

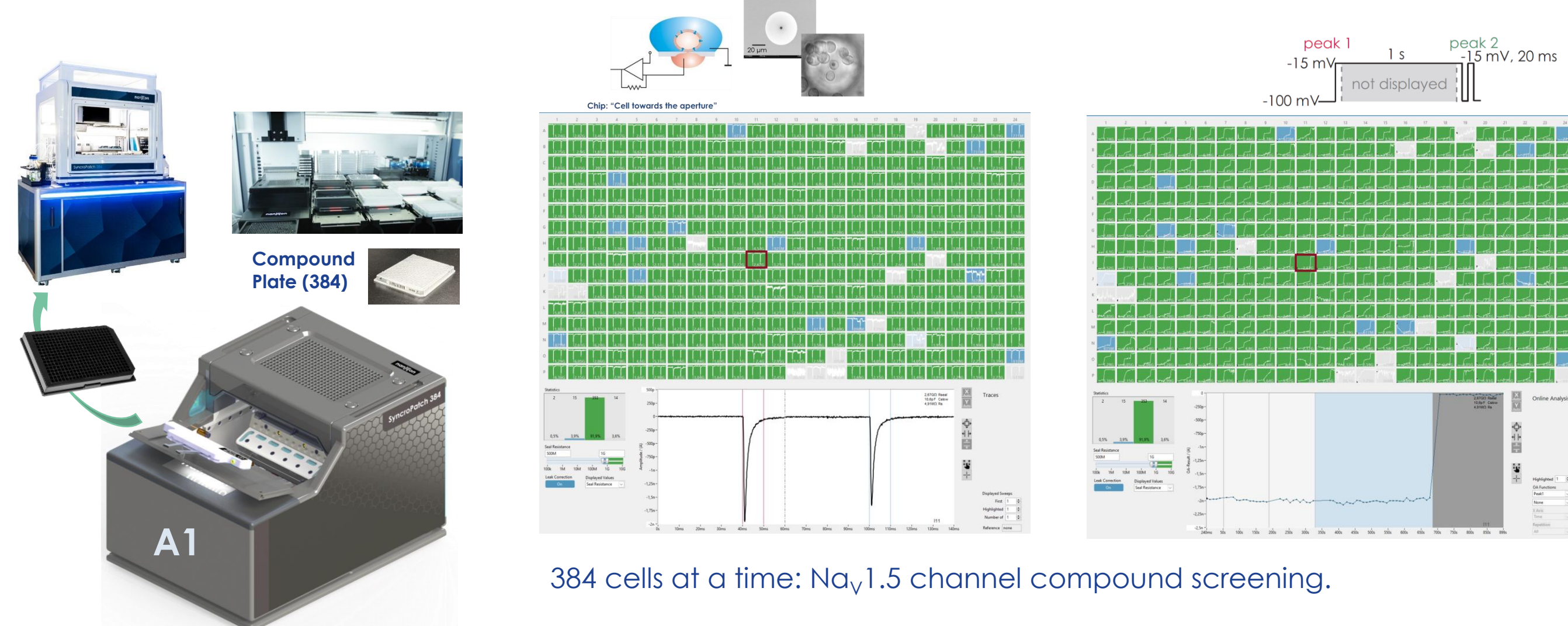
Studying state-dependent compound effects applying adaptive voltage clamp using a high throughput automated patch clamp

