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HT Automation for patch clamp based primary screen for $Na_V1.1$ using Qube 384

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Introduction

The voltage-gated sodium channel, $Na_V1.1$, highly expressed in fast spiking interneurons (FSIs), initiates the interneuron's action potentials and promotes γ-oscillations. This process is critical for memory encoding and other cognitive functions. An impaired function of FSIs is associated with disorders like autism, schizophrenia, Alzheimer's disease and others. Potentiators of $Na_V1.1$ can restore γ-oscillations by recovering reduced FSI function. These compounds were further shown to mitigate cognitive dysfunction in transgenic mice with decreased levels of $Na_V1.1$ expression in parvalbumin-positive neurons.

$Na_V1.1$ gating is complex, and activators of the ion channel can modulate various channel properties. Amongst many different screening techniques available, the patch clamp technique is the only one that reveals mode of action information. Recent advances of patch clamp devices have enabled the technology to be employed in large compound library screens. Qube 384 is a fully automated 384-well patch clamp device capable of testing thousands of compounds per day whilst providing true giga-ohm seal quality data. Using the Qube 384 in a drug discovery cascade enables acquisition of mode of action data simultaneous with hit detection during the primary screen, minimizing the need for many follow-up validation studies.

Conclusion

In this study, we used the automated patch clamp set-up Qube 384 (Sophion) to develop an assay for $Na_V1.1$ heterologously expressed in HEK293 cells (SB Drug Discovery).

The assay showed biophysical characteristics that are in line with literature values. Multi hole QChips usually provide higher success rates and here we confirmed again that this technology also provides correct biophysical values. These QChips are therefore well suited for a screen of a large compound library.

A pharmacological characterization of the assay was performed using the small molecule potentiator AA43279. A detailed analysis of the mode of action reconfirmed the compound`s action through impeding $Na_V1.1$ inactivation.

Finally, a fully unattended experiment, including 11 consecutively executed 384-well QChips, was performed that showed constantly high success rate over time. Making use of Qube 384`s automation capabilities it is possible to screen up to 6400 compounds per day using this assay. In summary, this work demonstrates that Qube 384 provides a robust method to accelerate the hit-to-lead time for ion channel drug discovery and helps to identify the most relevant compounds faster.

References

Barela, A, et al. (2006). An Epilepsy Mutation in the Sodium Channel SCN1A That Decreases Channel Excitability. Journal of Neuroscience, 26(10), 2714–2723.

Frederiksen, K, et al. (2017). A small molecule activator of Nav1.1 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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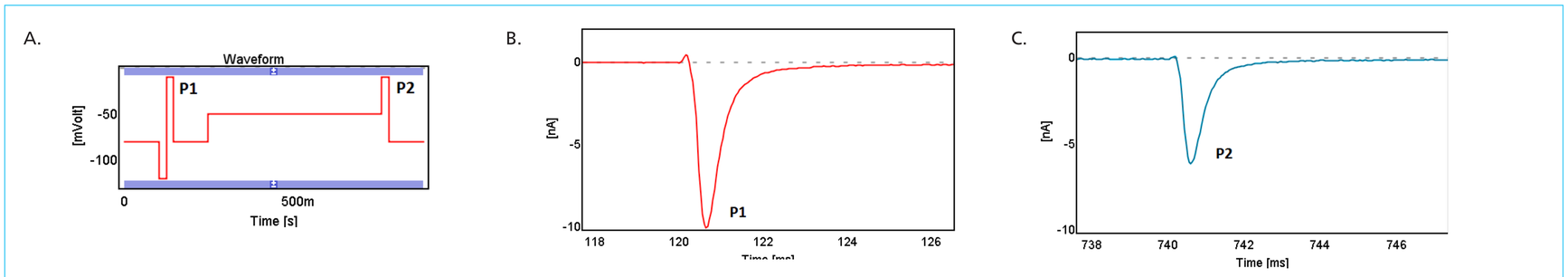


