Application Report:

CHO-$Na_V1.5$

Use-dependent blockers

The voltage dependent sodium channel, $Na_V1.5$, was tested on QPatch 16X in single-hole and multi-hole mode. In this study, we wanted to determine the best test protocol to distinguish between two antiarrythmic drugs with different modes of action.

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Use-dependent $Na_V1.5$ blockers on QPatch in single- and multi-hole mode

Introduction

The voltage dependent sodium channel, $Na_V1.5$, was tested on QPatch 16X in single-hole and multi-hole mode. Single-hole mode is a classic patch clamp experiment where one cell is in whole-cell configuration, whereas multi-hole mode comprises up to 10 cells in whole-cell configuration. The multi-hole mode therefore tests the summed current of up to ten cells.

In this study, we wanted to determine the best test protocol to distinguish between two antiarrythmic drugs with different modes of action: flecainide, which blocks open Nav channels, and lidocaine, which blocks inactivated Nav channels. Therefore four different voltage protocols were set up to test for: 1) steady-state inactivation, 2) open-channel block, 3) recovery from inactivation, 4) state- versus use-dependence. Each protocol was tested in both single-hole and multi-hole mode to compare the capabilities of the QPatch 16X in the two modes.

Materials and Methods

Cells

CHO-Nav1.5 QCells from B'SYS, optimized for QPatch experiments by Sophion Bioscience.

Solutions

Extracellular solution (in mM): 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 4 KCl, 145 NaCl, 10 Glucose, pH 7.2, 310 mOsm.

Intracellular solution (in mM): 135 CsF, 1/5 EGTA/CsOH, 10 HEPES, 10 NaCl, pH 7.3, 300 mOsm.

Drugs

Lidocaine and Flecainide (Sigma) were dissolved in an ethanol stock solution, such that the final ethanol concentration for the experiments did not exceed 0.1%.

Voltage protocols

V_{hold}= -100 mV. Data was sampled at 50 kHz, 8^{th} order Bessel filter, cut-off frequency 3 kHz, and, in single-hole mode, 80 % Rs compensation. P/n leak subtraction was employed.

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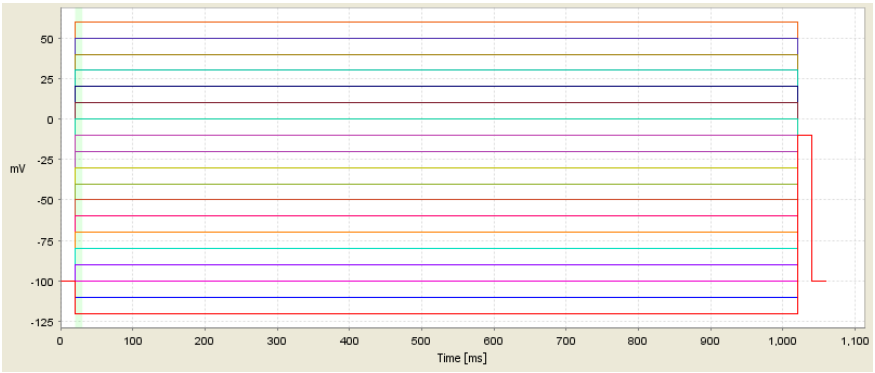
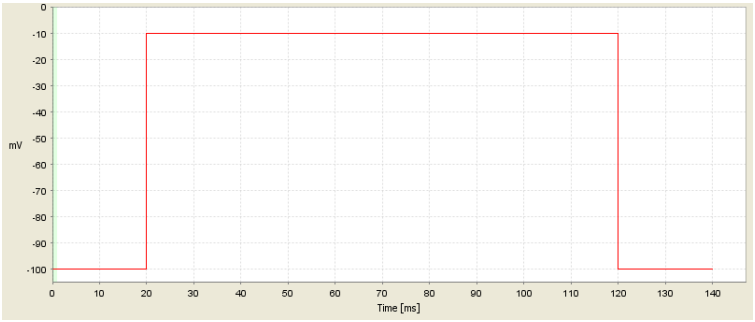
1) Steady-state inactivation



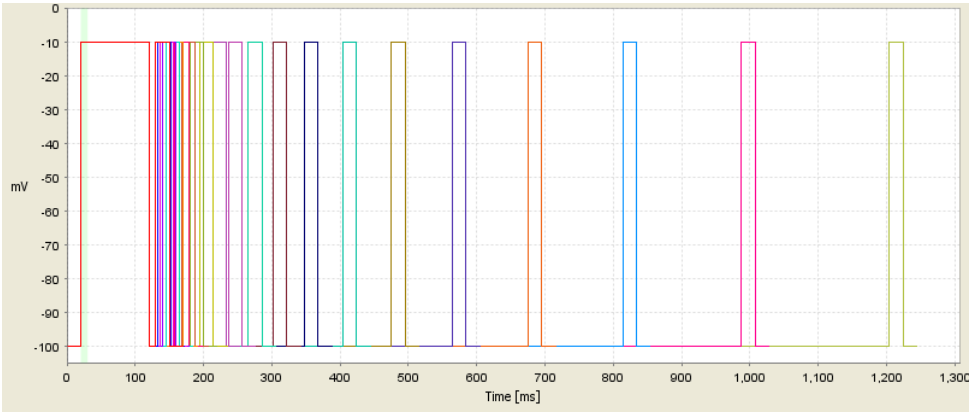
20 ms at V_{hold}, 1000 ms at potential ranging from -120 to +60 in 10 mV increments, 20 ms test potential at -10 mV.

2) Open-channel block



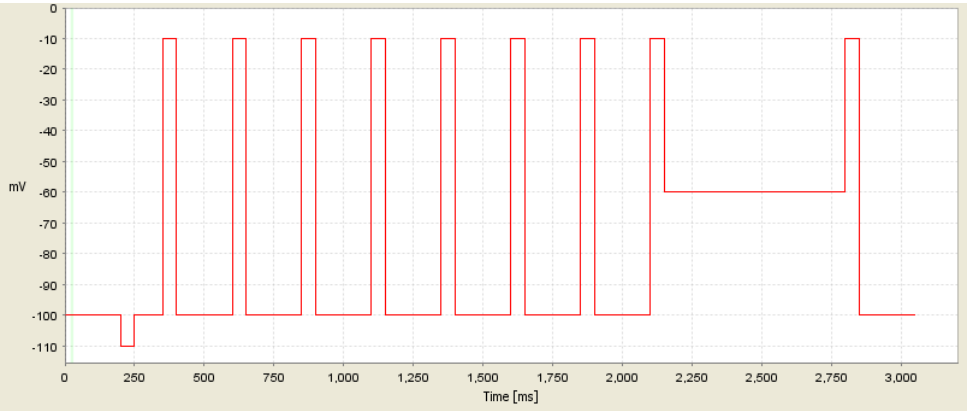
20 ms at V_{hold}, 100 ms at test potential of -10 mV.

