SOPHION BIOSCIENCE

Application Report

$Na_V1.1$ currents on Qube

Detection of channel activators using an HTS suitable assay with Qube, generating stable $hNa_V1.1$ currents for more than 7 hours with reliable pharmacology

Summary

- Success rates up to 97%
- Stable, unattended measurements over 7 hours
- Consistent current voltage relationship
- High reliability of detecting $Na_V1.1$ activators

Introduction

The sodium channel $Na_V1.1$, highly expressed in fast spiking interneurons (FSIs), initiates the interneuron's action potentials and promotes γ-oscillations, which are important for memory encoding and other cognitive functions. An impaired function of FSIs is associated with disorders like autism, schizophrenia, Alzheimer's and others. Potentiators of $Na_V1.1$ can recover reduced FSI function that might restore γ-oscillations. Therefore, these compounds hold the potential to mitigate cognitive dysfunction in transgenic mice with decreased levels of $Na_V1.1$ in parvalbumin-positive neurons.

Results and discussion

The following sets of experiments were carried out:

1. Sodium currents on single-hole and multi-hole QChips
2. Unattended run of $Na_V1.1$ and current-voltage relationship
3. Pharmacology of $Na_V1.1$

Sodium currents on single-hole and multi-hole QChips

Cells were clamped to -80 mV and for 10 sweeps sodium currents were evoked by application of a depolarization step to -10 mV for 20 ms after a 20 ms hyperpolarization to -120 mV (Fig.1 A). The time between sweep start was 15 seconds. In the next liquid period, $Na_V1.1$ was activated by a depolarization step to -10 mV for 20 ms after a 20 ms hyperpolarization to -120 mV as previously (P1), which was then followed by 100 ms at -80 mV, 0.5 s at -50 mV and 20 ms at -10 mV before another 100 ms at -80 mV (Fig.1 B). This liquid period was repeated in saline and then executed in the presence of a compound (Fig.2).

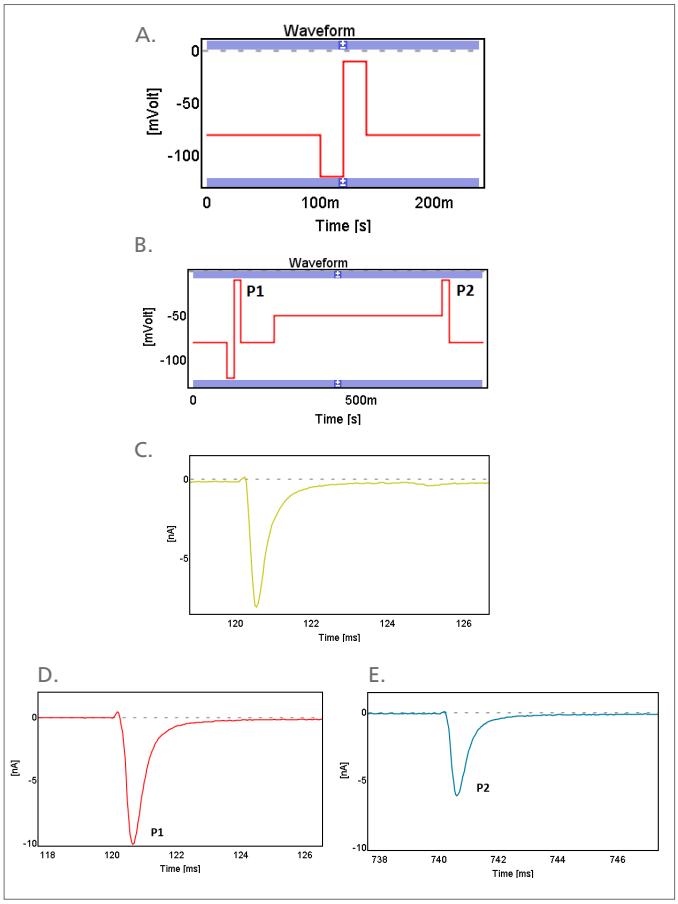


Fig. 1: Voltage protocol, showing the depolarization from -120 mV to -10 mV for 20 ms. B: Raw traces of a sodium current following the depolarization protocol from (A) in $Na_V1.1$-expressing cells on multi hole. C: Voltage protocol, showing the depolarization from -120 mV to -10 mV for 20 ms (P1), followed by 500 ms at -50 mV and 20 ms at -10 mV (P2) from protocol (C). Lower right: Raw traces of P1 (red) and P2 (blue) of the same well on multi hole.