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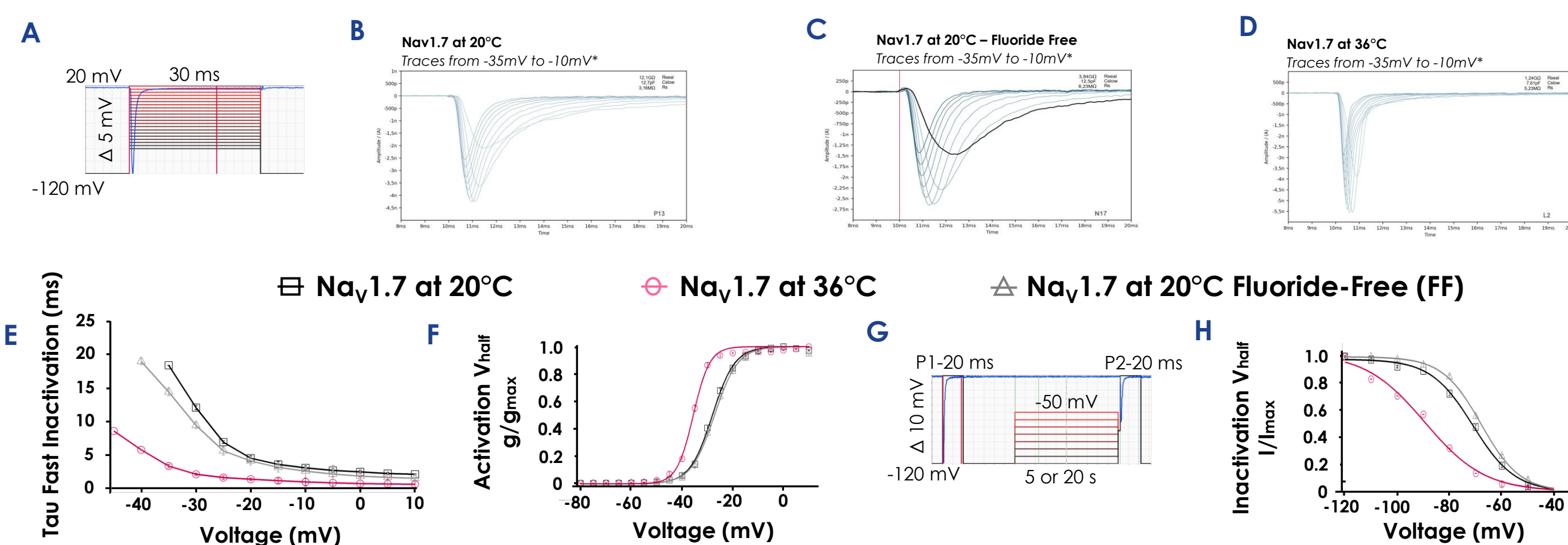
1 Introduction

Voltage-gated sodium channels (Na_v) represent important targets for the development of new medical strategies given their crucial importance in physiology and pathophysiology. Na_v channels exhibit complex gating characteristics involving transitions between open, closed, and inactivated states depending on membrane potential changes during an action potential. To find effective drugs targeting particular Na_v channels it is important to understand the molecular mechanisms of drug actions with respect to their gating transitions. A new technical development allows recording, automatic determination and feedback of each cell's individual half-maximal inactivation potential ($V_{\text{half,ind}}$) using a high throughput automated patch clamp device. Our aim was to determine the compound affinity at $V_{\text{half,ind}}$ compared with using a common (median) V_{half} for all cells. ($V_{\text{half,med}}$). This was investigated using cells expressing either $\text{Na}_v1.5$ or $\text{Na}_v1.7$ and currents were elicited from resting or inactivated states at different temperatures and in different internal solutions were recorded.