3) Recovery from inactivation



20 ms at V_{hold}, two pulses of 100 ms at test potential of -10 mV, with incremental increase in time between depolarizations starting at 10 ms and increasing by 25% per sweep.

4) State- versus use-dependence



8 depolarizations to -10 mV for 50 ms, at a frequency of 4 Hz, followed by a 650 ms step to -60 mV (approx. V½), and a final depolarization to -10 mV for 50 ms.

Results

Each of the voltage protocols described in Materials & Methods were employed on a QPatch 16X in both single- and multi-hole mode.

Figure 1 and

Figure **2** show IV plots for steady-state inactivation and activation in dose-response experiments with flecainide and lidocaine respectively (protocol 1). The figures show that both flecainide and lidocaine shift the voltage of half-maximal inactivation (V½) towards more hyperpolarized potentials, but that only flecainide has a significant effect on the maximal current amplitude.

Use-dependent $Na_V1.5$ blockers on QPatch in single- and multi-hole mode

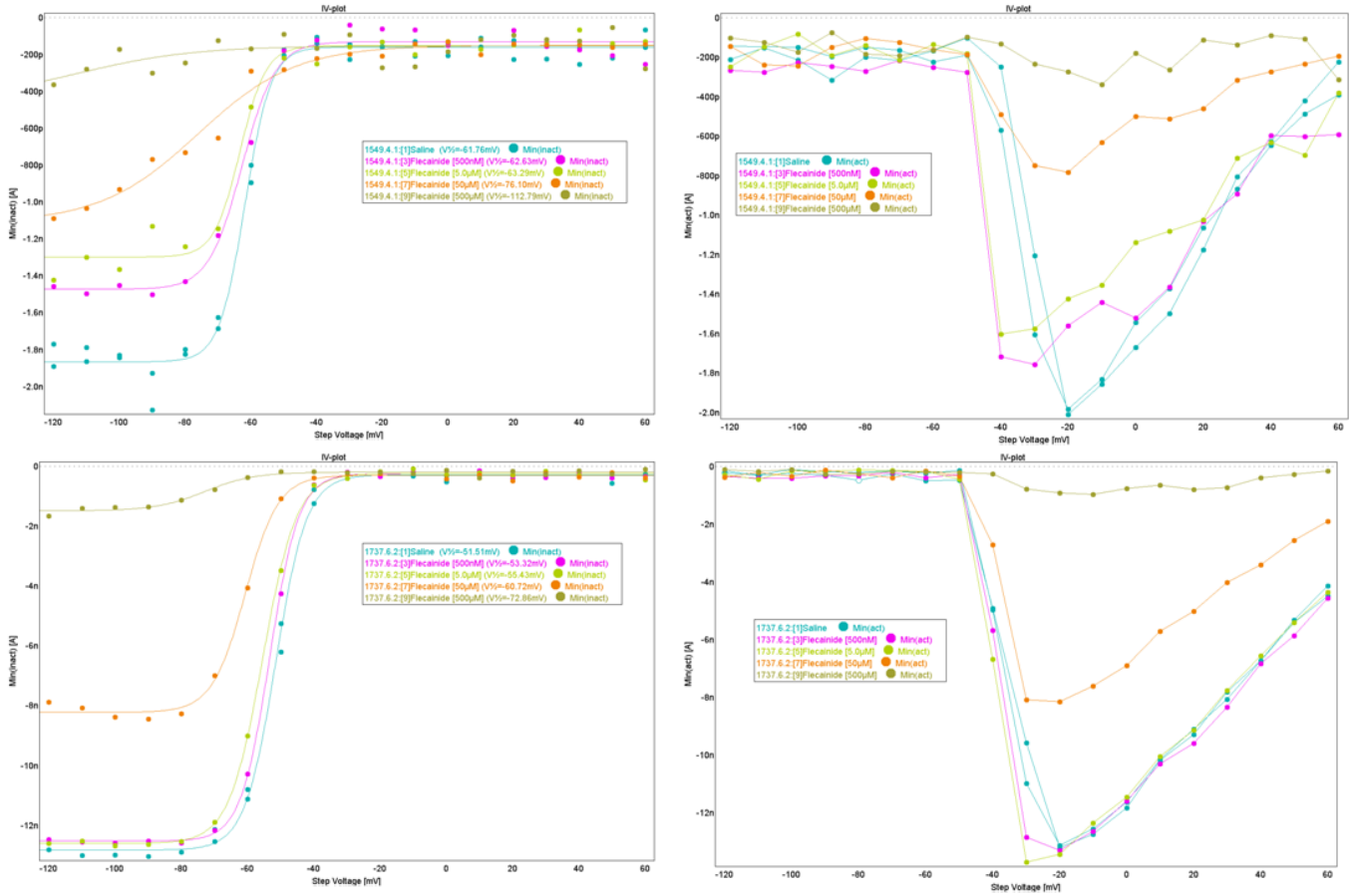


Figure 1 Steady-state inactivation (left) and IV for activation (right) for flecainide. Top is single-hole data, bottom is multi-hole data.

This is further emphasized in the next experiments shown in Figure 3 and Figure 4 where lidocaine and flecainide were tested with a standard protocol for open-channel block (protocol 2). Here it is very evident that flecainide produces a strong effect on the Nav1.5 ion channel already at 50 µM, whereas lidocaine does not have an effect until 500 µM. The data is summarized in Table 1.

It is also worth noting that the single-hole and multi-hole data is very similar. This is somewhat surprising for the IV-data, given that multi-hole experiments do not allow Rs compensation and we therefore could expect a systematic error in the voltage applied to the cells.

In a hypothetical example, say the Rseries on an individual cell is 5 MΩ and the current is 1 nA. This would result in a voltage error of 5 mV on the measurement site, in both single-hole and multi-hole mode. However, since only single-hole mode allows compensation of this, we expect shifts in the IV curves of multi-hole experiments; but we can see from this data that the shifts are very small.