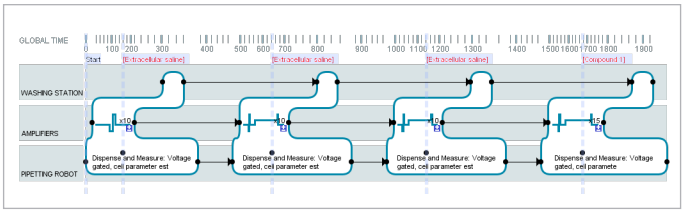


Fig. 2: Experiment scheme in Sophion Viewpoint software. The first block contains 10 single depolarizations and is followed by two blocks containing 10 inactivating depolarization protocols. Lastly, 15 inactivation stimulations are applied to the $Na_V1.1$-expressing cells.

The criteria for a successful recording were (per cell):

- I_{peak} < -200 pA on average in the first 10 sweeps (P1)
- $R_{membrane}$ > 100 MΩ per cell on average in the first 10 sweeps
- C_{total} > 4 pF per cell on average in the first 10 sweeps

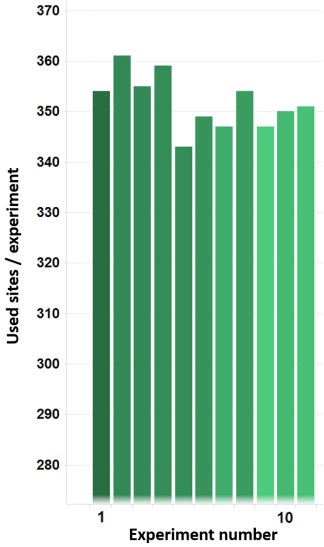
With 218 experiments passing the filter criteria, the success rate for single-hole experiments was 75%. For multi-hole, the success rate was up to 97%.

Unattended run of $Na_V1.1$ and current-voltage relationship

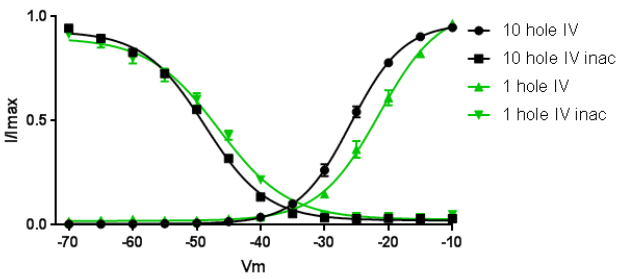
Over 11 consecutive, fully unattended runs with the stacker (7.15 hours), the $Na_V1.1$ cells performed consistently successful (88-94%) and with stable sealing (0.8 to 1.1 GΩ per cell) (Fig.3).

The voltage for half-maximal activation and inactivation ($V_{½}$) was determined using the protocols displayed in Fig.3 C. Half-maximal activation occurred at -21 mV on single-hole and -26 mV on multi-hole, while half-maximal inactivation was induced by application of -47 mV (single-hole) and -49 mV (multi-hole) (Fig.3).

A.



B.



	V50	
	Value	SEM
10 hole IV	-25.914	0.215
10 hole IV inac	-48.702	0.168
1 hole IV	-21.475	0.465
1 hole IV inac	-46.609	0.393

C.