2 Variability of V_{half} values in patch clamp recordings on Na_v - Channels

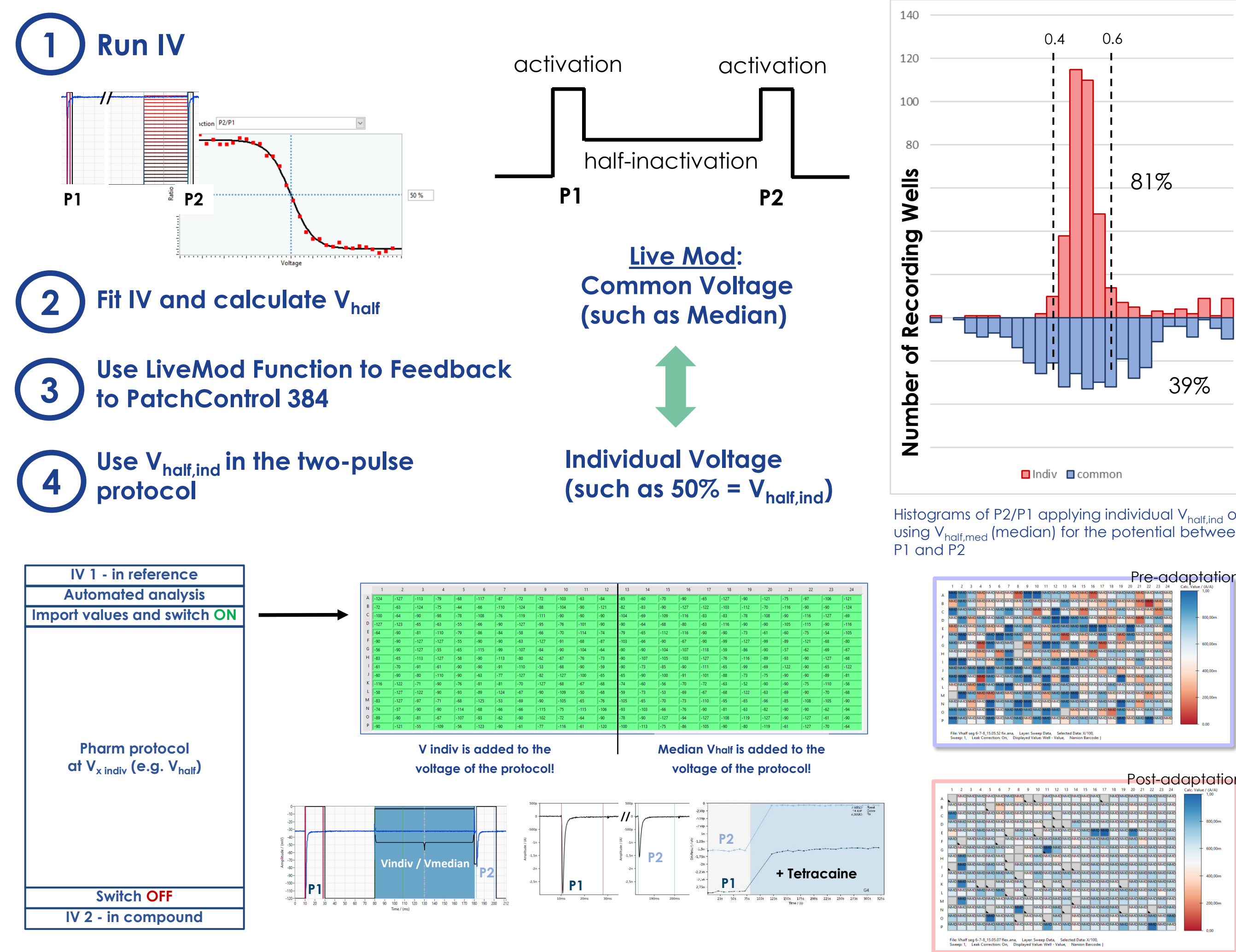
Impact on V_{half} by recording temperature and intracellular recording solutions with or without fluoride.