Use-dependent $Na_V1.5$ blockers on QPatch in single- and multi-hole mode

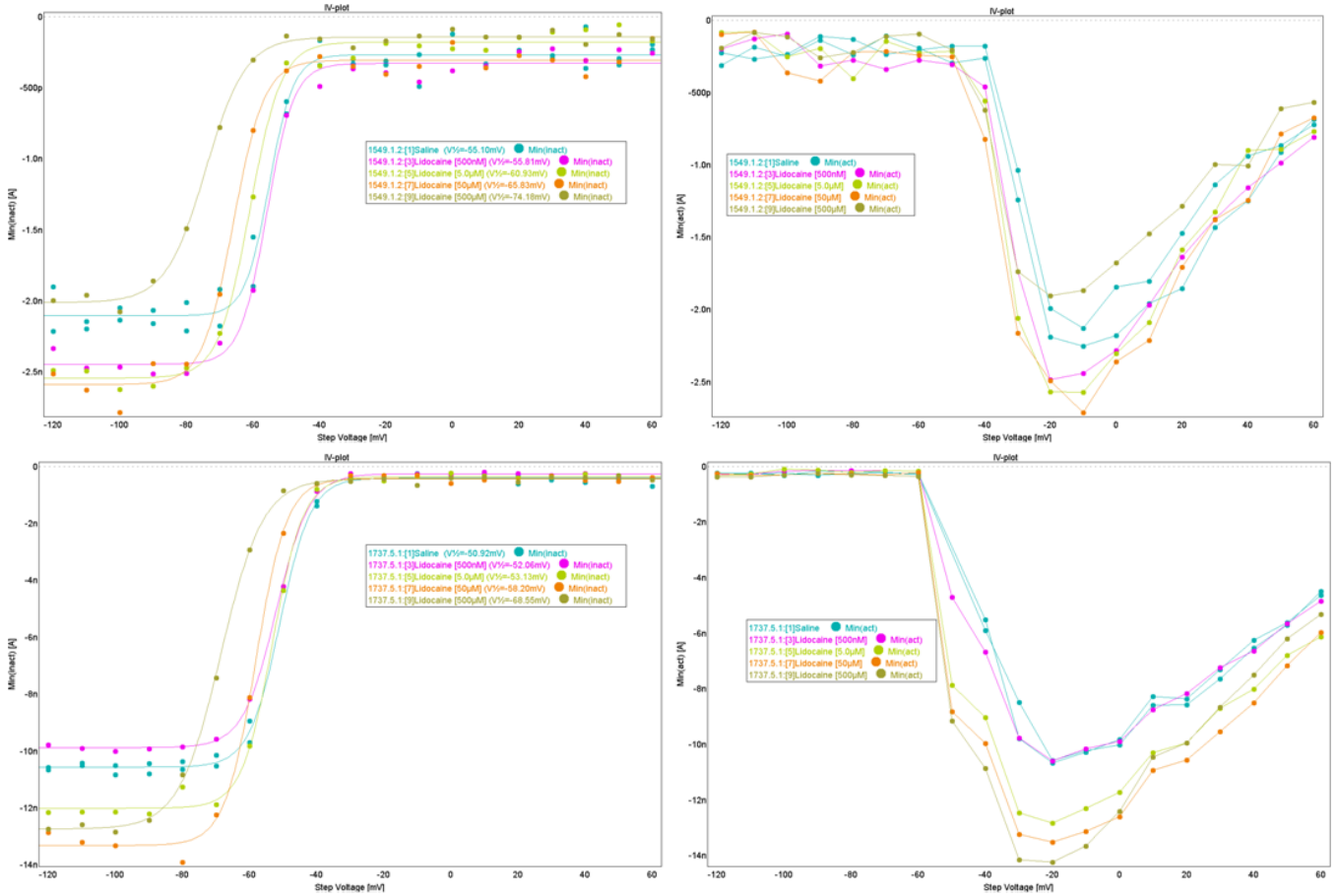


Figure 2 Steady-state inactivation (left) and IV for activation (right) for lidocaine. Top is single-hole data, bottom is multi-hole data.

