

Abstract

Voltage-gated sodium channels comprise nine family members, Na_v1.1-Na_v1.9, and are responsible for the generation and propagation of action potentials in excitable cells. One particular member, Na_v1.8 is of mounting interest as a druggable target for pain, and selective Na_v1.8 inhibitors have shown safety and efficacy in clinical trials in both acute and chronic pain indications. Sodium channel inhibitors can exhibit complex biophysical mechanisms of action where channel inhibition can be dependent on the resting membrane potential (state-dependent) and/or frequency of cell depolarization (use-dependent). When depolarization frequency diminishes pharmacologic inhibition, a reverse-use dependent relief of inhibition effect can be observed as has been reported for previously described Na_v1.8 inhibitors (A-887826 and A-803467)¹. We recently described a selective and state-independent Na_v1.8 inhibitor, LTGO-33, that showed minimal use-dependent relief of inhibition under physiological conditions². To extend this work, we developed a model system to evaluate the kinetic parameters of use-dependent relief of inhibition using an automated patch clamp (APC; Sophion QPatch II) platform to screen compounds. In this work we will describe the frequency-, voltage-, and time-dependency of use-dependent relief of inhibition by historical and newer selective Na_v1.8 inhibitors.

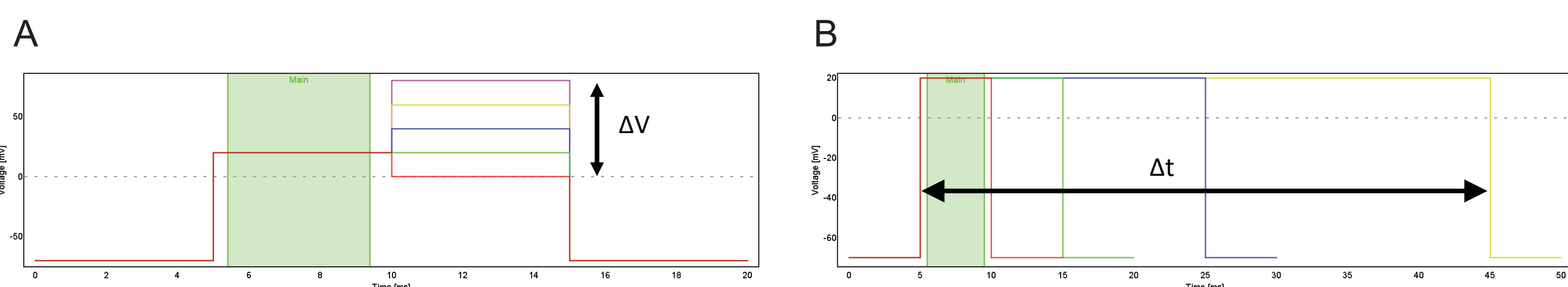
Methods

A HEK293 cell line stably expressing human Na_v1.8 and β1 subunits (Eurofins, DiscoverX) was obtained commercially and kept in continuous culture until use on the Sophion QPatch 48X platform. Cells destined for assay were incubated overnight at 30°C to increase expression level, lifted with Detachin and allowed to recover in the on-board QStirrer for at least 20 minutes prior to recording. Extracellular solution (in mM) consisted of 137 NaCl, 4 KCl, 3.8 CaCl₂, 1 MgCl₂, 10 HEPES, 10 Glucose; adjusted to pH 7.4 and supplemented with 500 nM tetrodotoxin. Intracellular solution (in mM) consisted of 50 CsCl, 90 CsF, 2 MgCl₂, 5 EGTA, 10 HEPES, adjusted to pH 7.2. Voltage protocols were designed and executed through Sophion Analyzer software in multihole configuration.

Equation 1:

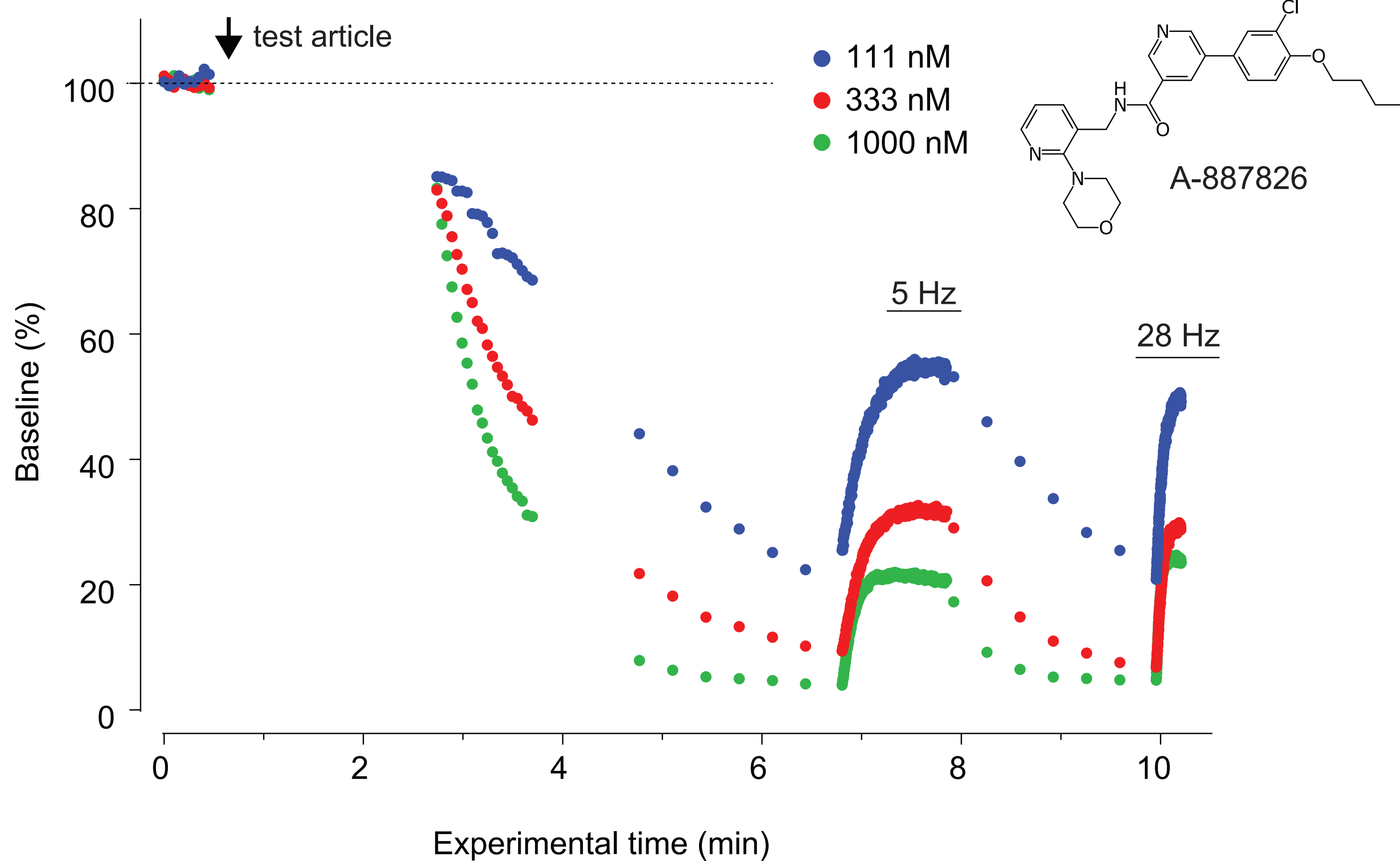
$$\% \text{ UDRI} = \frac{I_{\text{end}} - I_{\text{start}}}{1 - I_{\text{start}}} \times 100$$