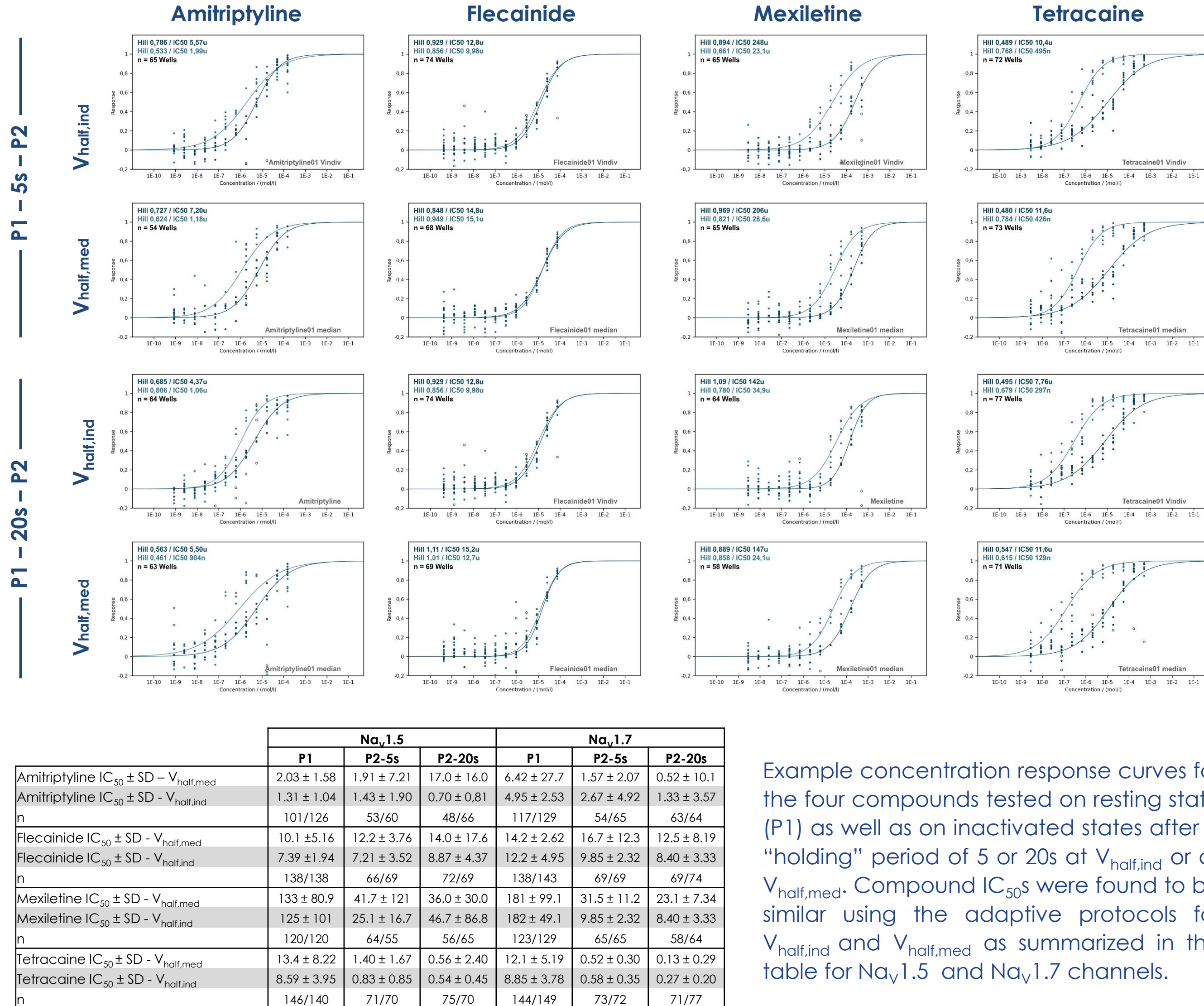
	Nav1.5			Nav1.7		
	Fluo 20°	Fluo 36°	FF 20°	Fluo 20°	Fluo 36°	FF 20°
Activation parameter						
V _{half} (mV)	-39.3 ± 6.7	-46.4 ± 6.8	-42.5 ± 6.9	-28.6 ± 5.7	-35.9 ± 5.4	-28.1 ± 5.7
n	140	139	66	153	143	153
Inactivation parameter						
V _{half} (mV)	-73.9 ± 6.9	-95.0 ± 18.9	-64.7 ± 6.1	-69.7 ± 6.6	-93.4 ± 11.1	-65.5 ± 6.8
n	278	449	91	291	350	41
Inactivation Kinetics						
Tau (ms) (0mV)	2.5 ± 0.3	1.1 ± 0.1	2.6 ± 0.4	2.0 ± 1.0	0.7 ± 0.2	1.9 ± 1.0
n	137	105	46	140	114	46

Top left: **A** Voltage protocol used to record voltage dependence of activation and kinetics of fast inactivation as highlighted in the top traces for the three conditions (**B**, **C**, **D**) tested and summarized in **E** & **F**. **G** Voltage protocol used to determine steady state inactivation of P2 and summarized in **H**.

