

DEVELOPMENT OF A Na_v1.5 ASSAY FOR PROLONGED RECORDING ON AN AUTOMATED ELECTROPHYSIOLOGY SYSTEM

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Introduction

The IonWorks Barracuda system allows simultaneous and continuous measurement of both ligand-gated ion channels (LGICs) and voltage-gated ion channels (VGICs) in 384 individual recording sites. To achieve this, IonWorks Barracuda system is equipped with 384 individual patch-clamp amplifiers together with a 384-channel fluidic pipettor. Similar to its predecessor, IonWorks Quattro, IonWorks Barracuda system measures cell membrane currents using the perforated patch clamp techniques on a polyimide substrate. Currents are measured using a single hole at each recording site or an array of 64 holes at each site (Population Patch Clamp or PPC, Finkel et al. 2006). In this study, we evaluated a new technique to further prolong the assay window for ionic currents on the IonWorks Barracuda instrument. Using Nav1.5 channel as an example, we demonstrated stable recording of Nav1.5 currents elicited by repetitive scans for 30 minutes or longer. This prolonged assay window, validated using multiple recording parameters, is about twice of that of IonWorks Quattro. This allows the researchers not only to precisely control the state of the channel by voltage clamp; but also to potentially analyze molecules with slow binding rate to the channel. To simulate the work flow in a drug screening, here we designed and validated a single-blind assay to first screen and then confirm use- and non use-dependent blockers of Nav1.5 channels. The high throughput, high sensitivity and the robustness of IonWorks Barracuda system makes it an ideal platform in screening use-dependent channel blockers in a drug discovery environment.

Material and Methods

Cells: Chinese hamster lung (CHL) cells stably expressing the human SCNA5 (Nav1.5) gene.

Reagents and buffers: Amphotericin (Sigma Cat. # A-4888), DMSO (Sigma Cat. # D-2650); Internal buffer contains (in mM): 100 K⁺ gluconate, 30 KCl, 3.2 MgCl₂, 5.0 EGTA, 5.0 HEPES, pH 7.25 with KOH; External buffer is Phosphate Buffered Saline (PBS, Gibco Cat. # 14040).

Compound management: All test compounds were obtained from Sigma-Aldrich, and freshly prepared everyday following a single-blinded procedure. The final test plate contains 0.1% DMSO.

Electrophysiology: All experiments were performed in the PPC mode, with voltage-clamp, and compound addition protocols described in each figure.

Data analysis: In addition to the native filter, two data filtering criteria were implemented, 1) seal resistance < 50 M Ω , and 2) seal resistance change > 50% (pre vs. post). The data were analyzed and plotted using Prism 5 software.

