Adaptive voltage protocols increase precision of voltage-gated ion channel measurements on high-throughput automated patch clamp platforms

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1 ABSTRACT

Voltage-gated sodium (Na_V) channels are studied extensively due to their potential as targets for several indications, such as pain, epilepsy, cardiac and muscle paralysis. Some Na_V channel modulators show state-dependence and bind preferentially to the inactivated state of the channel. The potency of state-dependent compounds are known to vary depending on the percentage inactivation of the channels.

To calculate accurate compound activity the precise value for the V_{Half} of inactivation should be used for each cell. The adaptive protocol block for the Sophion Qube 384-well automated patch clamp platform has made it possible to separately define the voltage applied to individual wells for both the activation and inactivation of the channels. This enables the generation of more precise data for voltage-gated ion channels.

2 MATERIALS AND METHODS

Cell Culture: HEK-Na_V1.1 cells were produced at Charles River Laboratories and are commercially available. Cells are grown according to their SOP as developed by Charles River. Cells were kept in a serum-free medium in the cell hotel on the Qube for up to 4 hours.
Solutions: Extracellular solution (mM): 145 NaCl. 4 KCl, 10 HEPES, 10 Glucose, 1 $MgCl_2$, 2 $CaCl_2$, pH7.4. Intracellular solution (mM): 120 CsF, 20 CsCl, 10 NaCl, 10 HEPES, 10 EGTA, pH7.2.
Qube experiments: Experiments were conducted using Sophion Qube software version 2.4.64 (Eagle) using Single-hole QChips. The experimental protocol was adapted from a previously used HTS screening protocol, which contained a voltage protocol aimed to inactivate the channels by 50%, using a pre-determined inactivation voltage of -50 mV for 500 ms. Series resistance compensation was applied at 70%. Currents were sampled at 25 kHz, with cut off at 5 kHz and Bessel filtering.
Analysis: Data analysis was performed using Qube Analyzer software, GraphPad Prism (7.0) and Vortex v2017.04.62496.96-s.

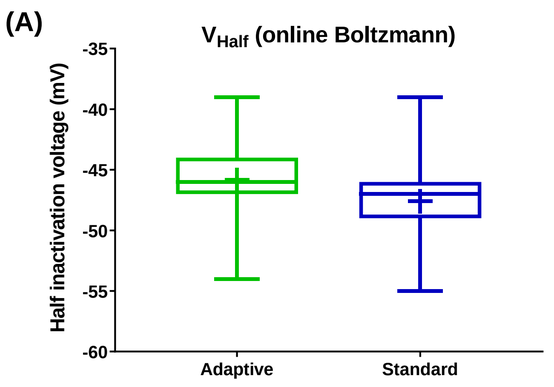
3 RESULTS

3.1 The adaptive protocol did not change the performance of the assay compared to the standard protocol.

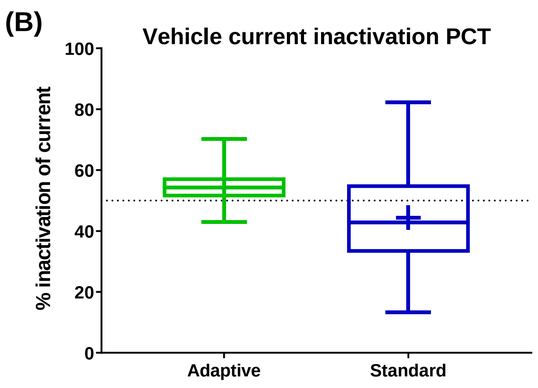
	Success rate	Seal resistance (GΩ)		Series resistance (MΩ)		Cell capacitance (pF)	
		Before	After	Before	After	Before	After
Standard	73 %	2.4 ± 1.2	3.1 ± 1.9	7.4 ± 2.2	8.6 ± 2.5	16.4 ± 6.2	16.4 ± 6.2
Adaptive	76 %	2.1 ± 1.9	2.5 ± 2.0	7.4 ± 2.7	8.1 ± 2.5	16.7 ± 5.5	16.3 ± 5.5

Table 1. Assay performance parameters. Parameters shown for before and after the online Boltzmann fit in the experimental protocol.