3 Individual voltage clamp - adaptive voltage protocols increase uniformity

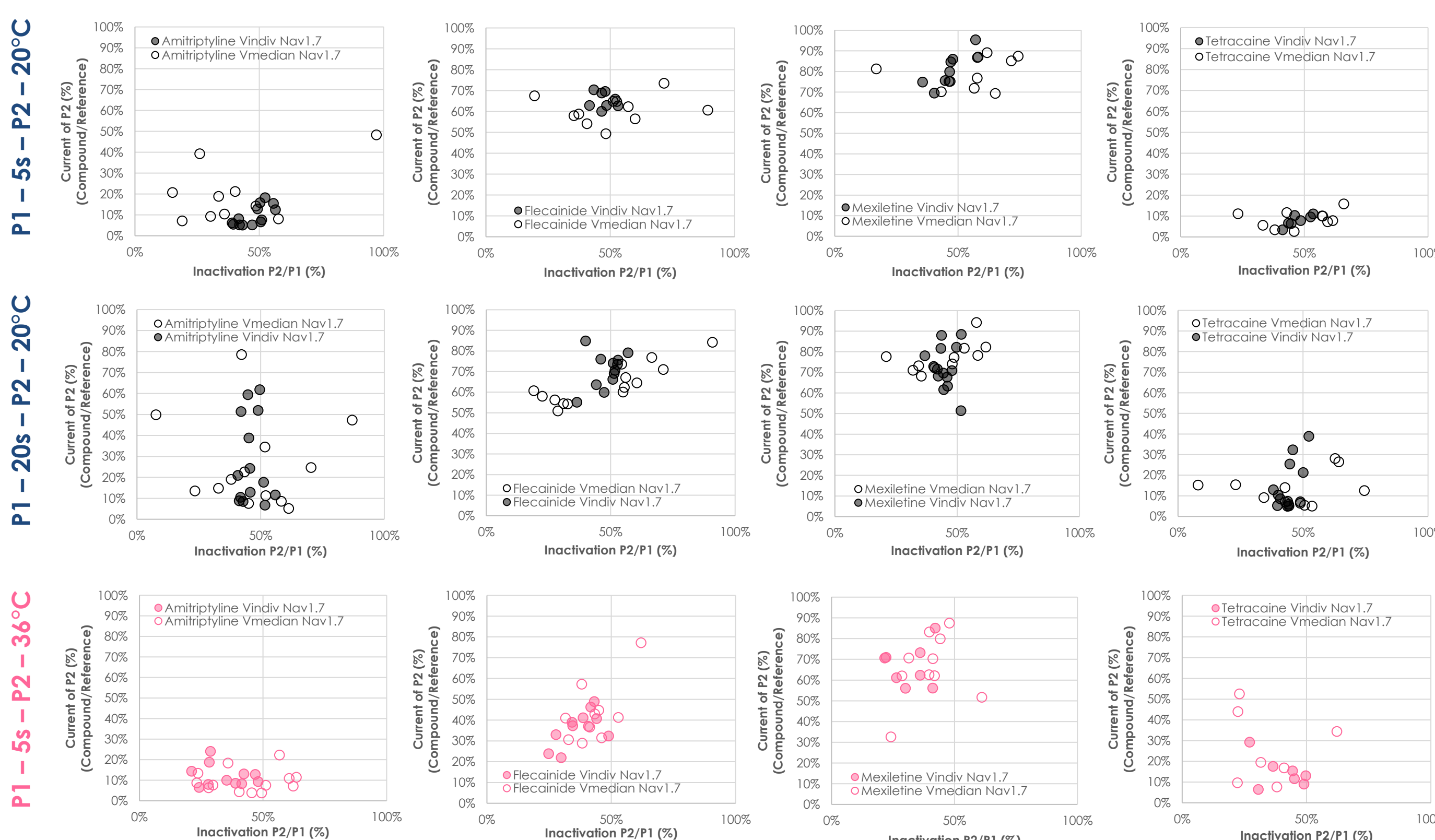


4 Compound affinity from resting - vs inactivated states of $\text{Na}_v1.5$ / $\text{Na}_v1.7$



Example concentration response curves for the four compounds tested on resting state (P1) as well as on inactivated states after a "holding" period of 5 or 20s at $V_{\text{half,ind}}$ or at $V_{\text{half,med}}$. Compound IC_{50} s were found to be similar using the adaptive protocols for $V_{\text{half,ind}}$ and $V_{\text{half,med}}$ as summarized in the table for $\text{Na}_v1.5$ and $\text{Na}_v1.7$ channels.

5 Variability of compound inhibition using different recording conditions



Single dose inhibition (10 μM) of P2 (inactivated state for the four test compounds as indicated with respect to ratio of inactivation (P2 over P1) close to V_{half} (50%). The time holding at $V_{\text{half,ind}}$ or $V_{\text{half,med}}$ was extended to 20s in the middle panel and lower panel shows same approach for 5s repeated at 36°C.

6 Summary

- Temperature (20°C vs 36°C) and the presence of fluoride in the intracellular solution affected kinetics of fast inactivation, voltage dependence of activation, and steady-state inactivation of $\text{Na}_v1.7$ and $\text{Na}_v1.5$ channels as previously reported^{1,2}.
- The test compounds affinities for activation from resting - and inactivated states were similar when using the adaptive protocol feature to apply either $V_{\text{half,ind}}$ or $V_{\text{half,med}}$ during the "inactivation" phase vs V_{hold} resting.
