

Reliable identification of cardiac liability in drug discovery using automated patch clamp: Considerations and best practices for high throughput recordings of Na_v1.5

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Introduction

For reliable identification of cardiac safety risk, compounds should be screened for effects on cardiac ion channels in addition to hERG, including Na_v1.5 and Ca_v1.2. Automated patch clamp (APC) instruments are increasingly adopted for cardiac safety measurements but cross-site and cross-platform comparisons of IC₅₀ values has identified the need for standardized protocols for reliable pharmacology. In this study, we identified different parameters that might affect IC₅₀s of compounds on Na_v1.5 peak and late currents recorded using APC.



Effect of voltage protocol on Na_v1.5

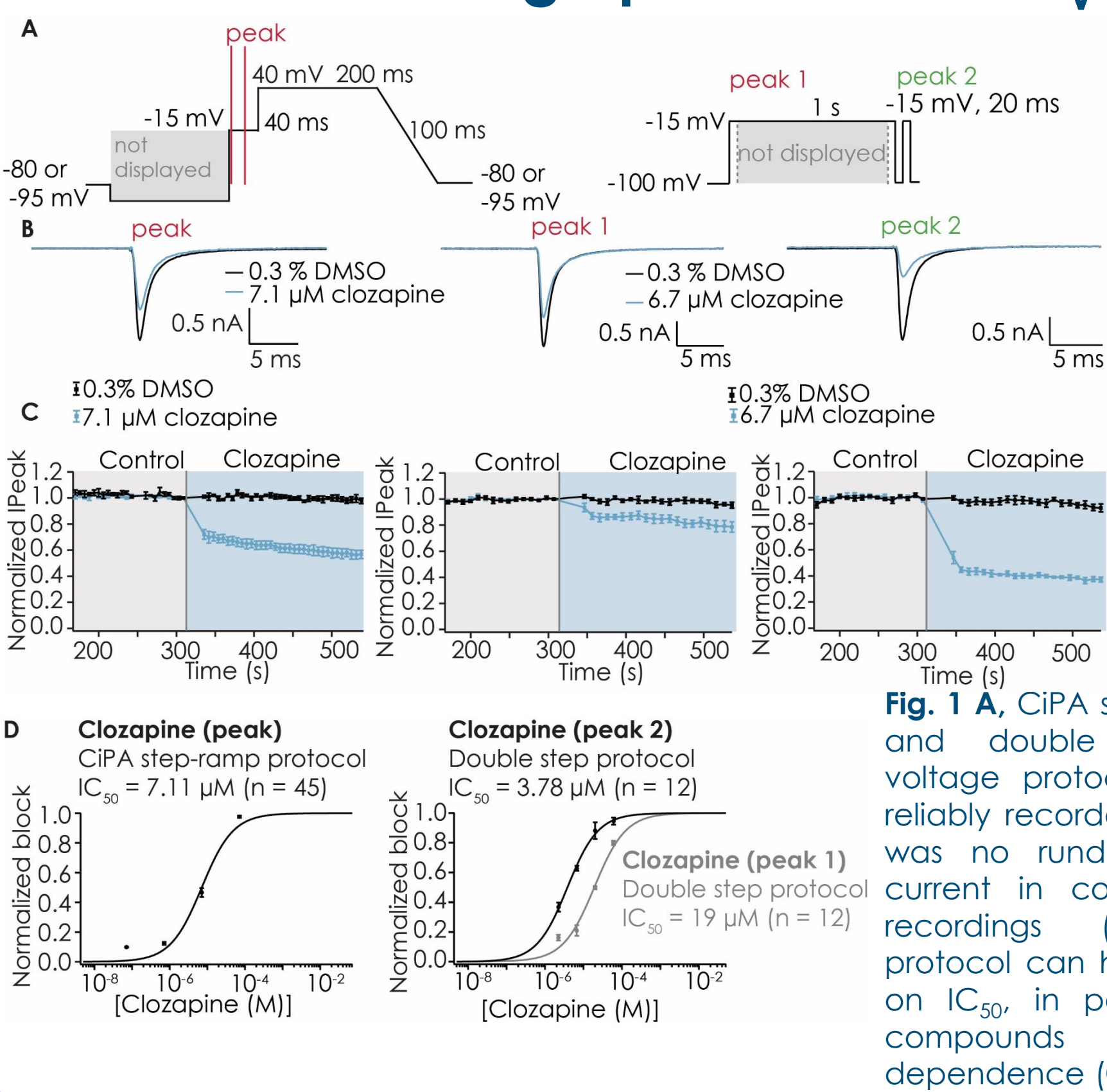


Fig. 1 A, CiPA step-ramp (left) and double step (right) voltage protocols. Na_v1.5 is reliably recorded (B,C), there was no rundown of peak current in control (vehicle) recordings (C). Voltage protocol can have an effect on IC₅₀, in particular when compounds display state dependence (C,D).

