the how inactivation can be analyzed using the Qube software. B, voltage protocol. Currents were measured at first and tenth (last) depolarization. C, dose-response relationships for $Na_V1.1$ (blue), $Na_V1.4$ (orange) and $Na_V1.8$ (grey) in response to increasing doses of tetracaine for the first pulse (dashed lines) and tenth pulse (solid lines).

Conclusion

This study presented biophysical and pharmacological characterization of eight different sodium channel expressing cell lines on Qube simultaneously. Multiple compounds and cell lines were used in parallel on Qube and the results for tetrodotoxin sensitivity, IV-relationship (activation and inactivation), and screening of use dependent sodium channel blockers were of high and consistent quality, which shows good voltage control and cell handling. This demonstrates the Qube's usefulness in screening for both biophysical properties and pharmacology for a set of challenging targets.

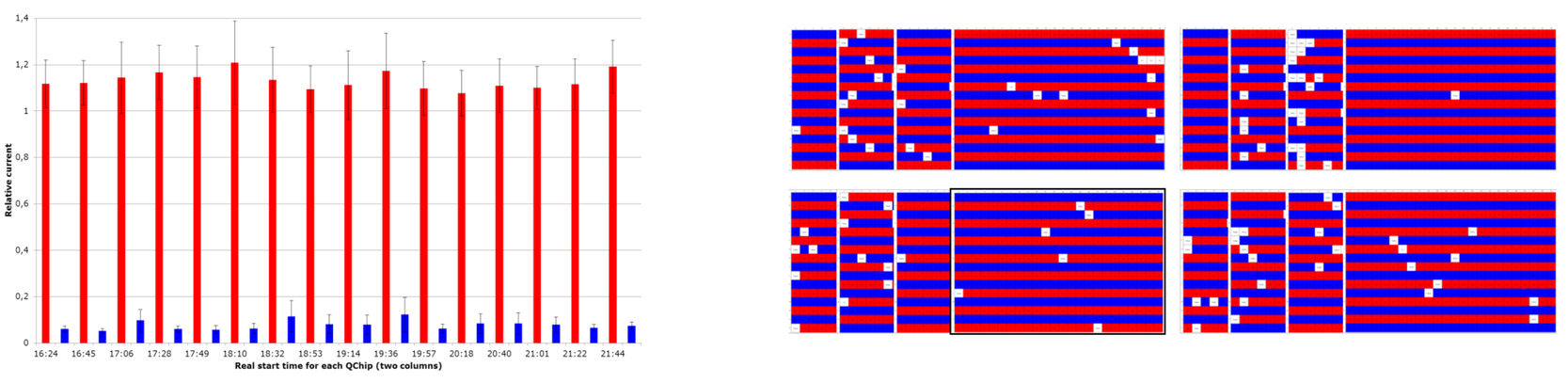
Materials and methods

Cells expressing sodium channel isoforms $Na_V1.1$ to $Na_V1.8$ were cultured according to the SOP for the respective cell line. $Na_V1.1$, $Na_V1.2$, $Na_V1.3$ and $Na_V1.4$ were from SB Drug Discovery (Glasgow, UK), $Na_V1.5$ was from B'SYS GmbH (Witterswil, CH), $Na_V1.6$ and $Na_V1.8$ from ChanTest (Cleveland, OH) and $Na_V1.7$ from Anaxon AG (Berne, CH). On the day of experiment the cells were harvested using detachin or trypsin and transferred to serum-free medium (EX-CELL® ACF CHO Medium, Sigma-Aldrich, Brøndby, DK) supplemented with HEPES 25 mM, 40 µg/ml trypsin inhibitor and P/S. At the time of the experiment, the cells were washed with EC solution and placed in the cell clone Cell Transfer Plate.

Solutions: Intracellular solution (IC) (in mM): CsF 135, NaCl 10, EGTA 5, HEPES 10, adjusted to pH 7.2 (CsOH). Extracellular solution (EC) (in mM): NaCl 145, KCl 4, $MgCl_2$ 1, CaCl2 2, HEPES 10, glucose 10, adjusted to pH 7.4 (NaOH).

Compounds: Tetrodotoxin was from Alomone labs (Jerusalem, Israel), all other compounds from Sigma-Aldrich.

Analysis was carried out using the Sophion Analyzer software, TIBCO Spotfire Cloud (www.spotfire.tibco.com) and the R package "drc" (www.R-project.org and www.bioassay.dk).



[Fig. 5]: Increased throughput using the Qube automation package. Using cells expressing $Na_V1.4$ 16 QChips were run in sequence without interruption using an integrated stacker. A, average normalized currents under control conditions (red) and in the presence of 30 µM tetracaine (blue). B, hit detection using qube software. Tetracaine (30 µM) was added to odd rows (red) and even rows are DMSO controls (blue) showing the possibility to visualize hits directly in Qube software. Average success rate was 93% across all plates.