Where I_{start} = sweep 1 of stimulus train, I_{end} = sweep 300



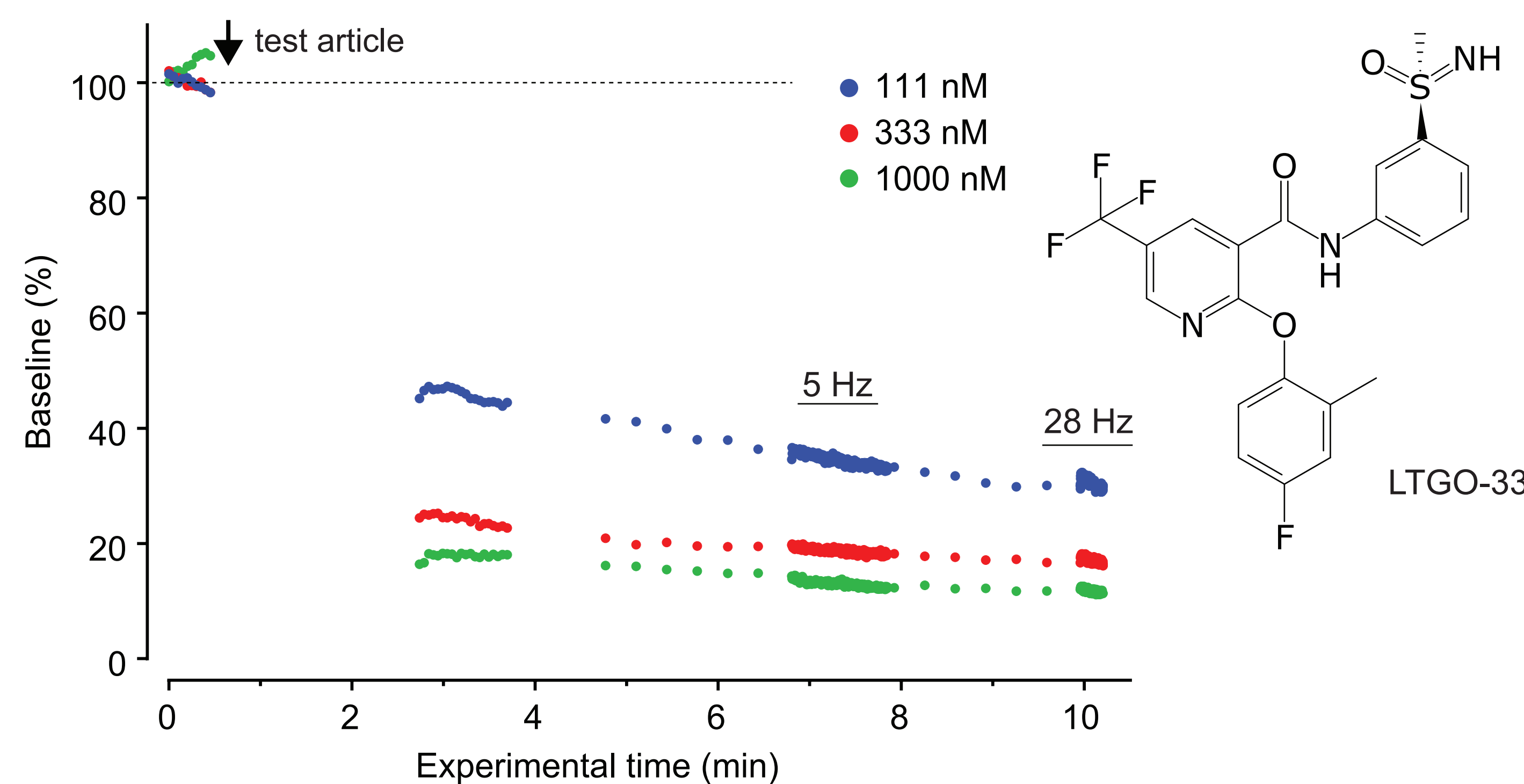
Graphic depiction of the voltage protocol utilized during the 5 Hz, 300 sweep stimulus train (A) and 5 Hz pulse train (B). Green rectangle shows area of peak inward current detection.