Cultured cells versus frozen stocks and correlation across platforms

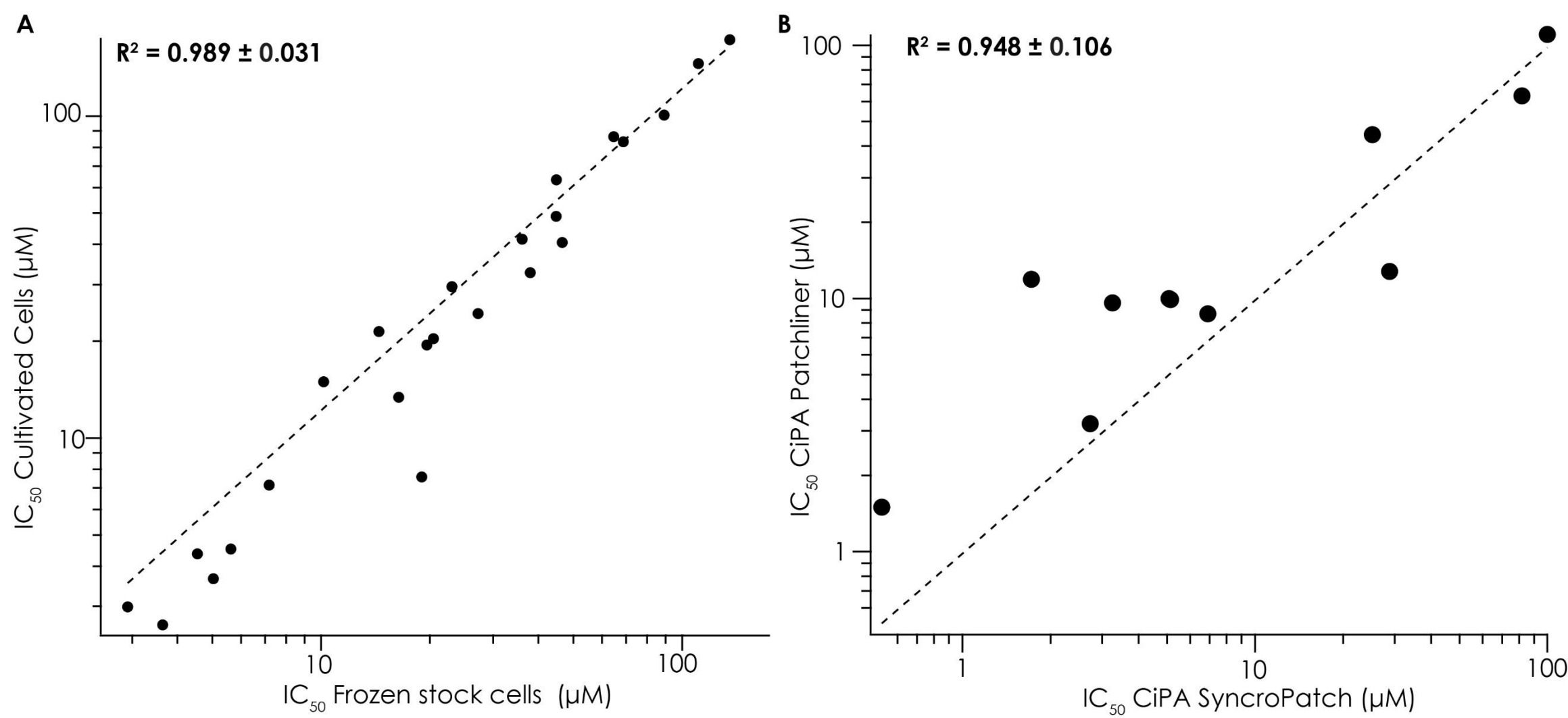


Fig. 2 A, IC₅₀s for 24 compounds calculated using cultured cells or frozen stocks correlated well with a Pearson's correlation coefficient, R² = 0.989 ± 0.031. B, IC₅₀s of 11 compounds obtained on the Patchliner or SyncroPatch 384i correlated well, with an R² = 0.948 ± 0.106.