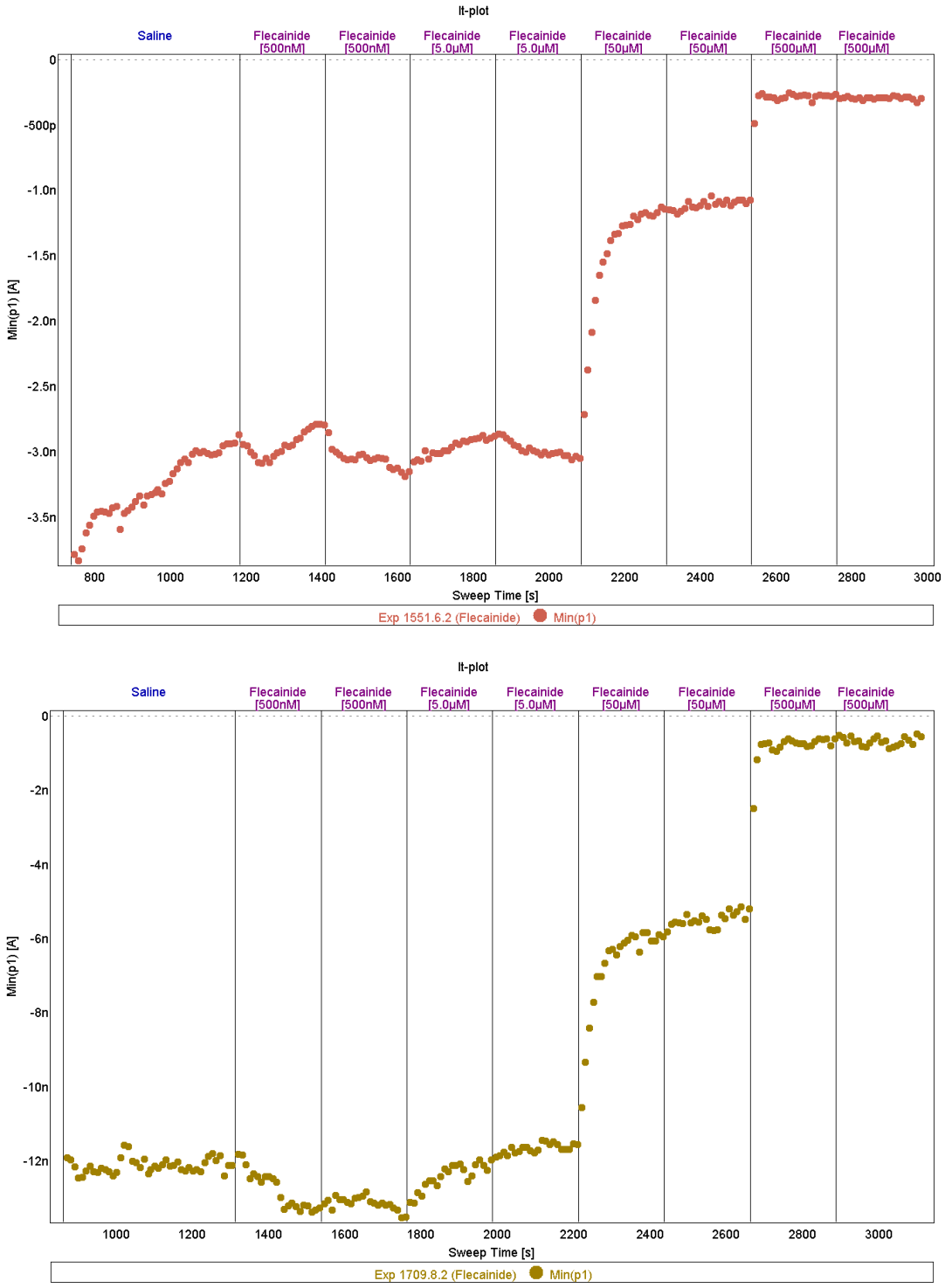


Figure 3 IT plot for open-channel block for flecainide (simple depolarization to -10 mV), top is single-hole and bottom is multi-hole data.

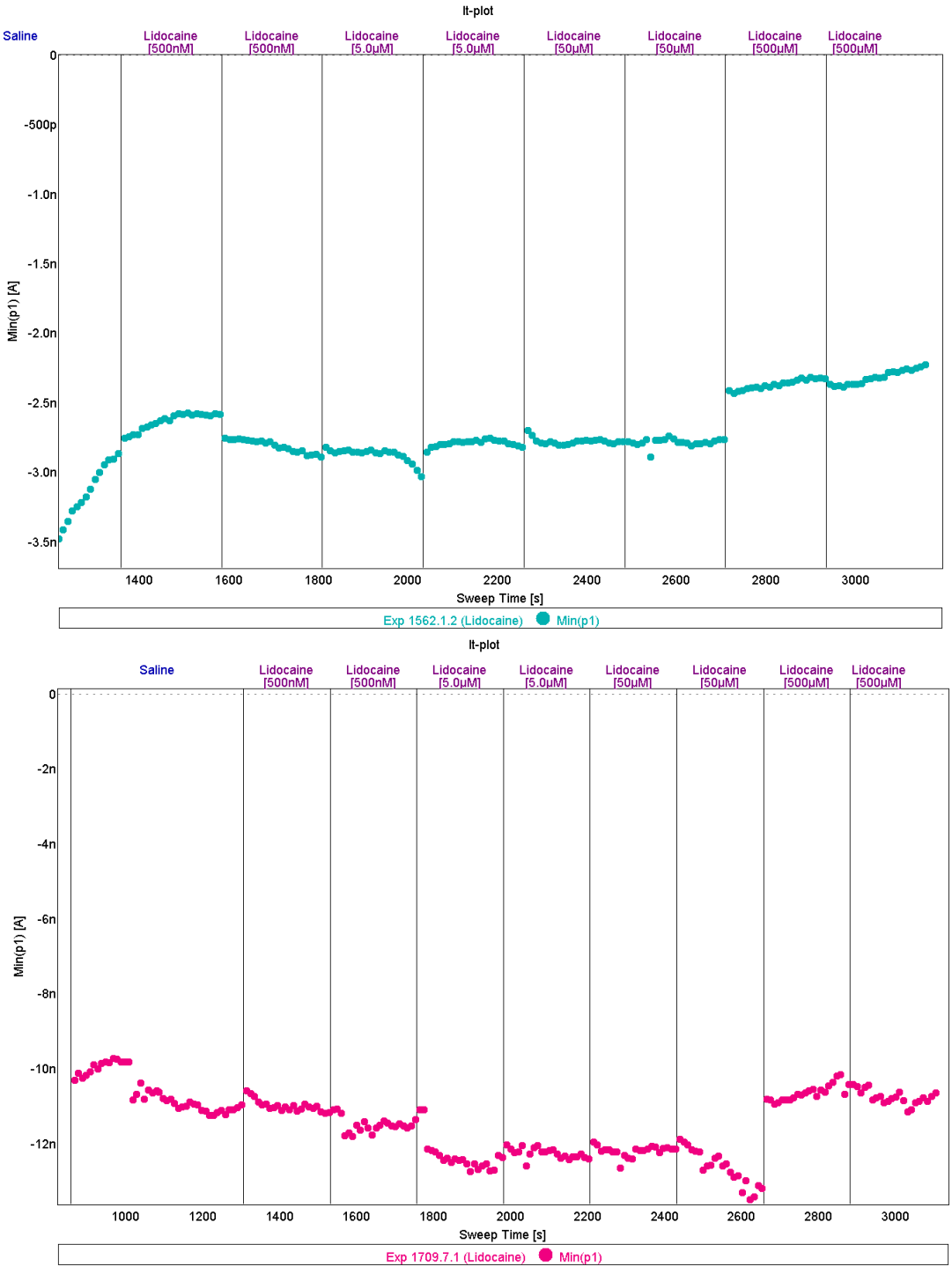


Figure 4 IT plot for open-channel block for lidocaine (simple depolarization to -10 mV), top is single-hole and bottom is multi-hole data.

Next, in Figure 5 and Figure 6, we tried a voltage protcol to test for recovery from inactivation (protocol 3). The protocol is made up of two depolarizations where the time between them is increased by 25% with each sweep. The ratio between the last and first peak (peak2/peak1) is plotted as a function of the time between them, and

the data is fitted to exponential equation to produce a time constant for recovery from inactivation. The time constants are summarized in **Table 1**.