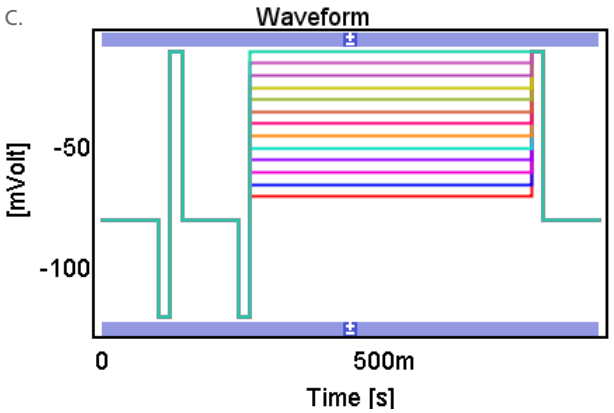


Fig. 3: A: Unattended 7 hour run of 11 multi-hole QChips with success rates between 88 and 94%. Dark green indicates the highest average wholecell resistance QChip of the set (1.1 GΩ/cell), light green the lowest (0.9 GΩ/cell). B: Comparison of V½ of activation and inactivation on single-hole (1x, green) and multi-hole (10x, black). C: Voltage protocol used for the $V_{½}$ comparison above.

For the current-voltage curve (Fig.4 A), cells were clamped to -80 mV and hyperpolarized to -120 mV for 20 ms. The following 20 ms depolarization, occurring in steps of 10 mV from -80 mV to +80 mV was finalized by stepping back to -80 mV (Fig.4 B).

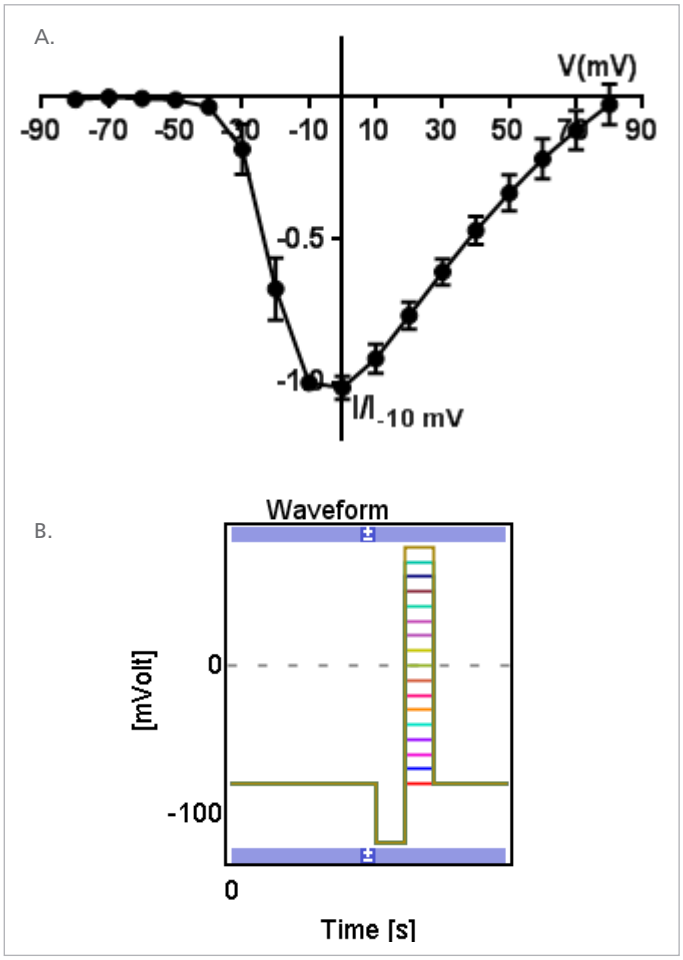
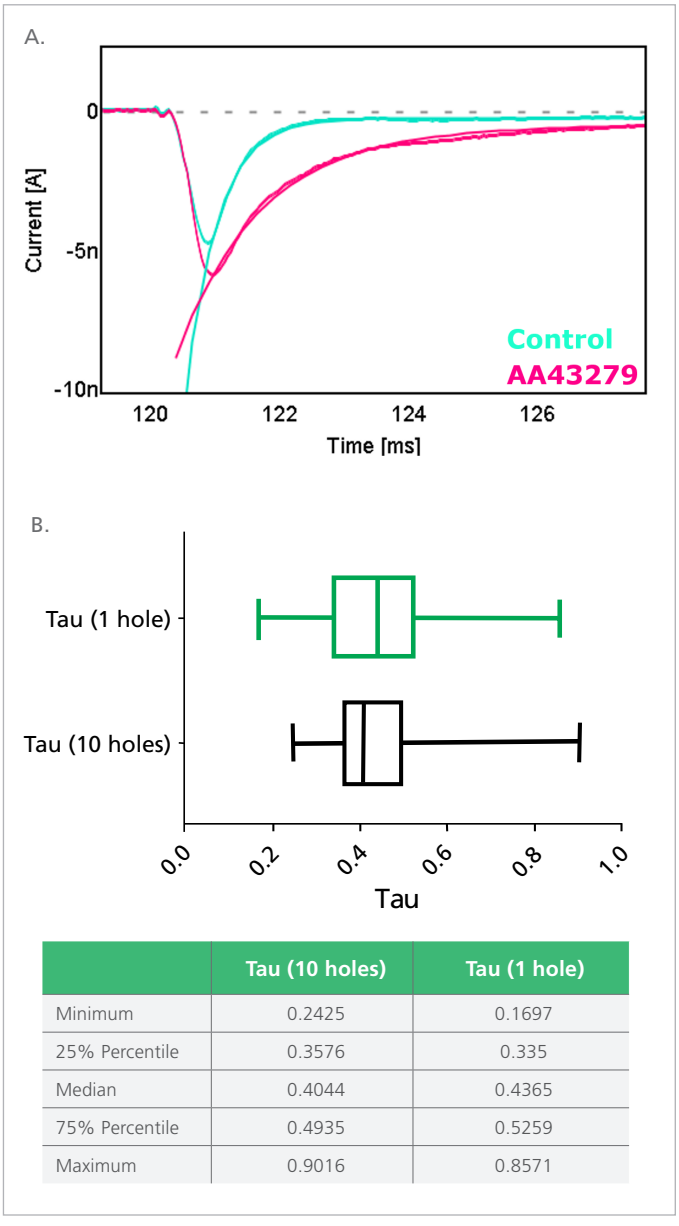