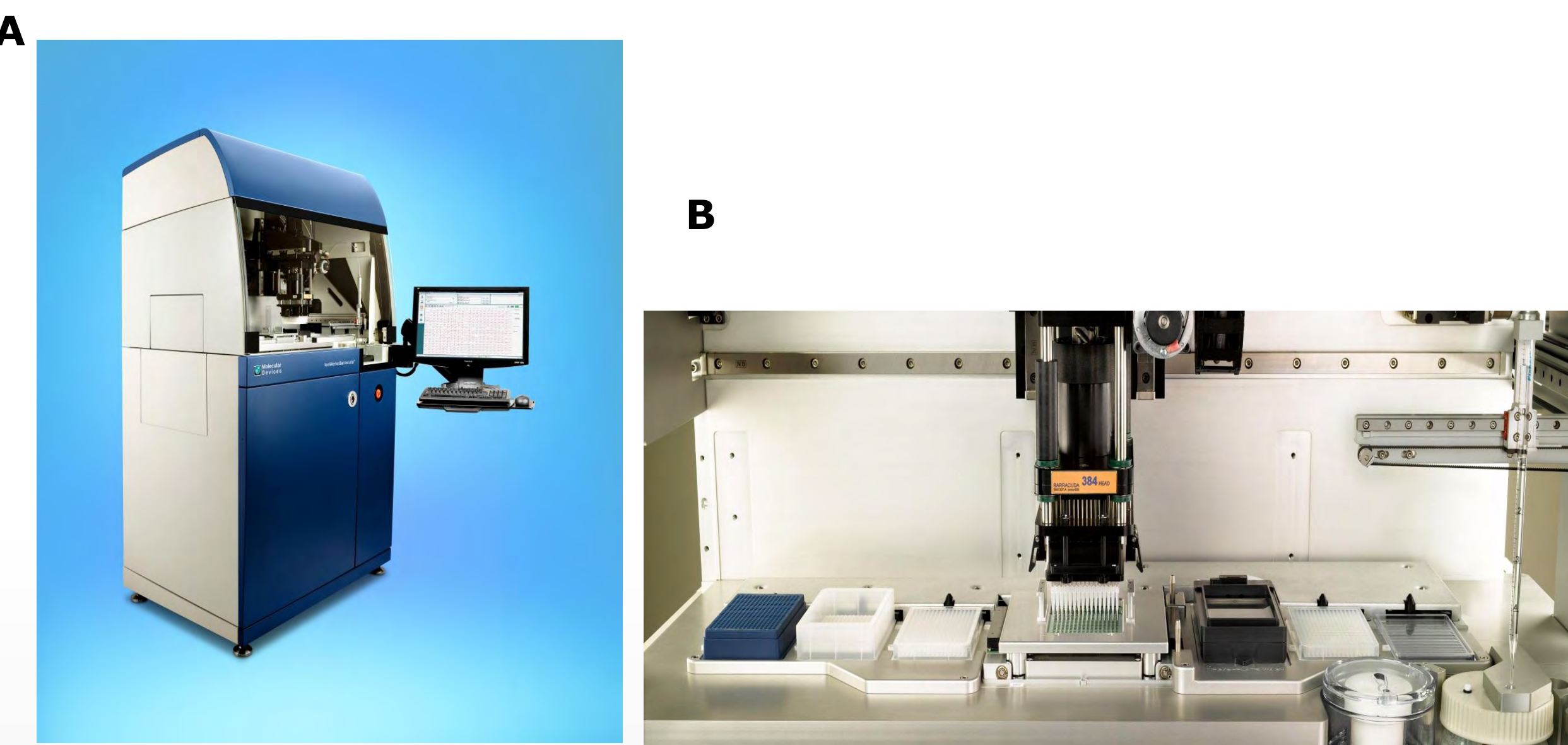


Fig. 1. IonWorks Barracuda™ automated patch clamp system records ion channel currents continuously and simultaneously, from 384 parallel recording sites. **A)** External view of the system; **B)** Front view of the process deck.