Persistent and late Na_v1.5 current

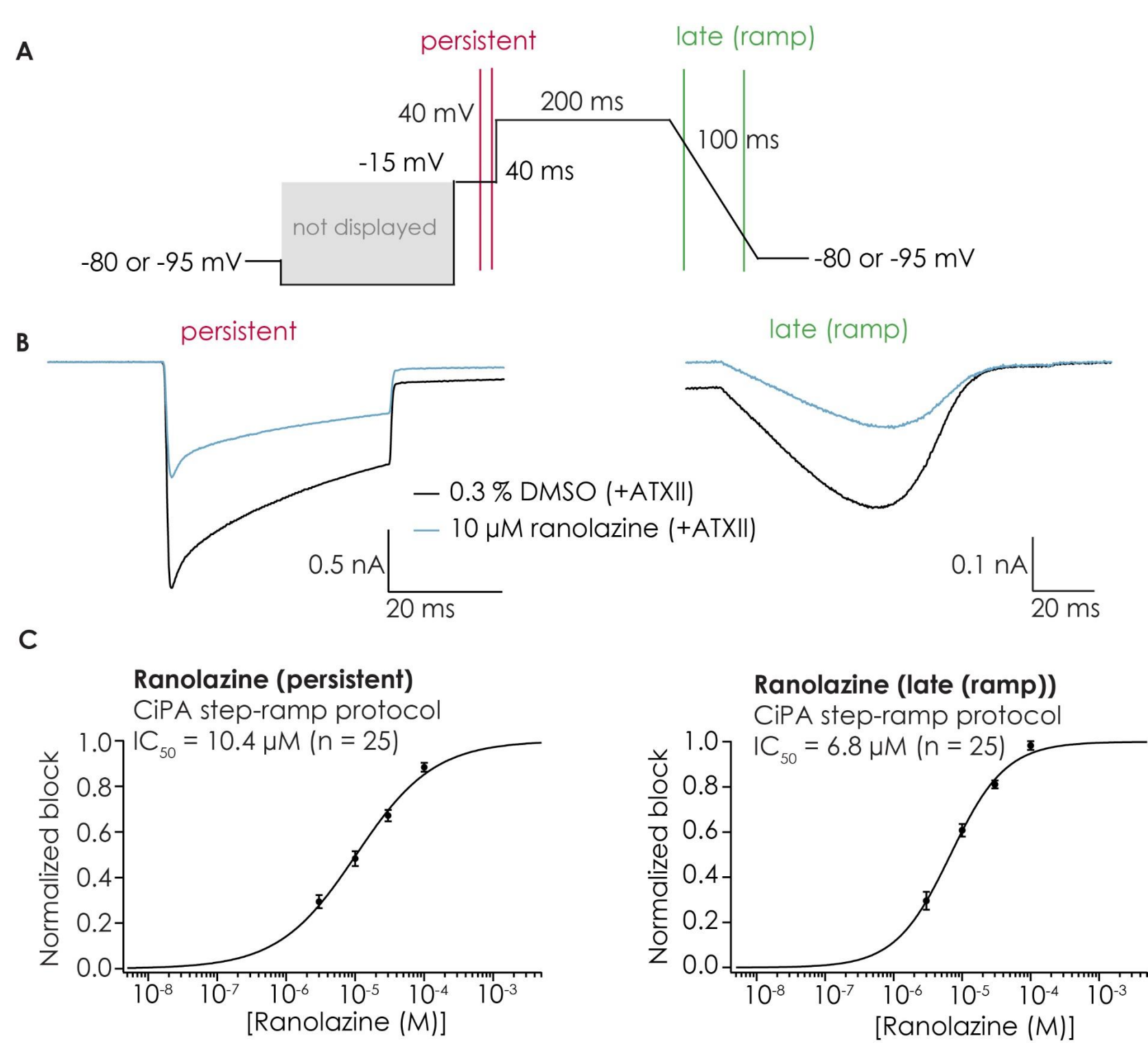


Fig. 3 A, Voltage protocol used to record persistent and late current. B, Example traces of persistent (left) and late (right) current activated by ATXII and blocked by ranolazine. C, IC₅₀ of block of ranolazine was similar if obtained using the persistent or ramp phase of the voltage protocol. Despite the relative small current size of the late Na_v1.5 current compared with peak current, it can have a significant effect on action potential duration and arrhythmia and therefore is an important factor to consider in safety pharmacology.