3.2 V_{Half} of inactivation values derived from online Boltzmann fits were comparable for both experiments, but the resulting current inactivation percentage less variable using the adaptive protocol



	Adaptive	Standard
Mean	-45.84	-47.59
Std. Deviation	2.613	2.870



	Adaptive	Standard
Mean	54.36	44.35
Std. Deviation	5.032	14.94

Figure 1. V_{Half} of inactivation and current inactivation values. (A) V_{Half} of inactivation values derived from the online Boltzmann experiment block for the experiments using either the standard voltage protocol or the adaptive voltage protocol. (B) % inactivation of the Na_V1.1 current in the vehicle period, calculated as a ratio of the inactivated state current amplitude to the resting state current amplitude. The mean values are indicated by the '+' symbol, median values by the line in the box.

3.3 Example Na_V1.1 traces.

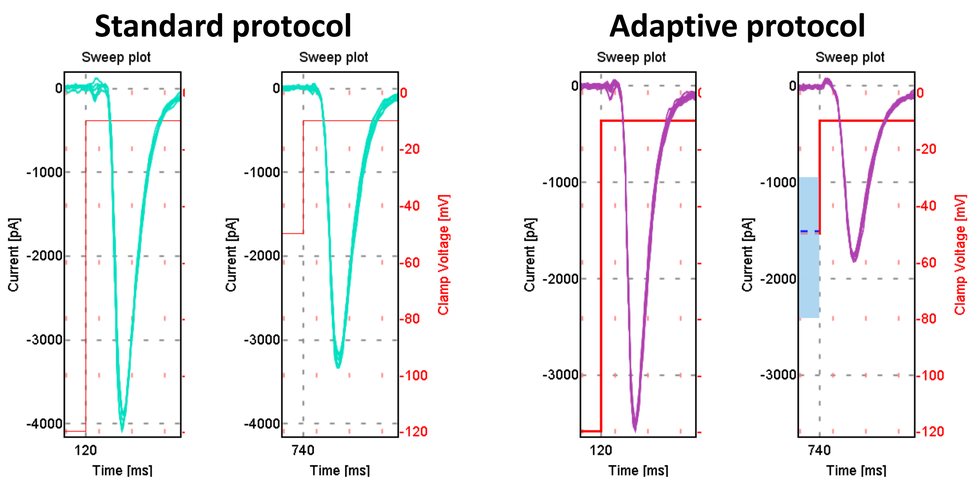


Figure 2. Example Na_V1.1 current traces. Example traces from the resting state and inactivated state for the standard protocol, where a fixed inactivation voltage of -50 mV was applied, and the adaptive protocol, where the V_{Half} inactivation voltage was determined by the online Boltzmann fit.

3.4 Histogram showing reduced variability of current inactivation percentage values when using the adaptive protocol.

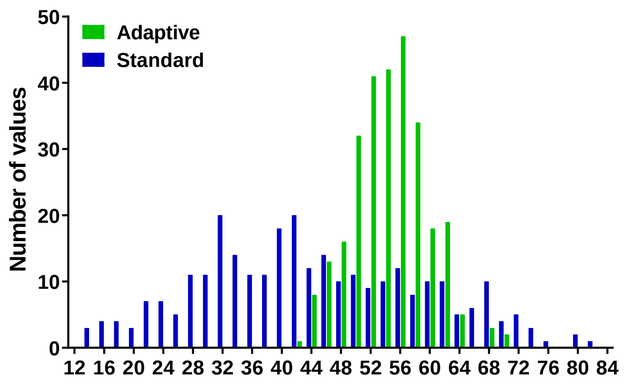
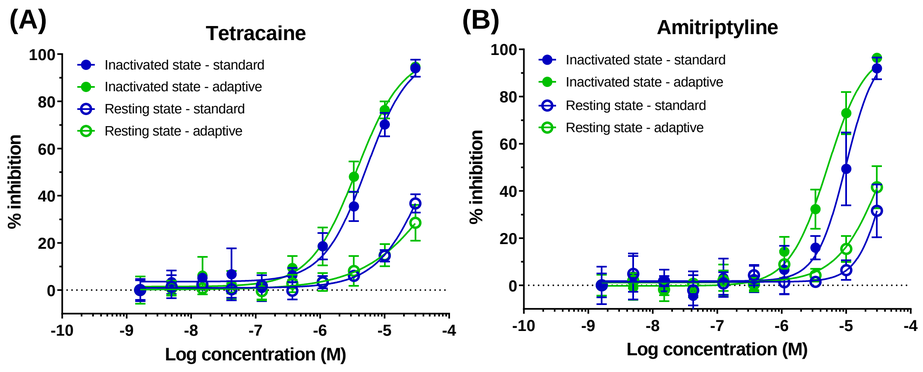