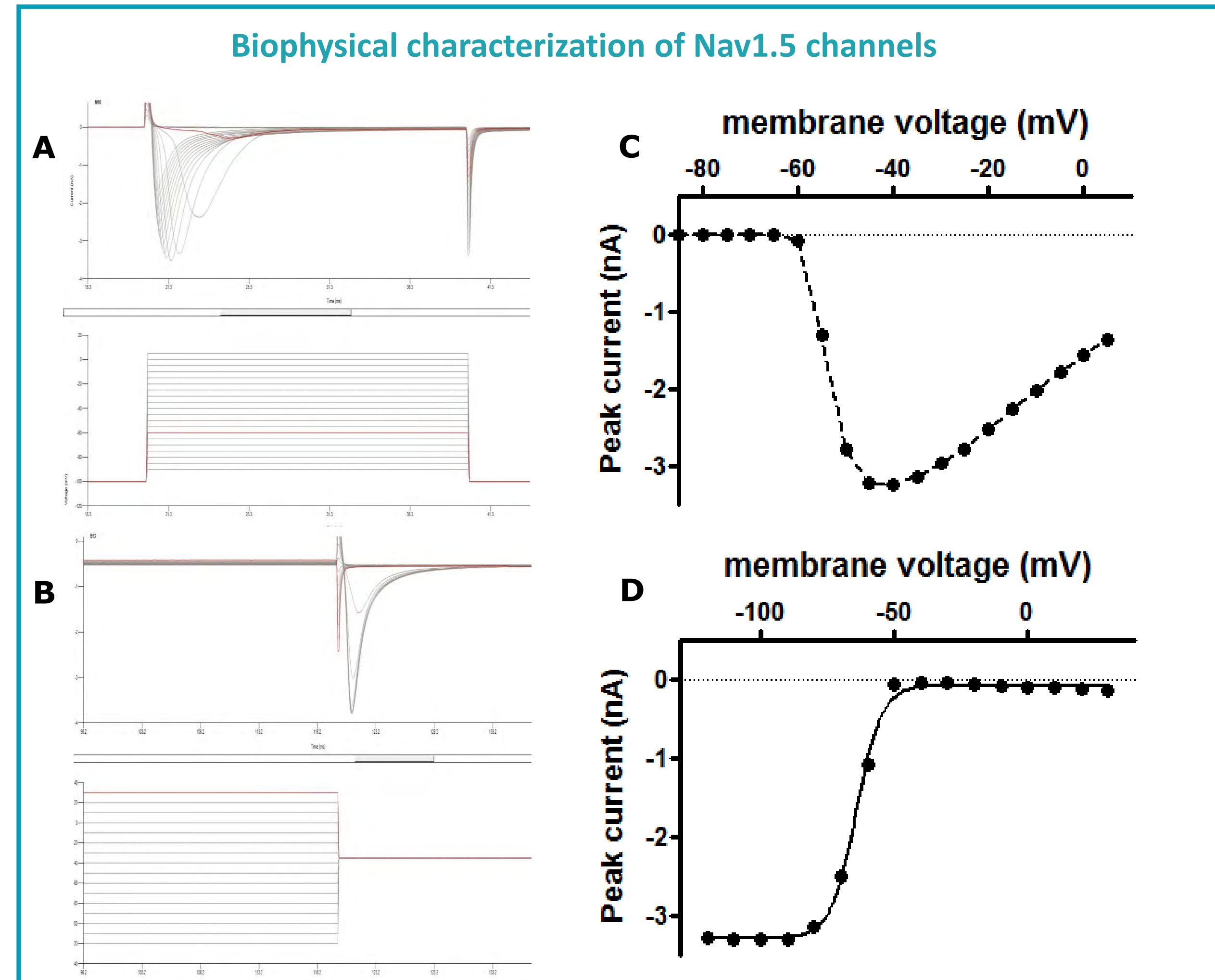


Fig. 2. The voltage-dependent activation and voltage dependence of inactivation of Nav1.5 channels were examined on the IonWorks Barracuda system, in the PPC mode. **A)** and **B)**, representative Nav1.5 currents elicited from the same well (top traces), using different voltage protocols (bottom); **C)** and **D)**, the current-voltage relationships for both activation and inactivation of Nav1.5 channels, data was collect from one experiment (mean \pm SEM, n = 382 wells, 2 wells filtered out).