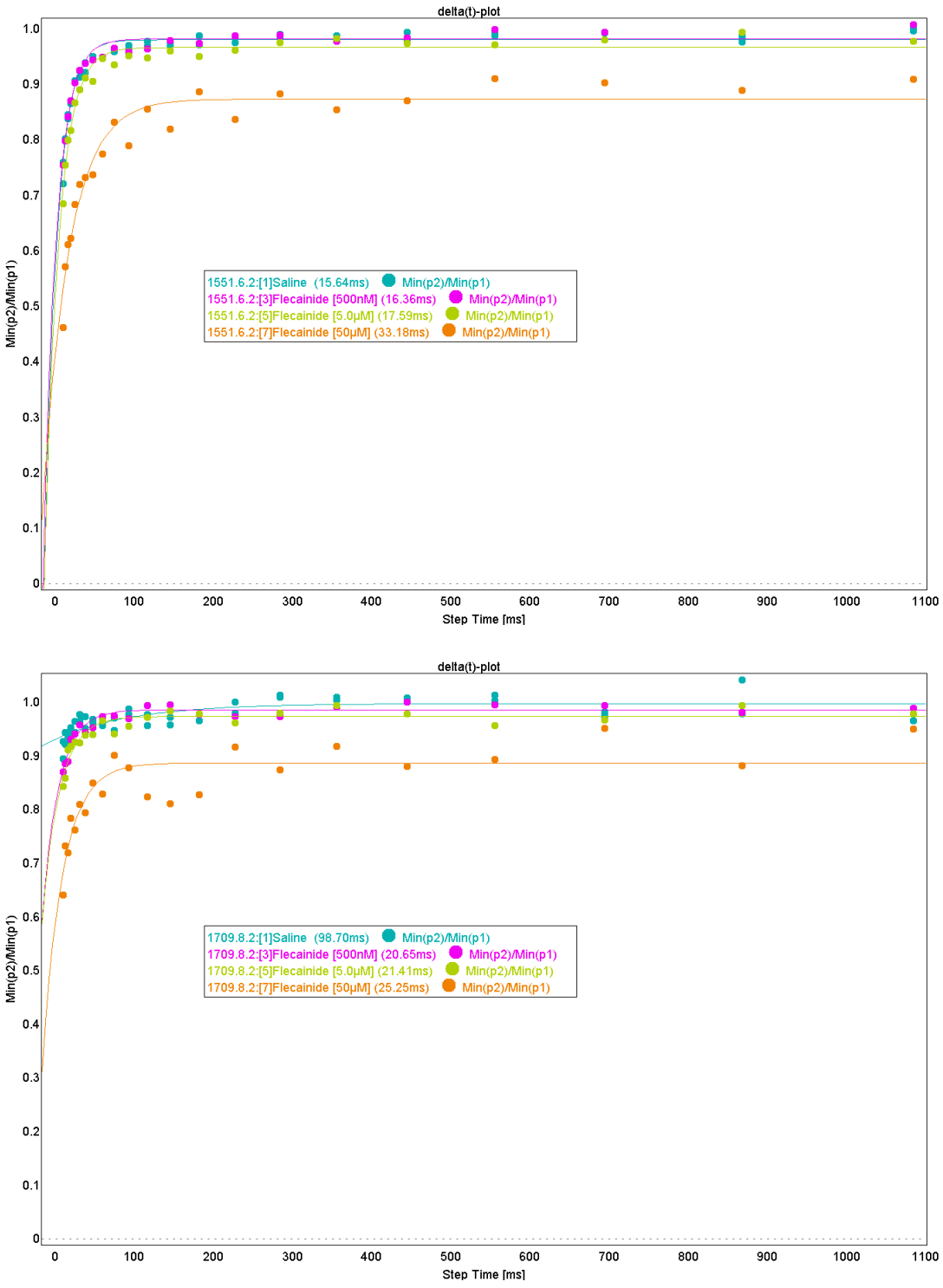


Figure 5 Recovery from inactivation for flecainide. Peak 2/peak 1 is plotted as a function of the time increment between peaks. Top is single-hole data, bottom is multi-hole data.

It can be seen in Figure 6 that lidocaine increases the time constant for recovery from inactivation, suggesting that lidocaine keeps the ion channels in the inactivated state. Flecainide (Figure 5) does not have as strong an effect, consistent with its reported affinity for open channels.

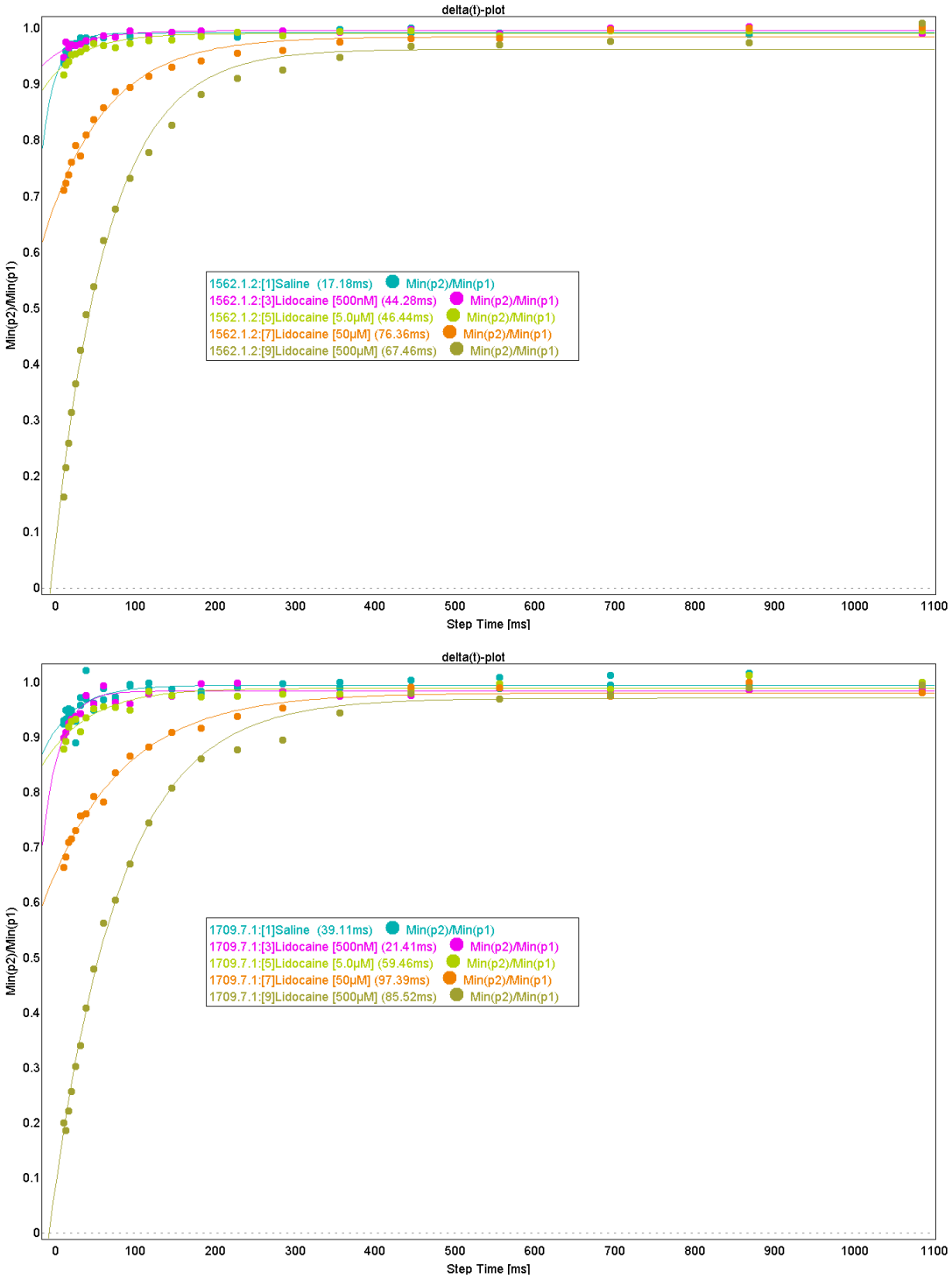


Figure 6 Recovery from inactivation for lidocaine. Peak 2/peak 1 is plotted as a function of the time increment between peaks. Top is single-hole data, bottom is multi-hole data.