Fig. 1: Voltage protocol and representative $Na_V1.1$ current traces. A: Voltage protocol to discriminate state-dependent inhibitors. A depolarization from -120 mv (all channels in the closed state) to -10 mV for 20 ms (P1) caused an activation of all channels. In a next step, the voltage was clamped to -50 mV for 500 ms. At this potential, one portion of the channels was in the inactivated and the other in the closed state. A 20 ms voltage step to -10 mV (P2) activated all channels that were in the closed state. B and C: Raw traces of P1 (red) and P2 (blue) recorded from the same well on a multi-hole QChip.

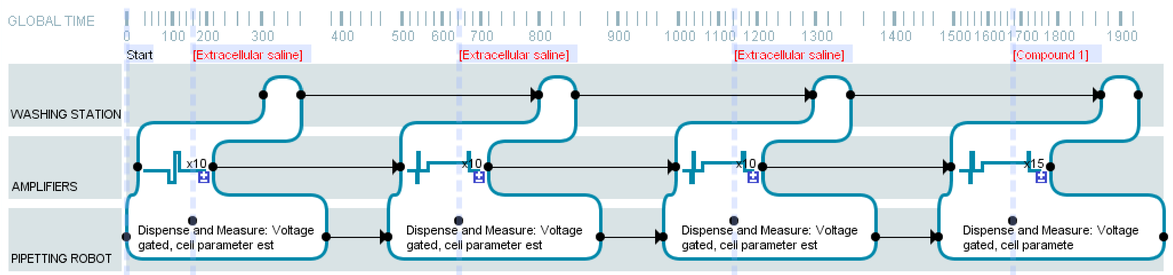
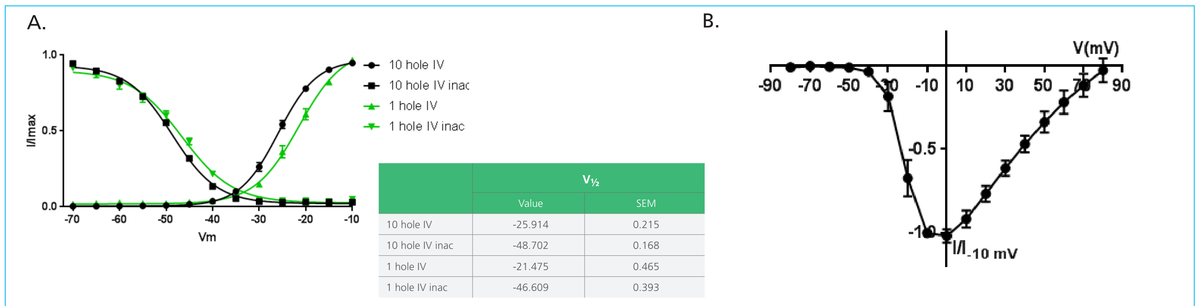
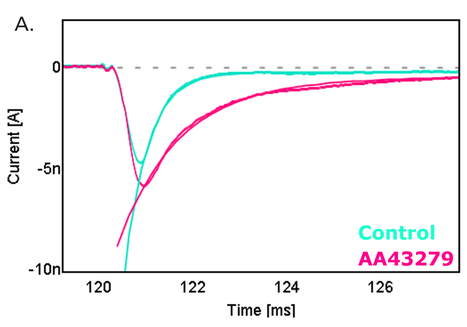


Fig. 2: Experimental set up in Sophion Viewpoint software. At the beginning of each block (liquid period), a defined solution was added to the cells. Following this addition, a voltage protocol was executed. Saline was added during the first 3 liquid periods. The liquid addition was followed by an application of a voltage protocol. 10 single depolarization steps were applied in the first, and a double pulse protocol as shown in figure 1 was applied to the in the second and third liquid period. Compound effects were assessed during the last period.