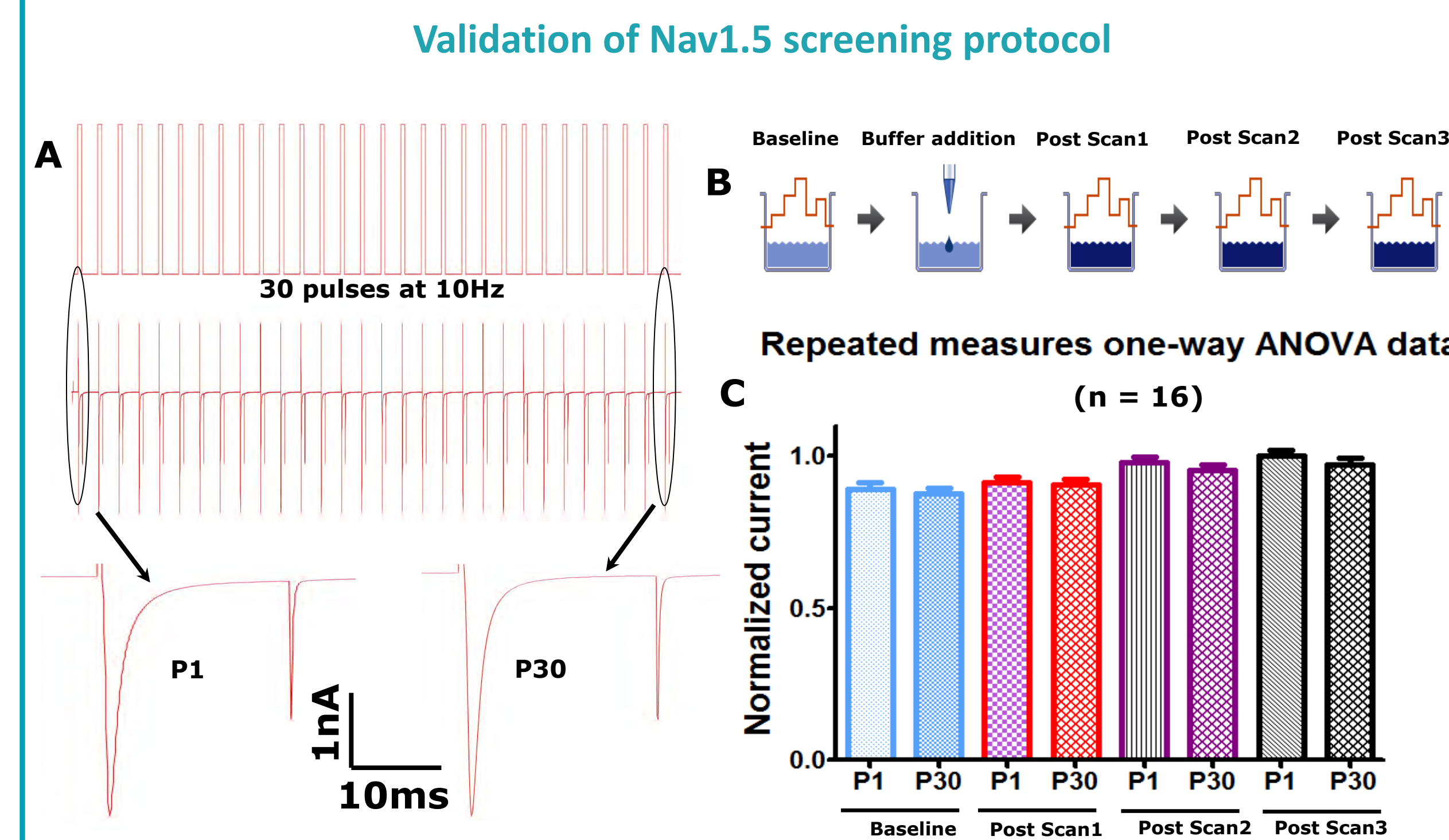


Fig. 3. To screen for use- and non-use dependent compounds, a train protocol was designed and validated based on the biophysical properties of the channel. **A)** Top: diagram of the voltage protocol, $V_h = -100$ mV, test voltage = -35 mV for 20ms; Middle: representative Nav1.5 currents elicited from one well, in PPC mode; Bottom: zoom-in view of the currents elicited by the first (P1) and last (P30) pulses. **B)** Description of the screening process: after baseline measurement, in each assay 10 μ l of compound (1X concentration) or buffer were introduced into PatchPlate well at medium position, with no mixing. Three post-compound scans were collected at 0s, 90s, and 180s after compound addition. **C)** The current amplitudes of test pulse #1 and #30, for all scans, were examined by repeated measures one-way ANOVA analysis (n = 16). There is no statistical difference among all groups, indicating the stability of currents over-time, and after compound (buffer in this case) addition.