Use-Dependent Relief of Inhibition of hNa_v1.8 by A-887826



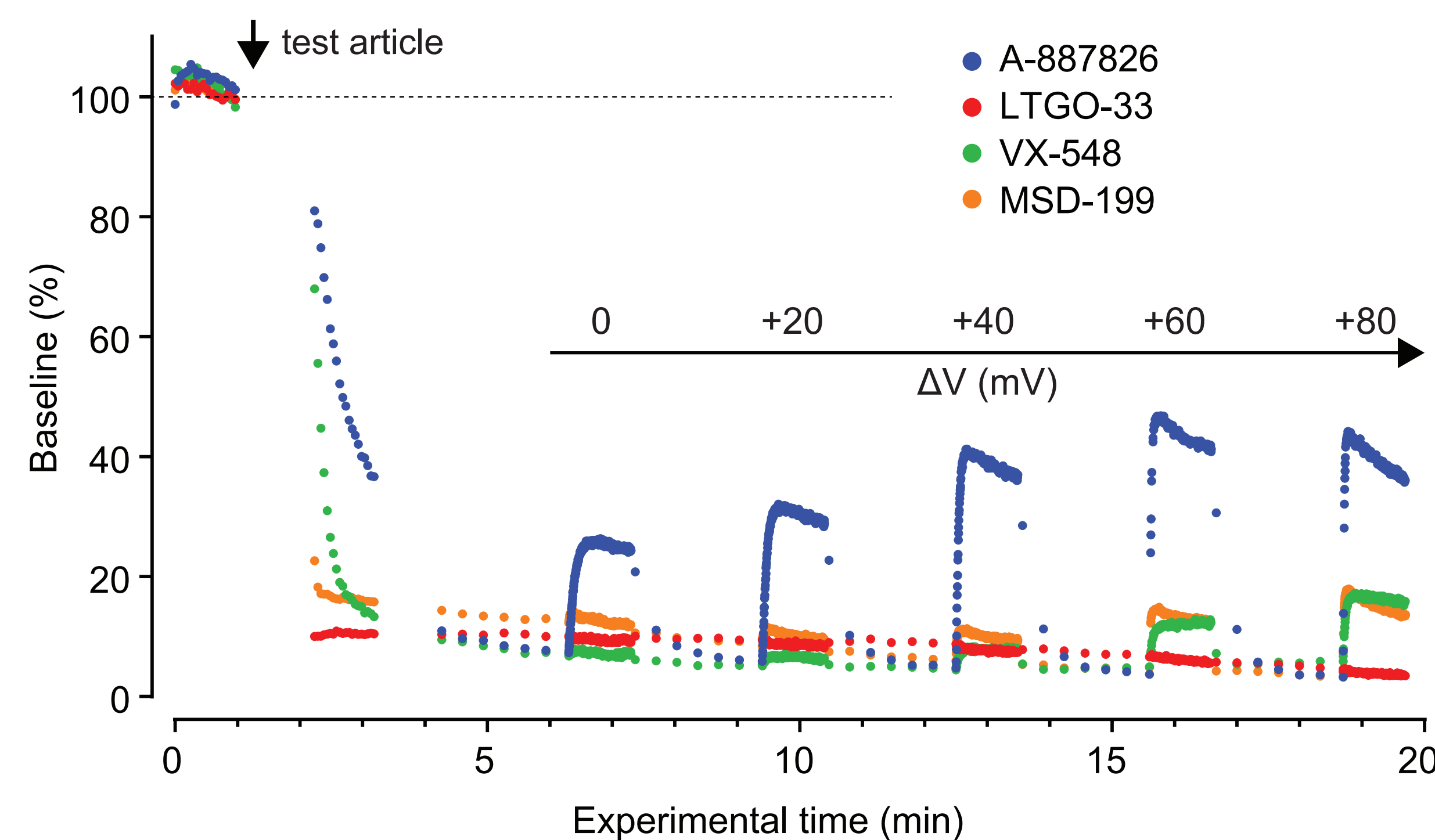
A-887826 (structure shown) inhibition of hNa_v1.8 currents was partially relieved by stimulations at both 5 and 28 Hz. Currents were elicited by a train of 300 sweeps with depolarizations to +20 mV (5 ms) from a holding potential of -70 mV. Intervening periods were stimulated at 0.05 Hz to allow rebinding of compound.