Effect of temperature on biophysical properties and IC₅₀

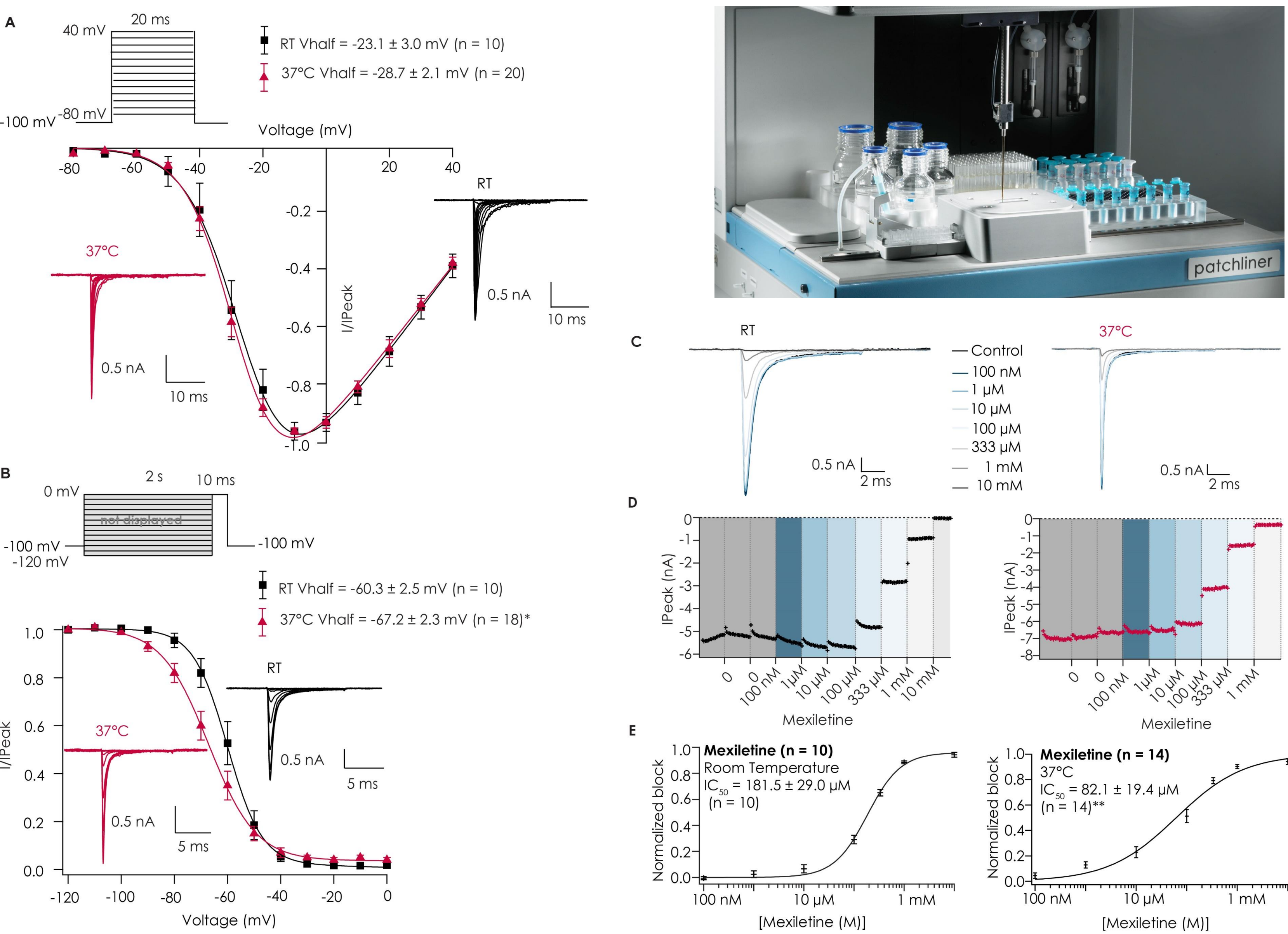


Fig. 4 Effect of temperature on activation and inactivation IV and pharmacology of Na_v1.5. A, V_{half} of activation is unchanged by temperature but the V_{half} of inactivation (B) is shifted by approximately 7 mV to more hyperpolarized potentials (* p<0.05, unpaired Student's t test). C – E, Temperature can affect IC₅₀ of mexiletine, IC₅₀ is approximately 2-fold higher at room temperature compared with 37°C.