Fig. 4: Top: Current voltage relationship derived from multi hole experiments. Values are average ± SD. Bottom : Current voltage step protocol. Cells were clamped to -80 mV and hyperpolarized to -120 mV for 20 ms. 20 ms depolarization in steps of 10 mV from -80 mV to +80 mV was concluded by stepping back to -80 mV.

Pharmacology of $Na_V1.1$

AA43279 is a small molecule activator of $Na_V1.1$, which impairs the fast inactivation of the channel and is a potential tool for further investigating the role of $Na_V1.1$ in cell culture, tissues and organisms 1. The compound was applied after a baseline period in saline, using the voltage and application protocol from Fig.2. 30 µM AA43279 was applied to 198 wells in total during 32 different experiments. To obtain the time constant of inactivation (tau), the current trace was fitted with a mono-exponential fit (Fig.5A) for P1 and P2 (see Fig.1). With a median of 0.40 ms (multi-hole) and 0.44 ms (single-hole), tau values were indistinguishable between multi-hole and single-hole QChips (Fig.5B). In addition, the area under the curve (AUC) was estimated with an internal algorithm and the peak currents were compared (Tab.1). The assay performed stable both intra and inter day with a throughput of up to 20 plates / 6400 compounds screened per day.



	Tau (10 holes)	Tau (1 hole)
Minimum	0.2425	0.1697
25% Percentile	0.3576	0.335
Median	0.4044	0.4365
75% Percentile	0.4935	0.5259
Maximum	0.9016	0.8571

Fig. 5: A: Control current of $Na_V1.1$ (light blue) and current after compound addition (pink) with the according extrapolated tau fit in the same colour. B: Tau values for single-hole (1 hole) and multi-hole (10 holes).