Lack of Use-Dependent Relief of Inhibition of hNa_v1.8 by LTGO-33



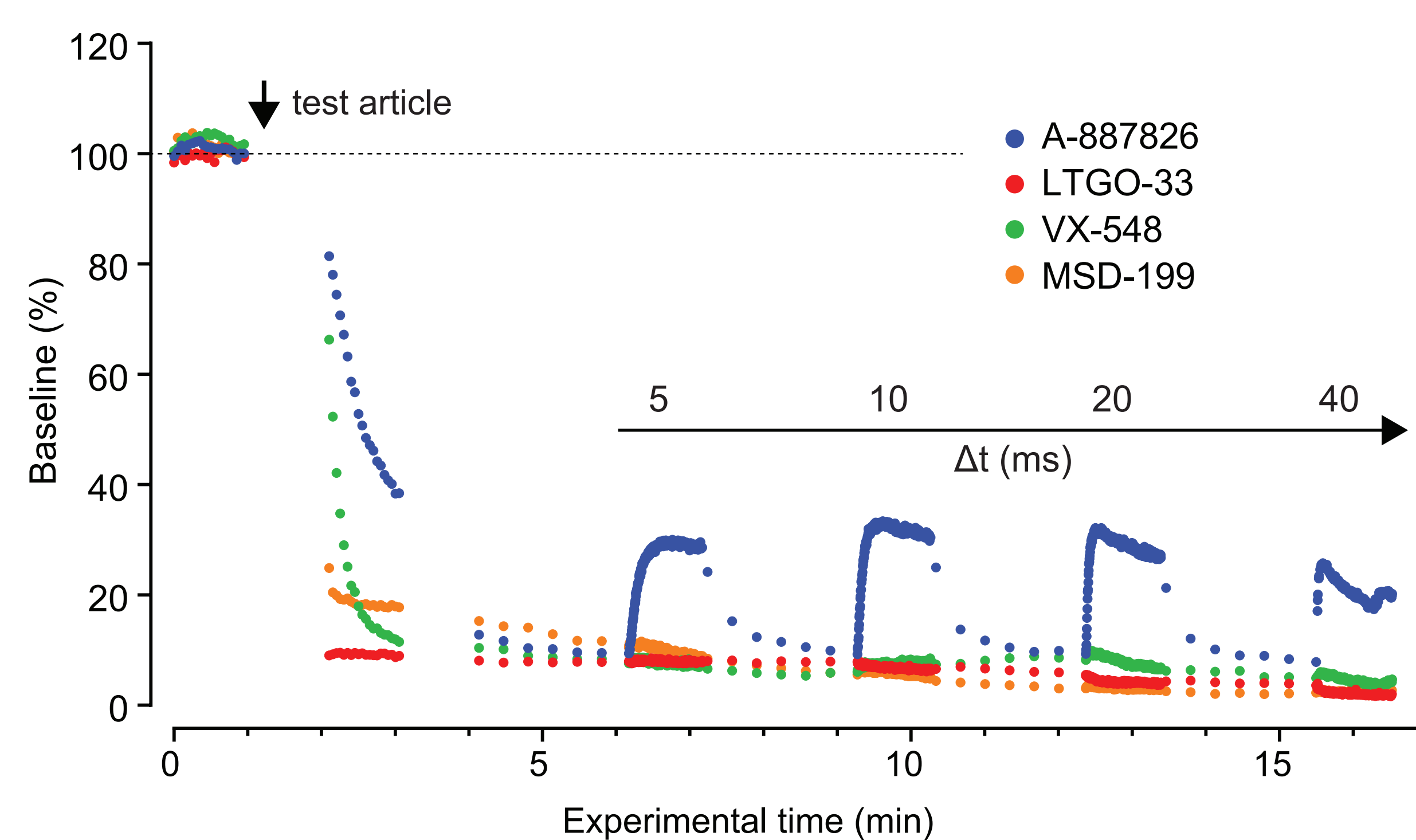
LTGO-33 (structure shown) inhibition of hNa_v1.8 currents was not relieved by stimulations at either 5 or 28 Hz. Currents were elicited by a train of 300 sweeps with depolarizations to +20 mV (5 ms) from a holding potential of -70 mV. Intervening periods were stimulated at 0.05 Hz to allow rebinding of compound.