Figure 3. Histogram of the % inactivation of the Na_V1.1 current. In the standard protocol experiment approximately 80% of the wells had % inactivation of the current between 26 and 66. In the adaptive protocol experiment, the % inactivation of the current was between 47 and 61 for 80% of the wells.

3.5 Concentration-response curves for Tetracaine and Amitriptyline.



(C)

Tetracaine	Resting	Inactivated
Standard	> 30 µM	6.7 µM
Adaptive	> 30 µM	4.1 µM

Amitriptyline	Resting	Inactivated
Standard	> 30 µM	15.2 µM
Adaptive	> 30 µM	6.3 µM

Figure 4. Example concentration-response curves for (A) Amitriptyline and (B) Tetracaine. Data from the resting and inactivated state pulses using either the standard (blue) or adaptive (green) protocol. (C) Tables show IC_{50} values for both protocols.

3.6 Percentage inhibition data for compounds at 10 µM was less variable using the adaptive protocol.

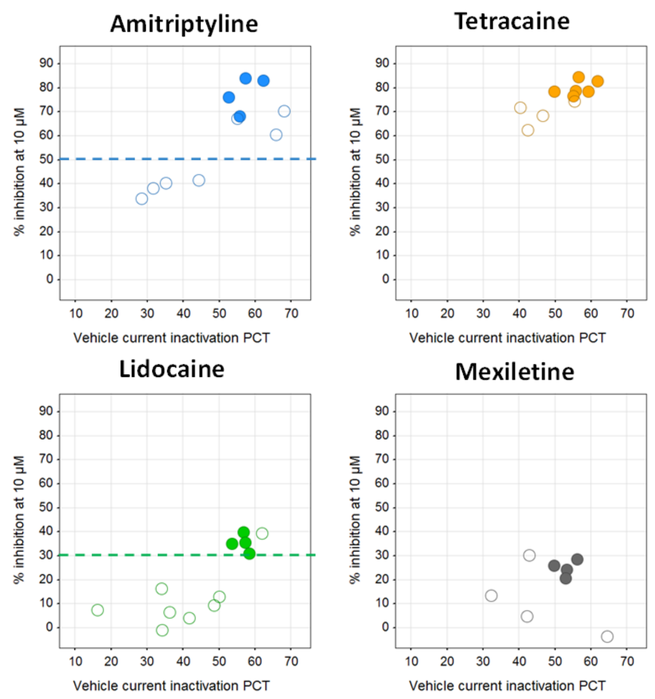


Figure 5. Compound % inhibition data for 10 µM Amitriptyline, Tetracaine, Lidocaine and Mexiletine. Data were collected using either the standard experiment (empty circles) or adaptive experiment protocol (full circles). The % inhibition data for all compounds was less variable using the adaptive experiment protocol. If the hit limit had been set to 50% inhibition (blue dashed line), some of the 10 µM Amitriptyline wells would have been missed using the standard protocol. Similarly, if the hit limit had been set to 30% inhibition (green dashed line), most of the 10 µM Lidocaine wells would have been missed using the standard protocol.

4 SUMMARY

The incorporation of the adaptive protocol did not change the performance of our Na_V1.1 assay compared to the standard protocol. The adaptive protocol significantly decreased the variability of the percent current inactivation. In the standard protocol experiment approximately 80% of the wells had percent current inactivation between 26-66%, whereas in the adaptive protocol experiment this was between 47-61%.

Four known state-dependent compounds were tested as concentration-response curves against Na_V1.1 in both protocols, with compound potencies found to be similar. However, the compound data at 10 µM was found to be much less variable in the adaptive protocol experiment. In a high throughput screen this reduced variability should lead to increased confidence in the results.

In summary, the new adaptive protocol enables increased control of the state that voltage-gated channels during an experiment on a 384-well high throughput automated patch clamp platform, which leads to reduced data variability and increased confidence in compound testing results.

Acknowledgements
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