Table 1: The parameters current, area under curve (AUC) and tau were compared before and after compound addition. "Compound" either represents AA43279 or DMSO control. The ratio of the average values ± SEM is displayed below.

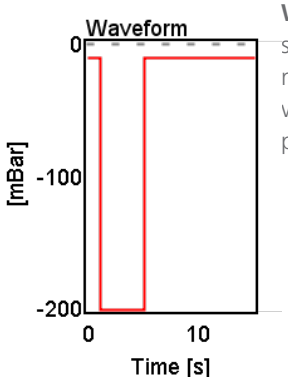
Parameter	Pre-compound	AA43279	Pre-compound	DMSO control
Current P1 [nA]	**-8.03** ± 2.97	**-9.22** ± 3.22	**-7.89** ± 3.07	**-7.95** ± 3.05
AUC P1 [pC]	**-8.58** ± 3.57	**-18.61** ± 6.22	**-8.58** ± 3.04	**-8.96** ± 3.14
Tau P1 [ms]	**0.47** ± 0.16	**1.12** ± 0.25	**0.48** ± 0.22	**0.49** ± 0.22
Current P2 [nA]	**-4.31** ± 1.99	**-5.43** ± 2.36	**-4.29** ± 2.18	**-4.09** ± 2.12
AUC P2 [pC]	**-6.30** ± 3.61	**-13.29** ± 5.53	**-6.46** ± 3.29	**-6.29** ± 3.17
Tau P2 [ms]	**0.43** ± 0.10	**1.25** ±0.25	**0.43** ± 0.10	**0.44** ± 0.12

Parameter	Ratio AA43279 n = 186	Ratio DMSO control n = 464
Current P1	**1.16** ± 0.14	**1.02** ± 0.16
AUC P1	**2.25** ± 0.52	**1.08** ± 0.48
Tau P1	**2.49** ± 0.54	**1.04** ± 0.14
Current P2	**1.28** ± 0.23	**0.97** ± 0.36
AUC P2	**2.23** ± 0.56	**1.01** ± 0.35
Tau P2	**2.97** ± 0.49	**1.04** ± 0.18

Material and methods

HEK cells expressing human $Na_V1.1$ were kindly provided by SB Drug Discovery and cultured and harvested according to Sophion standard procedures.

For Worktable, Cell preparation and Cleanup, Qube default protocols were used.



Whole-cell protocol: A four second suction pulse from -10 mbar to -200 mbar was applied in order to reach whole-cell configuration. For more parameters, see Fig.6.

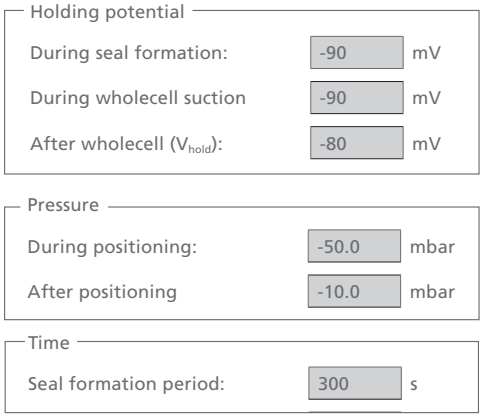


Fig. 6: Detailed parameters of the whole-cell protocol.

References:

Frederiksen K, Lu D, Yang J, Jensen HS, Bastlund JF, Larsen PH, Liu H, Crestey F, Dekermendjian K, Badolo L, Laursen M, Hougaard C, Yang C, Svenstrup N, Grunnet M, 2017. A small molecule activator of Na_V 1.1 channels increases fast-spiking interneuron excitability and GABAergic transmission *in vitro* and has anti-convulsive effects *in vivo*. Eur J Neurosci.;46(3):1887-1896.

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