Finally, we tested the pulse-train protocol for determining state- and use-dependence (protocol 4), in both single-hole and multi-hole mode.

The protocol employs 8 depolarizations to -10 mV at a frequency of 4 Hz (i.e. peaks 1-8). This is followed by a 650 ms step to -60 mV (approx. V½), and a final depolarization to -10 mV (peak 9). Thus, "peak 1" -current is comparable to the simple open channel block (protocol 2), whereas the current at "peak 8" is a measure of use-dependent block, and peak 9 determines state dependency.

The peak currents from the first, eighth (use dependence) and ninth peak (state dependence) are plotted in Figure 7 for flecainide and Figure 9 for lidocaine.

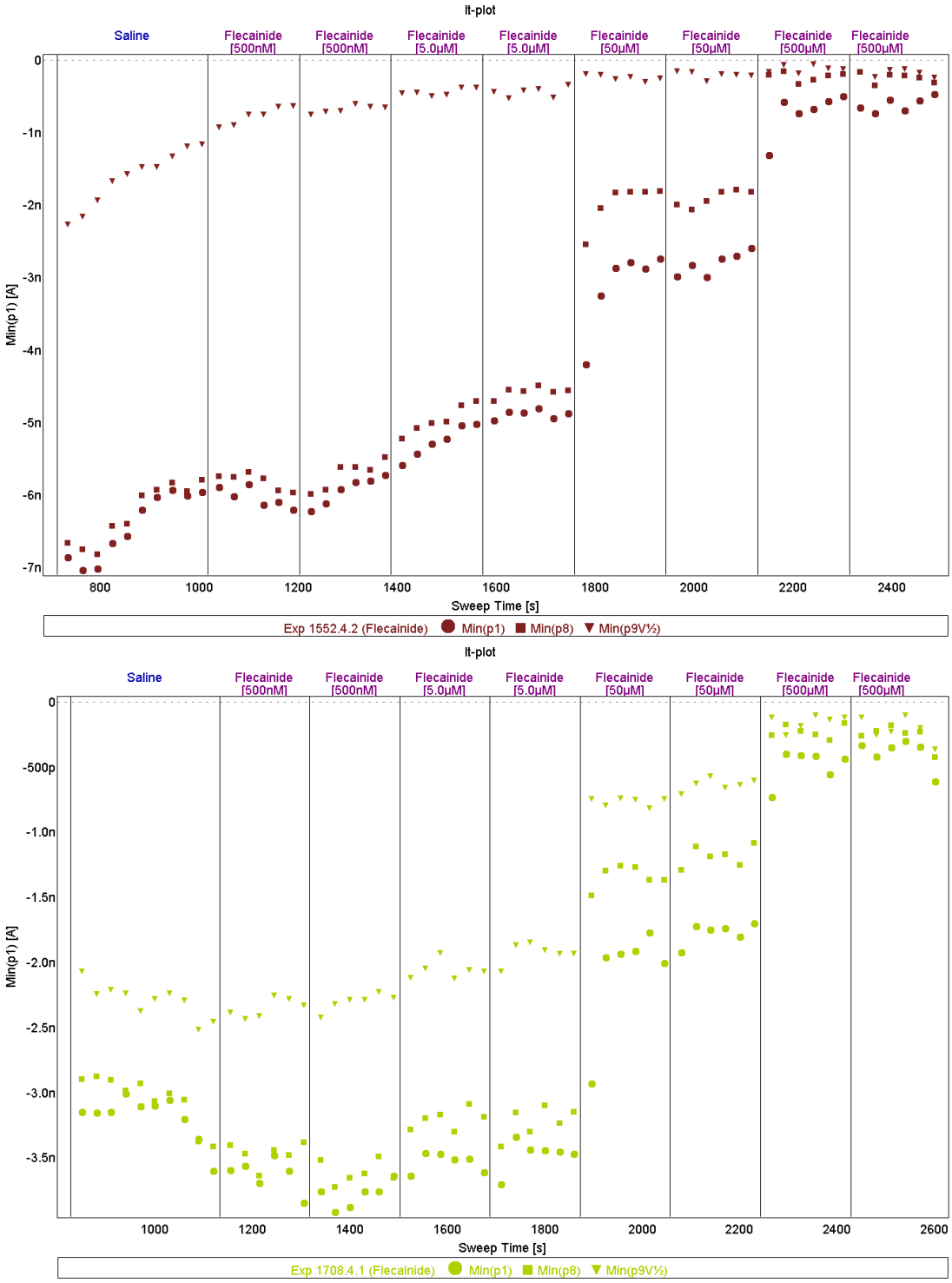


Figure 7 IT-plot of peak 1 (circles) , peak 8 (squares) and peak 9 (triangles) in a dose-response experiment with flecainide. Top: single-hole data, bottom: multi-hole data.