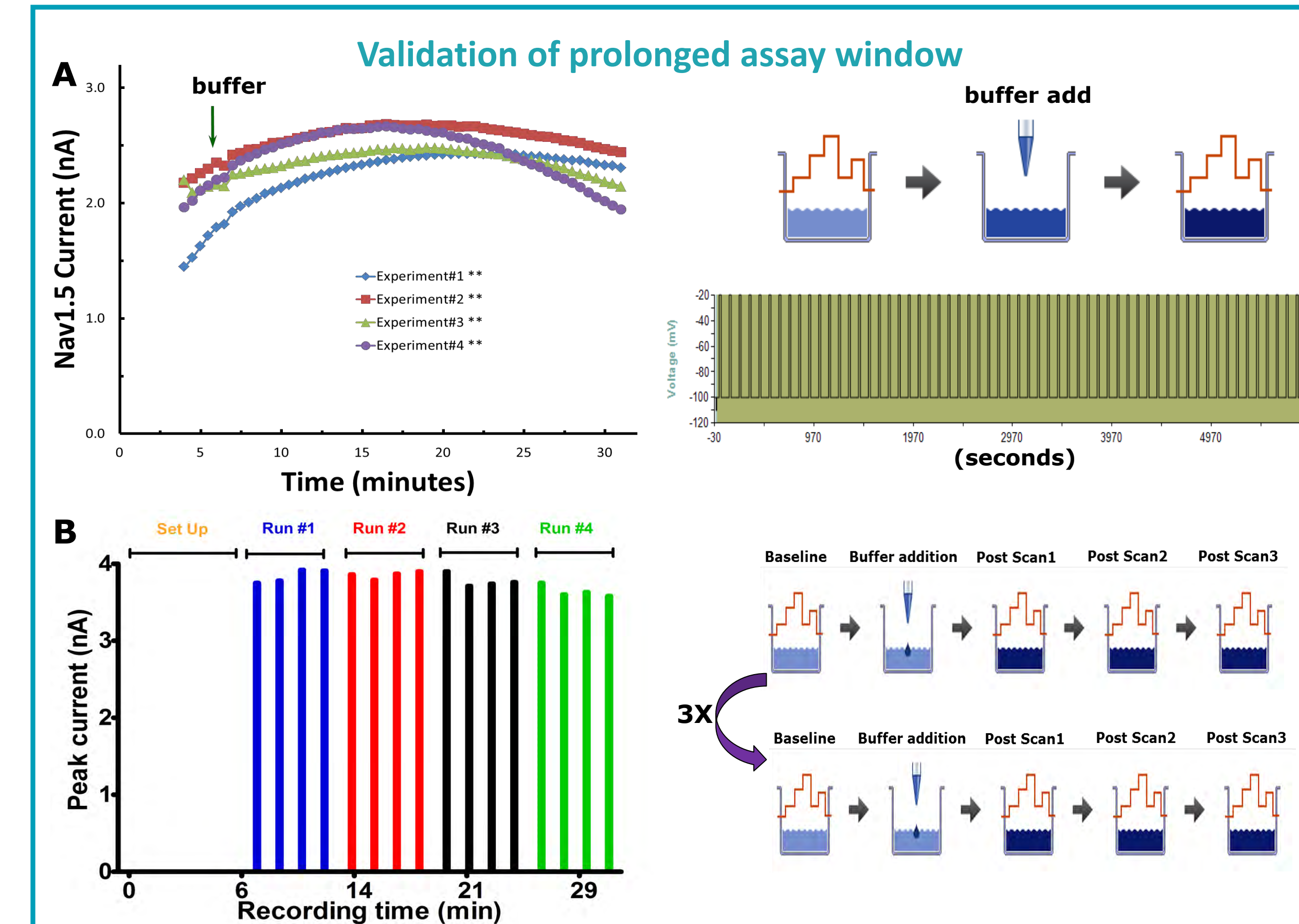


Fig. 4. Evaluation of assay window for Nav1.5 channels, and recording stabilities among multiple experiments. **A)**, plotting of peak Nav1.5 currents against time, from 4 consecutive experiments in one day. Currents were elicited by a train of test pulses each separated by 30 seconds, with a buffer addition 3 minutes into each experiment. **B)**, peak current stability with the cells subjected to 4 repetitions of the screening protocol (n = 16).