- As expected, the inactivation ration of P2/P1 was less variable when $V_{\text{half,ind}}$ was used compared with $V_{\text{half,med}}$. Also, the compound inhibition of inactivated states was less variable for some compounds (amitriptyline, tetracaine) comparing $V_{\text{half,ind}}$ vs $V_{\text{half,med}}$. However, when holding at $V_{\text{half,ind}}$ or $V_{\text{half,med}}$ for a longer time period (20 s vs 5 s) compound inhibition was more variable for those two compounds. This was not obvious for flecainide or mexiletine indicating preferential interference of amitriptyline and tetracaine with pronounced inactivated states.
- At 36°C the inactivation ration (P2/P1) was decreased compared with 20°C for $V_{\text{half,ind}}$ and $V_{\text{half,med}}$, in line with the leftward shift in the IVs of inactivation at 36°C. Flecainide showed increased inhibition at 36°C, whereas the other compounds inhibited $\text{Na}_v1.7$ at comparable levels at 36°C vs 20°C recording temperature.
- The new adaptive voltage clamp feature of the SyncroPatch 384 supports more homogenous distribution at 50% of inactivation for the two Nav - channels tested ($\text{Na}_v1.5/\text{Na}_v1.7$) and can thus give increased confidence in compound potency and should lower occurrence of false positives/negatives in drug discovery at V_{half}
- Methods: Nanion standard solutions were used with decreased external Na^+ gradient (NMDG60) and 110 mM CsF for fluoride containing as well as 110 mM K-Gluconate for fluoride free experiments. Voltage protocols as indicated with sampling frequency of 20 KHz. Error bars represent SEM unless otherwise indicated. The following QC filters were applied: 1. Rseal > 0.5 Gohm at Start/End, 2 Rseries < 15 Mohm at Start/End, Current for P1 < -500 pA/ P2 < -300 pA.
- References: 1) Rapedius et al., *Front. Mol. Neurosci.*, 22. 2) Kriegeskorte et al., *JGP.*, 23.