Voltage-Dependence of Use-Dependent Relief of Inhibition



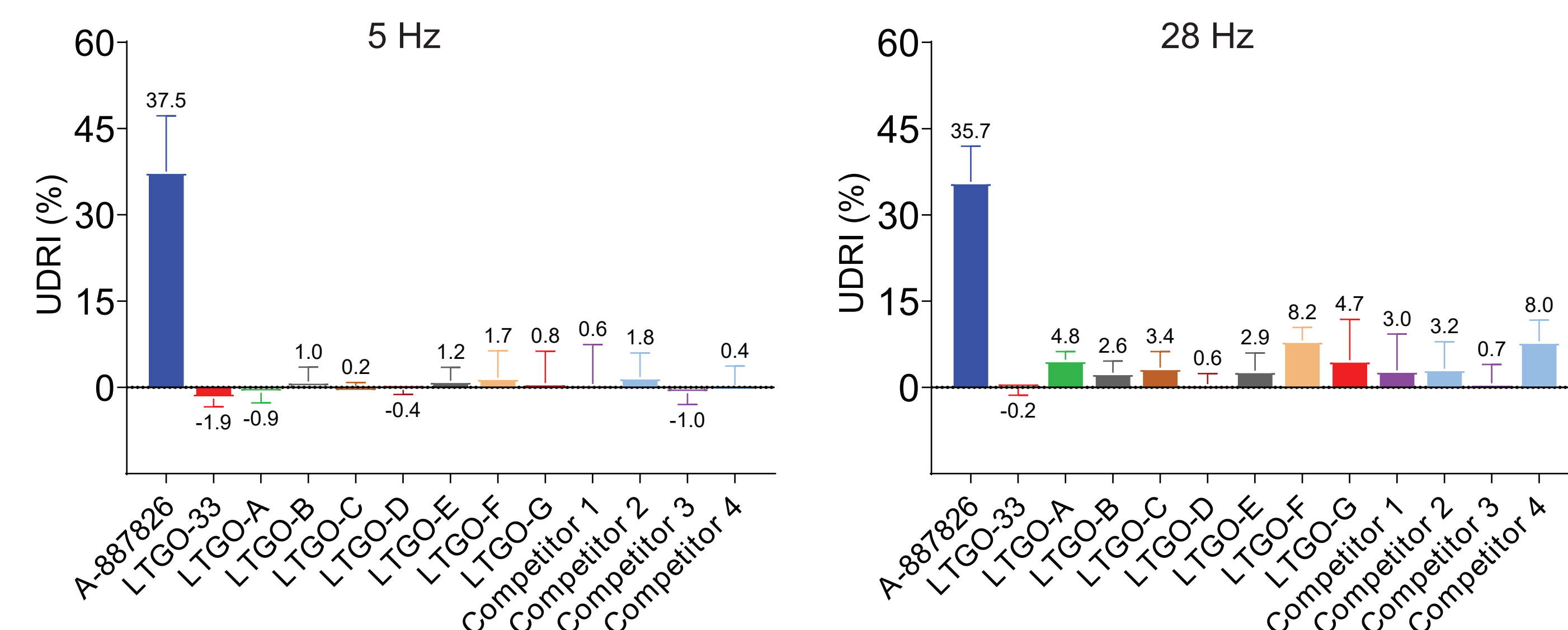
A) Evaluation of four inhibitors tested at concentrations approximating 90% inhibition of hNa_v1.8 currents. 5 Hz UDRI was generally greater at more depolarized potentials. A-887826 in blue demonstrated the highest amount of UDRI. Currents were elicited by 5 ms depolarizations to +20 mV from a holding potential of -70 mV, immediately followed by a 5 ms interval at varying voltages (0 to +80 mV with 20 mV intervals). Intervening periods were stimulated at 0.05 Hz to allow rebinding of compound. (n ≥ 3 wells each condition).

Lack of Time-Dependence of UDRI with Depolarizations to ≤ 40 ms



A) Evaluation of four inhibitors approximating 90% inhibition of hNa_v1.8 currents. A-887826 demonstrated UDRI that was similar with different pulse widths (5 to 40 ms). Currents were elicited by 5 ms depolarizations to +20 mV from a holding potential of -70 mV and sustained for varying durations (5 to 40 ms). Stimulus trains in each epoch remained constant at 300 sweeps and 5 Hz. Intervening periods were stimulated at 0.05 Hz to allow rebinding of compound (n ≥ 3 wells each condition).