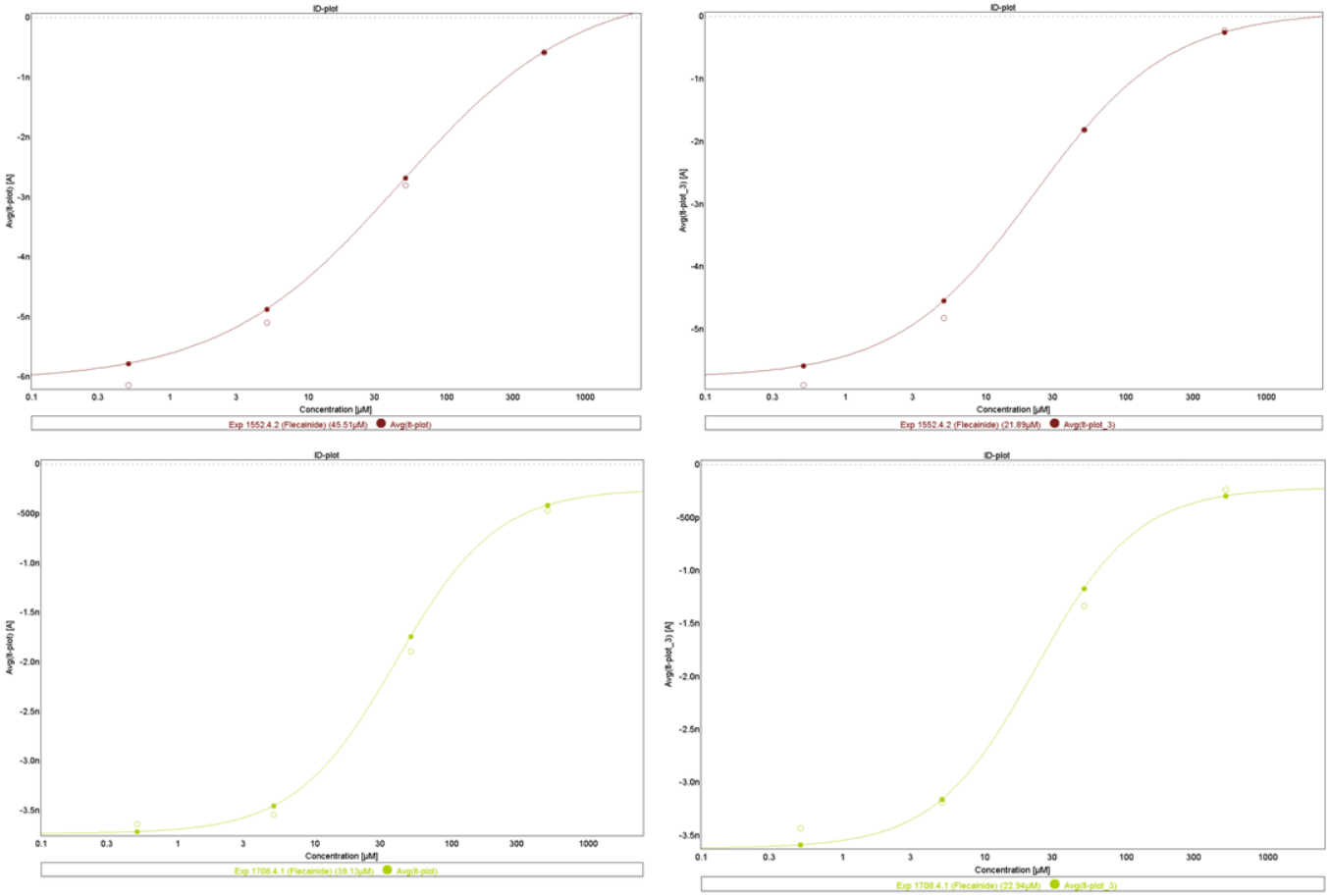


Figure 8 Hill fits for flecainide. Left are fits for current at peak 1, right are fits for current at peak 8. Top: single-hole data, bottom: multi-hole data.

Figure 8 shows dose-response plots with Hill fits for peaks 1 and 8 for flecainide (summarized in Table 1). It is evident that flecainide is a lot more potent at peak 8, after the pulsetrain, than at peak 1. The effect is not, however, further enhanced after the V½ step.

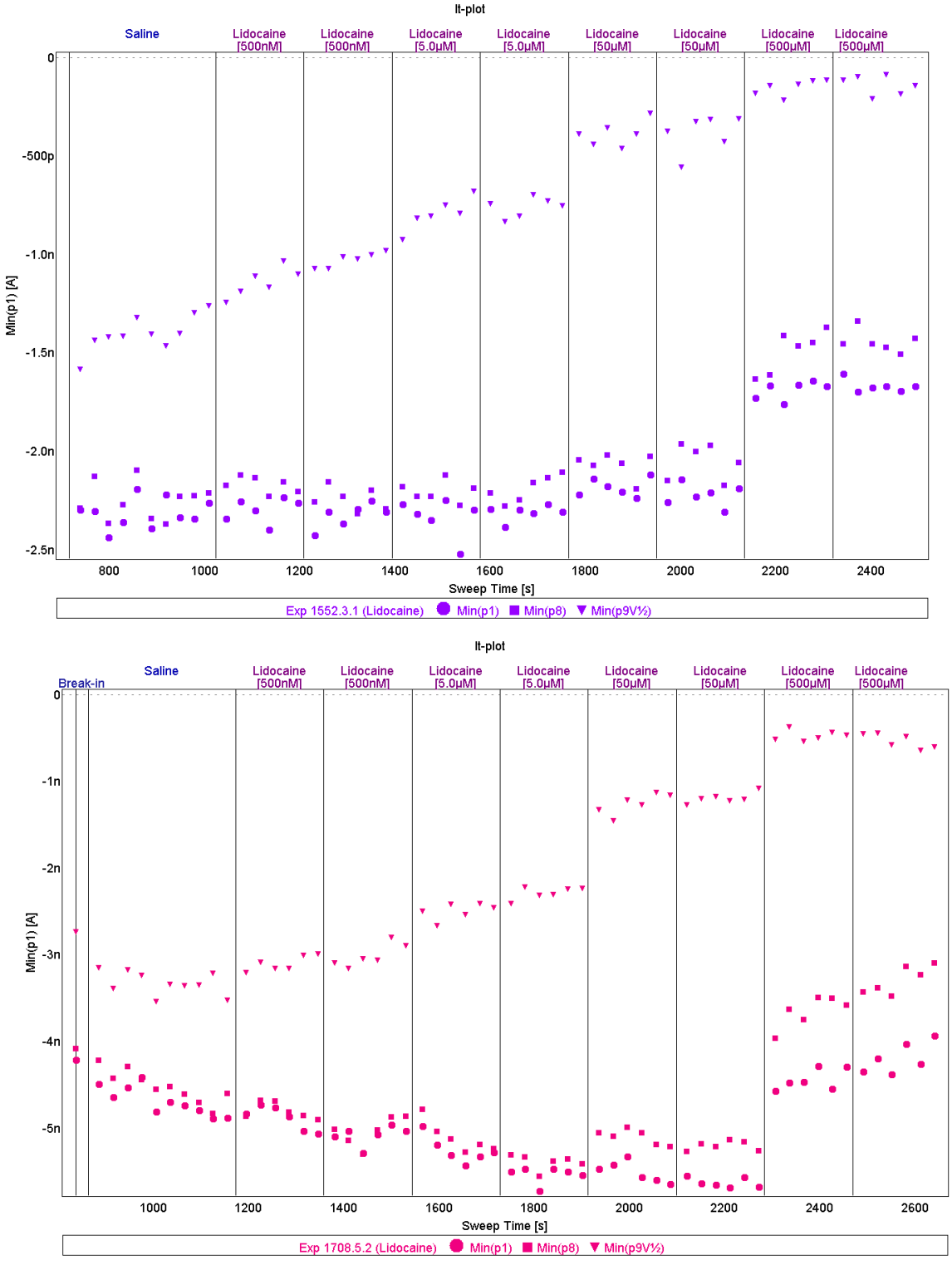


Figure 9 IT-plot of peak 1 (circles), peak 8 (squares) and peak 9 (triangles) in a dose-response experiment with lidocaine. Top: single-hole data, bottom: multi-hole data.