	$V_{½}$	
	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

Fig. 3: Biophysical characterization of $Na_V1.1$. QChips with multiple holes in each well usually result in higher success rates. To confirm that both single- and multi-hole QChips provide the same results, we investigated $Na_V1.1$ gating using both technologies. Activation and inactivation kinetics were assessed by sending a family of voltage steps from -80 to +80 mV with 500 ms duration to the cells followed by a 20 ms segment at +80 mV. A: Activation and inactivation on single-hole (1 hole/well, green) and multi-hole (10 holes/well, black). No significant difference between the single- and the multi-hole experiment was observed. $V_{½}$ values of these experiments are shown in the insert and all values are in good agreement with the literature (Barela, 2006). B: Current voltage relationship of a multi-hole experiments.



	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. 4: Analysis of the inactivation kinetics. To further validate the use of the multi-hole technology, we analyzed inactivation kinetics of $Na_V1.1$ using both single- and multi-hole QChips. Using Sophion`s Analyzer software, it is possible to fit the data with all commonly used functions, and to extract biophysical parameters such as time constants in an exponential decay (tau values). A: Representative current trace of $Na_V1.1$ in control conditions (light blue) and currents following exposure to the $Na_V1.1$ potentiator AA43279 (pink) with the respective mono-exponential fit shown in the same color. B: Tau values for single-hole and multi-hole (10 holes) experiments. No significant difference was observed between the single- and the multi-hole technology.

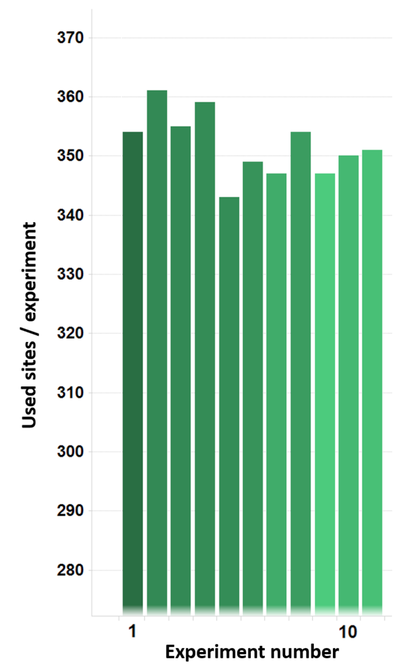


Fig. 5: Testing up to 20 plates (6400 compounds) per day: Unattended 7 hour run of 11 multi-hole QChips with success rates between 88 and 94%. The criteria for a successful recording were: I_{peak} < -200 pA/cell (P1), R_{total} > 100 MΩ/cell and C_{total} > 4 pF/cell. The highest average wholecell resistance of the entire set of experiments was 1.1 GΩ/cell (darkest green) the lowest was 0.9 GΩ/cell (lightest green).

Table 1: Mode of action study. It is known that AA43279 potentiates Na^+ current by impeding $Na_V1.1$ inactivation (Frederiksen, 2017). Using Sophion Analyzer software it is possible to rapidly extract relevant parameters for mode of action studies. Here, peak current, area under the curve (AUC) and time constant inactivation (tau) were compared before and after compound addition. Application of the compound significantly prolonged tau both at P1 and P2. Peak current values, however, remained unaffected. The effect on AUC did not reach significance due to the relatively large variation in the data. Taken together, this data reconfirms that AA43279 increases Na^+ current by impeding $Na_V1.1$ inactivation. Data is presented as mean ± SEM. *** represents p<0.001 in a ANOVA test.

Parameter	Relative activation [%] AA43279 (30 µM) n = 186	Relative activation [%] DMSO (0.3%) n = 464
Peak current P1	116 ± 14	102 ± 16
AUC P1	225 ± 52	108 ± 48
Tau P1	249 ± 54***	104 ± 14
Peak current P2	128 ± 23	97 ± 36
AUC P2	223 ± 56	101 ± 35
Tau P2	297 ± 49***	104 ± 18