Incubation time and reproducibility

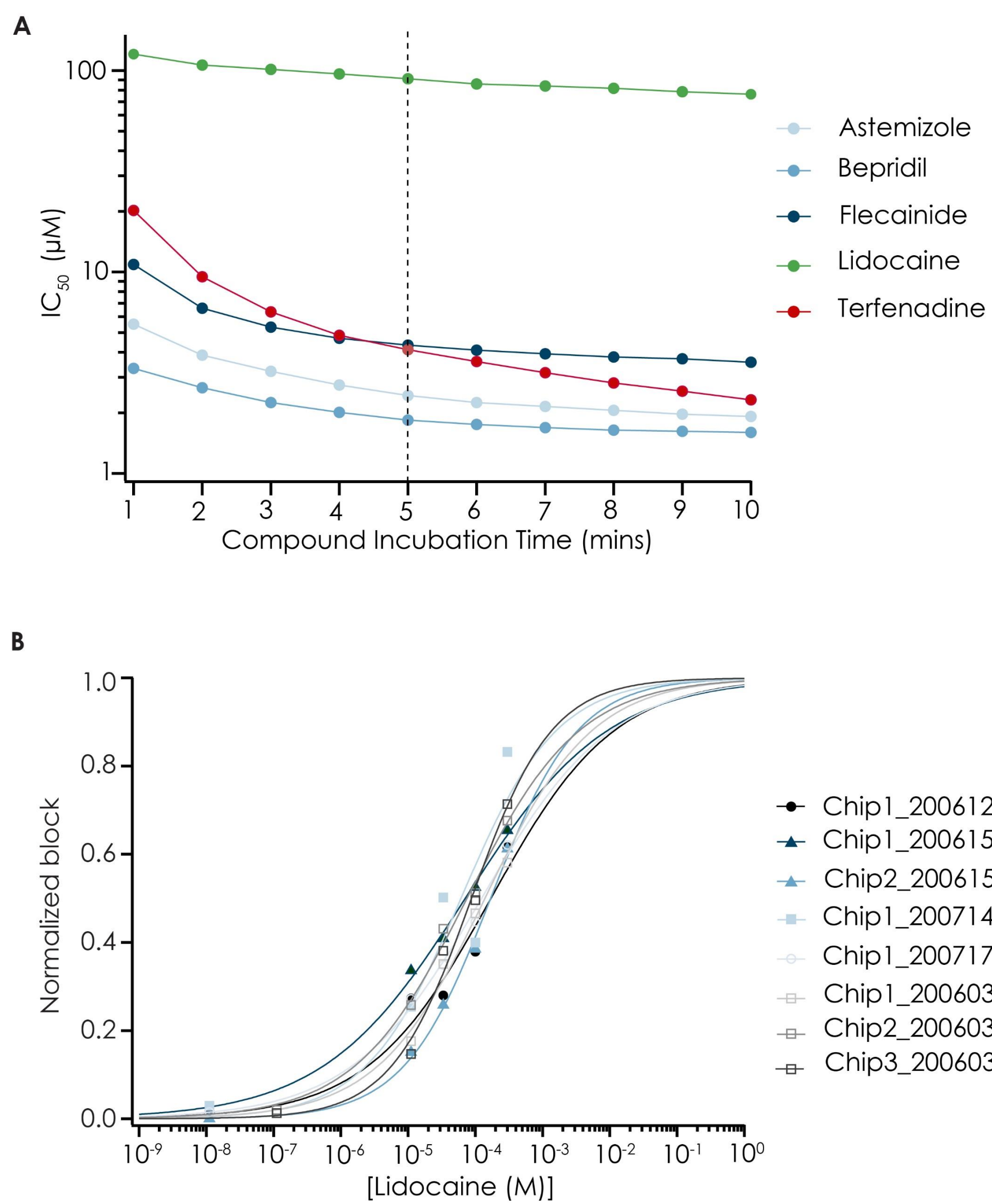


Fig. 5 A, Effect of incubation time on calculated IC₅₀ values for 5 compounds. For most compounds, 5 min incubation in compound was sufficient to reach steady state but for sticky compounds, such as terfenadine, 9 or 10 min incubation was required. B, Data was reproducible: concentration response curves for lidocaine from different chips and different days overlaid almost exactly.