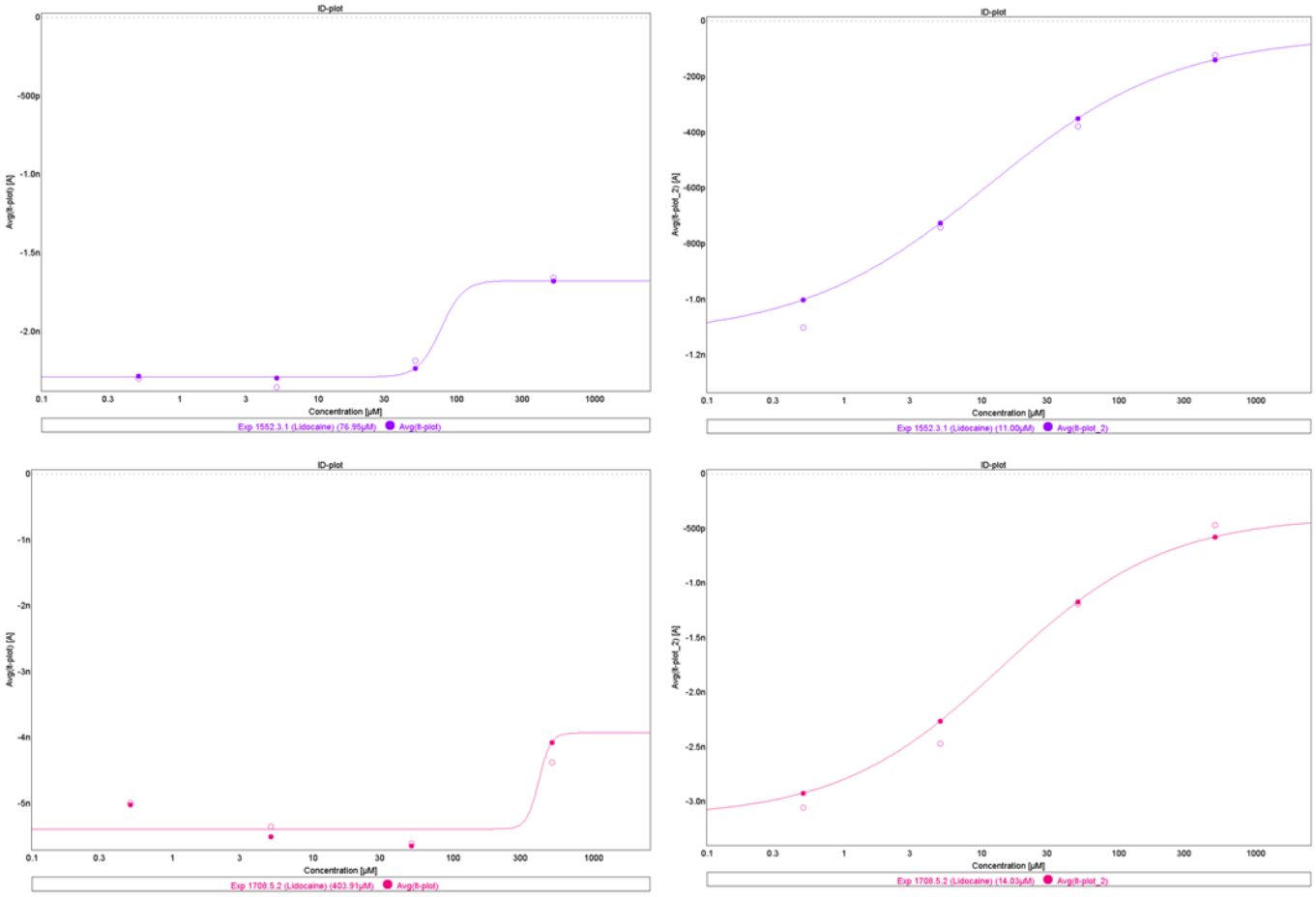


Figure 10 Hill fits for lidocaine. Left are fits for current at peak 1, right are fits for current at peak 9. Top: single-hole data, bottom: multi-hole data.

As is evident in Figure 10 and Table 1, the effects of lidocaine are not strong until pulse 9, after the V½-step. The IC50 values shown for lidocaine at peak 1 and peak 8 are not good estimates (as indicated by the asterisks in the table), because of the small drug effect.

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Voltage protocol		Single-hole		Multi-hole	
		Flecainide	Lidocaine	Flecainide	Lidocaine
Steady-state inactivation (V½ in mV, Boltzmann fit)	Control	-61.8	-55.1	-51.5	-51.0
	500 nM	-62.6	-55.8	-53.3	-52.0
	5 µM	-63.3	-60.9	-55.4	-53.1
	50 µM	-76.1	-65.8	-60.7	-58.2
	500 µM	-	-74.2	-72.7	-68.6
Open channel block (% of block by chain of single depolarization)	500 nM	0	0	0	0
	5 µM	0	0	0	0
	50 µM	60	0	60	0
	500 µM	90	20	100	30
Recovery from inactivation (ms, time constant)	Control	15.6	17.2	-	39.1
	500 nM	16.4	44.3	20.7	21.4
	5 µM	17.6	46.4	21.4	59.5
	50 µM	33.2	76.4	25.3	97.4
	500 µM	-	67.5	-	85.5
State versus use dependence (µM, IC50)	Peak 1	45.5	77.0*	39.1	403.9*
	Peak 8	21.9	82.6*	22.9	497.8*
	Peak 9	19.9	11.0	17.5	14.0

Table 1 Summary of data from all figures

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Conclusion

The data presented here shows that the QPatch is capable of producing data in multi-hole mode that is fully comparable to those in single-hole mode, both with regard to biophysical and pharmacological characteristics.

The two compounds tested clearly have different modes of action on the $Na_V1.5$ ion channel, and these differences are evident in all of the protocols used. The data is fully consistent with flecainide being a use-dependent blocker of open channels, and lidocaine a state-dependent blocker of inactivated channels.

In a screening scenario where one wishes to distinguish between these two modes of action, we would prefer to use protocol 4 (state- versus use-dependence).