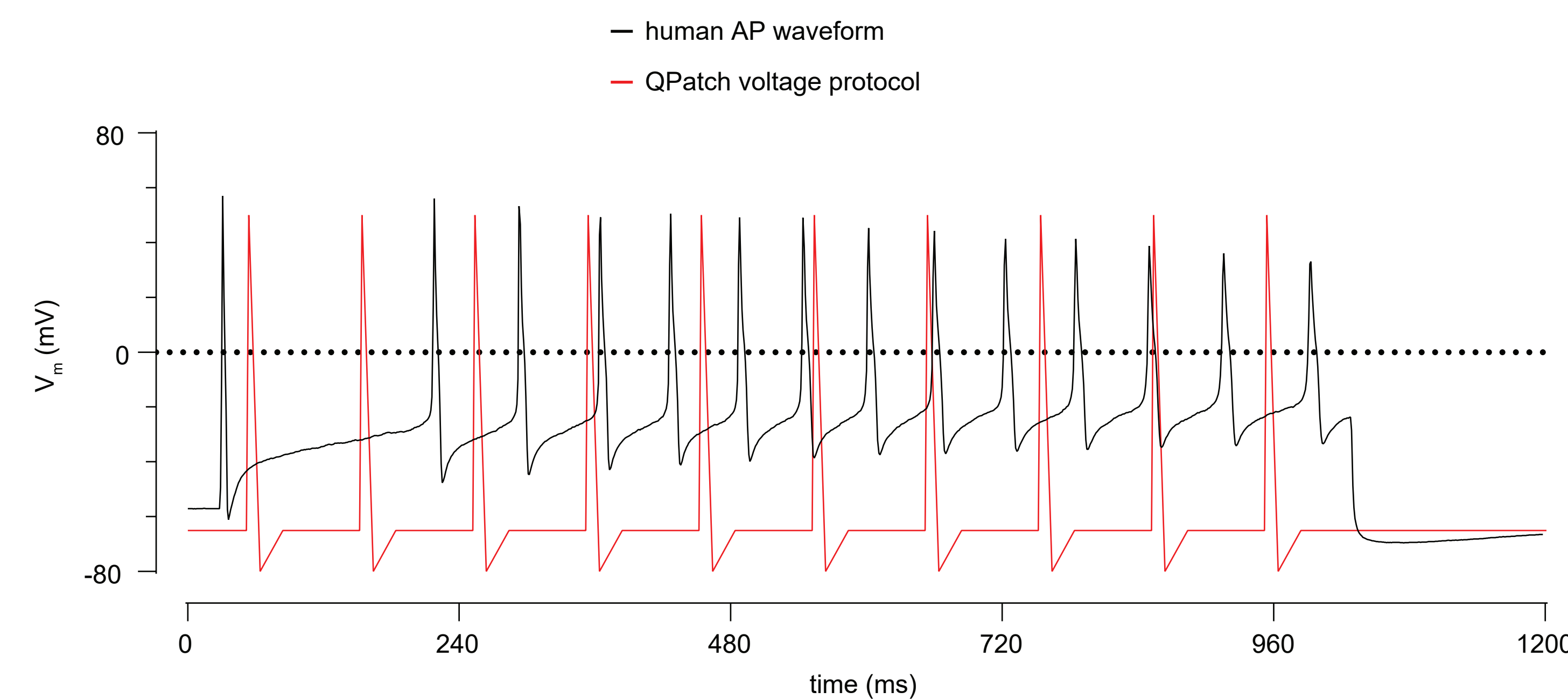
Screening for Use-Dependent Relief of Inhibition



Magnitude of UDRI observed at either 5 or 28 Hz for a panel of compounds either commercially available or synthesized internally. Currents were elicited by 5 ms depolarizations to +20 mV from a holding potential of -70 mV. Degree of UDRI was determined by normalizing to baseline current and calculated using the following equation for each frequency.

Results

- A-887826 inhibited human Na_v1.8 in a concentration-dependent manner. UDRI of the current was observed upon repeated stimulation to +20 mV (5 ms) at both 5 Hz and 28 Hz.
- LTGO-33 inhibited human Na_v1.8 in a concentration-dependent manner. However, upon repeated stimulation at 5 Hz and 28 Hz, negligible UDRI was observed.
- Additional voltage protocols were designed to investigate both voltage- and time-dependence of UDRI. A distinct pattern of greater relief of inhibition with stronger depolarizations was observed.
- Evaluation of a larger panel of Na_v1.8 inhibitors revealed pronounced UDRI only with A-887826.
- Biophysical voltage protocols utilizing square pulses to interrogate ion channel function are non-physiological and not experienced by neurons in vivo. Further investigation into UDRI could employ an action potential waveform as stimulus



References

- Jo, Sooyeon, Han-Xiong Bear Zhang, and Bruce P. Bean. "Use-dependent relief of inhibition of Nav1.8 channels by A-887826." *Molecular Pharmacology* 103, no. 4 (2023): 221-229.
- Gilchrist, J. M., Yang, N. D., Jiang, V., & Moyer, B. D. (2024). Pharmacologic Characterization of LTGO-33, a Selective Small Molecule Inhibitor of the Voltage-Gated Sodium Channel Na_v1.8 with a Unique Mechanism of Action. *Molecular Pharmacology*, 105(3), 233-249.

Acknowledgements

Appreciation is extended to Daniel Jong for providing cell culture assistance and to the Latigo Biotherapeutics team for discussions, feedback, and support.