Comparison with literature

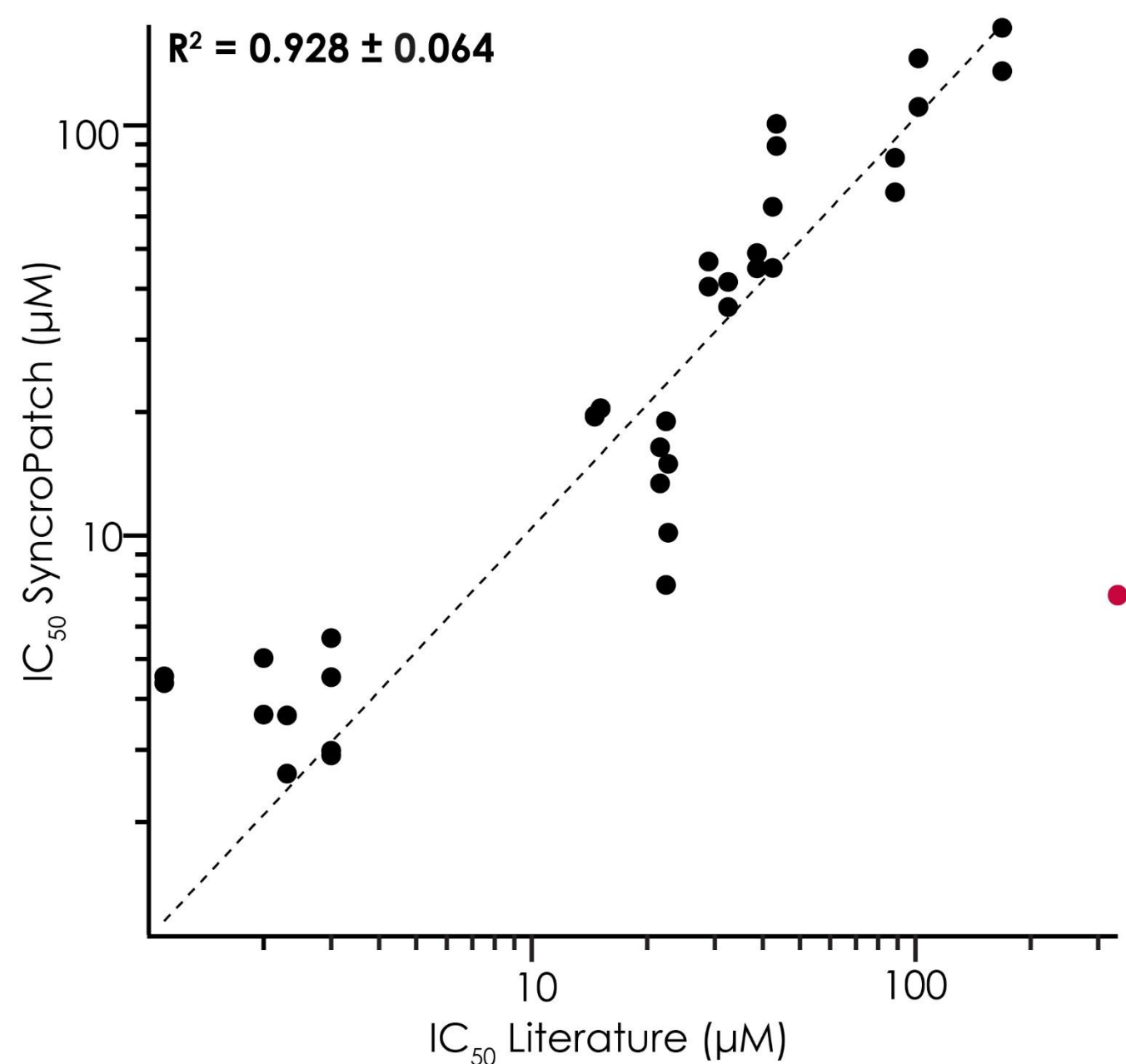


Fig. 6 IC₅₀ values for 18 compounds obtained using the SyncroPatch 384 correlated well with literature values except cisapride shown in red which was not used for the correlation.

Conclusions

- CiPA step-ramp protocol should be used for safety pharmacology testing. This allows effects on both peak and late current to be studied.
- Temperature affects biophysical properties and pharmacology and, at the very least, should be carefully controlled and at best, experiments should be performed at physiological temperature (where possible).
- A minimum compound incubation time of 5 min is required for reliable IC₅₀ measurement, longer is preferred for sticky compounds.
- The use of standardized protocols for APC experiments e.g. voltage protocol, incubation time, replicate number etc, is critical to be able to compare IC₅₀ values across platforms, sites and with the literature.

References: Data shown in this poster has been accepted into JPTM pending revisions.