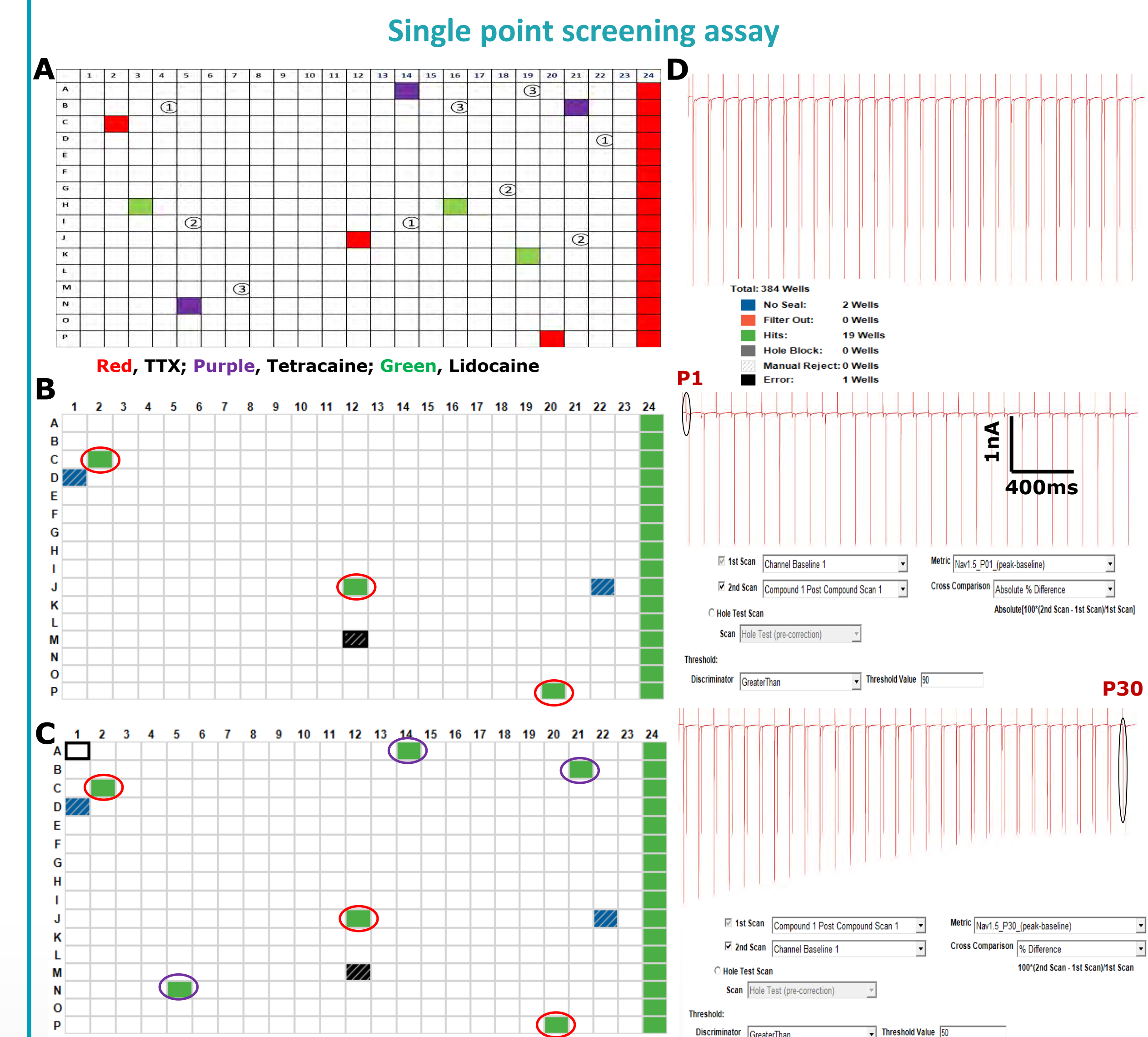


Fig. 5. Screening for use- (lidocaine and tetracaine) and non use-dependent (tetrodotoxin, TTX) blockers for Nav1.5 channels. **A)**, plate map of the single point compound plate, with 10 μ M final concentration in 0.1% DMSO. In circles are negative controls compounds (①, 4-AP; ②, cisapride; ③, nifedipine). **B)**, plate view of wells with pulse 1 current reduced by more than 90%; **C)**, plate view of wells with pulse 30 current reduced by more than 50%; **D)**, representative currents recorded from control (top), TTX (middle), and tetracaine (bottom) wells; with filter and "heat map" settings in the IonWorks Barracuda software, Z' = 0.6.

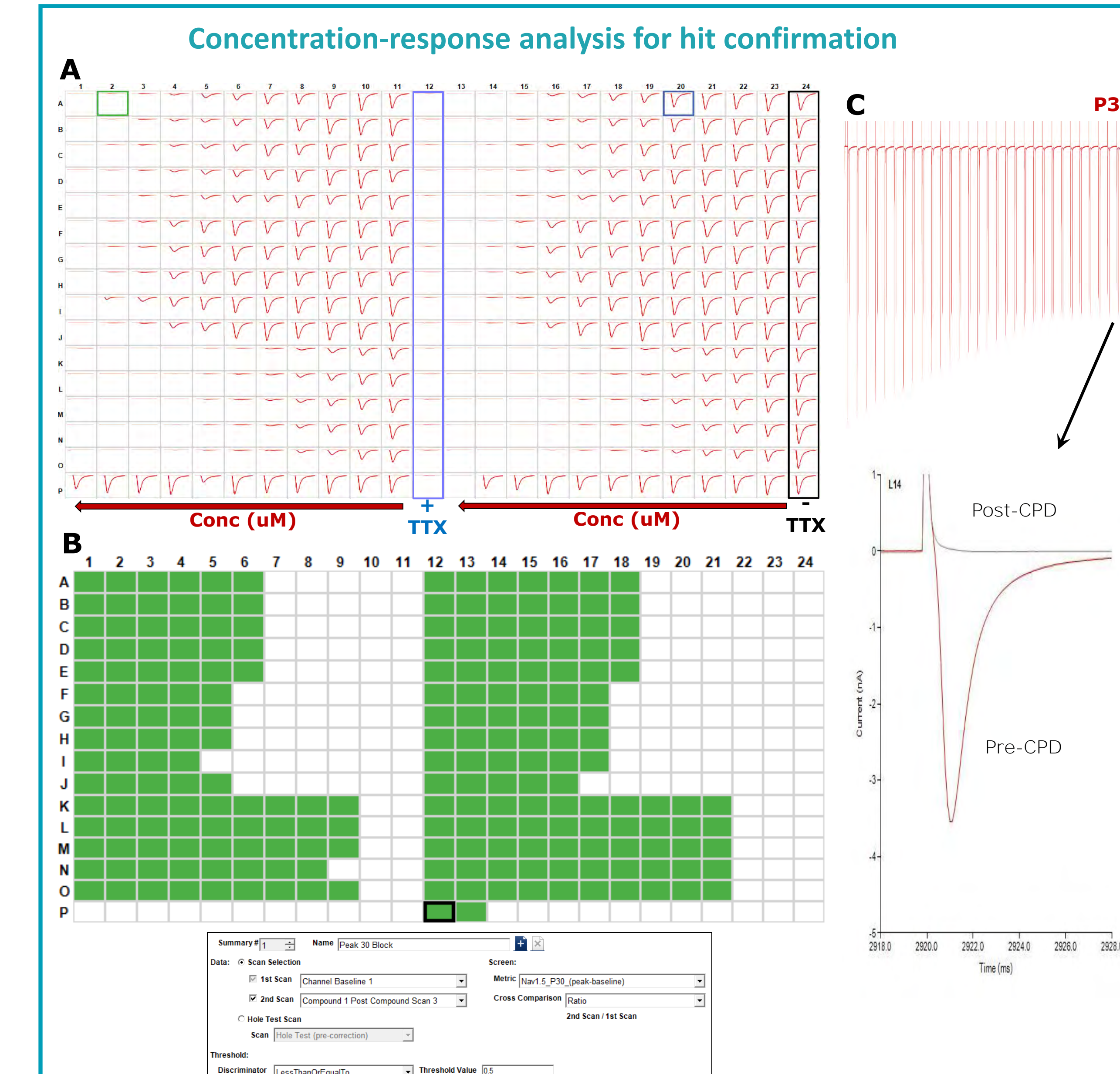


Fig. 6. Pharmacological characterization of concentration-response relationship, for confirmation of screening hits. **A)**, plate view of Nav1.5 currents (pulse 30 only) in response to different concentrations of compounds (TTX, tetracaine, lidocaine) or buffer; **B)**, heat map view of the wells with more than 50% inhibition of pulse 30 currents; **C)**, representative currents (top) and the overlay of pre- and post-compound currents (bottom) from the same well.

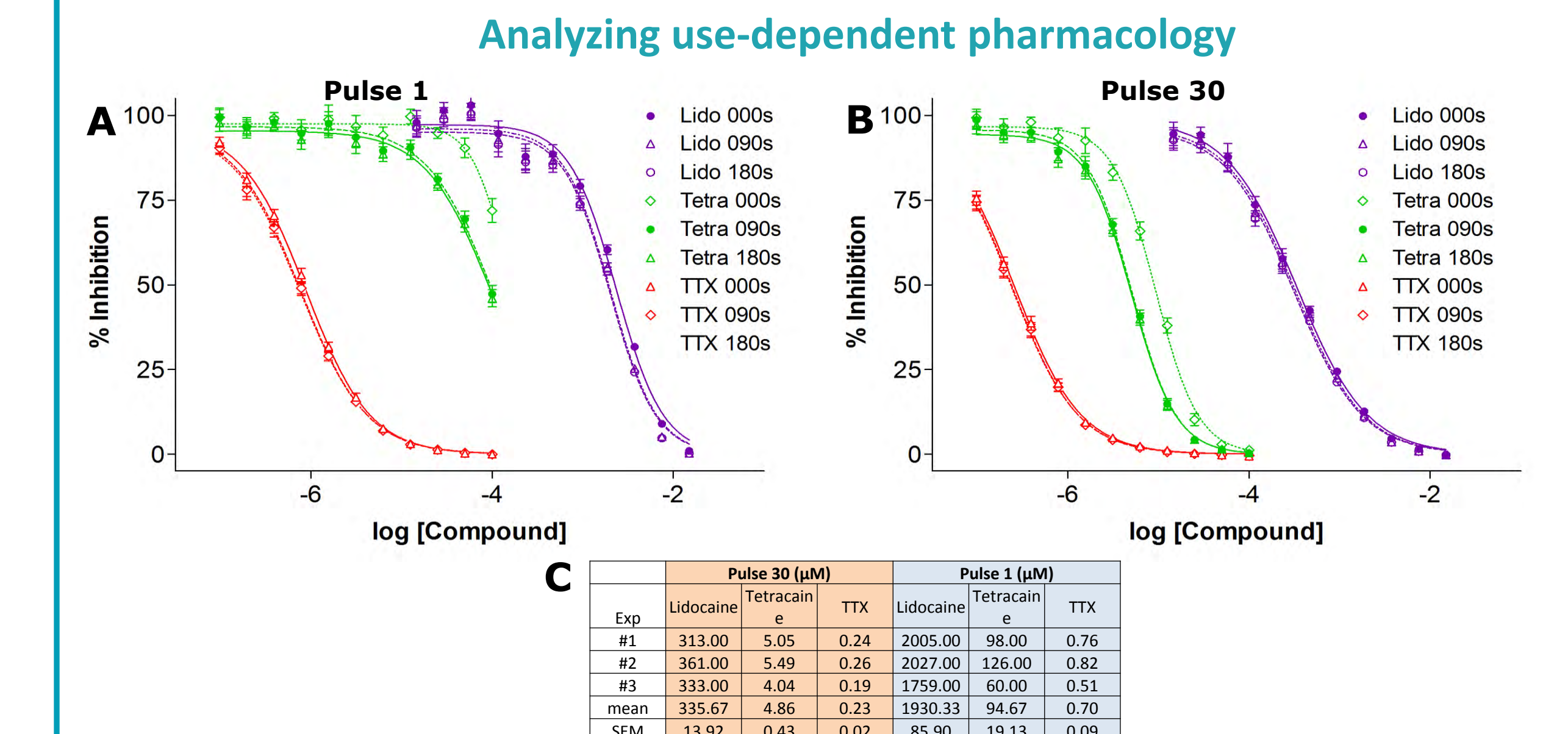


Fig. 7. Identification of use-dependent compounds by comparing concentration-response curves analyzed at pulse 1 (**A)** and pulse 30 (**B)**), and at different time points. **C)**, data scatter from three different experiment, indicating highly consistent results.

Summary

- In this study we designed and validated a high-throughput electrophysiology assay for screening and confirming blockers of a voltage-gated sodium channel. The pharmacology data collected on IonWorks Barracuda system is in good agreement with literature reports.
- The IonWorks Barracuda system enables continuous voltage clamp, sophisticated voltage protocols, flexible recording parameters, and prolonged assay window, for identification of use-dependent compounds in a drug screening setting.