Late $Na_V1.5$ current

- Assay validation -

To test the effect of compounds on late voltage gated sodium currents, their delayed inactivation is mandatory. For this purpose, ATXII -a 47 amino acid peptidyl toxin, originally isolated from Anemonia sulcata sea anemone venom- can be used.

Concentrations between 0.1 nM and 30 nM were used to determine the most suitable ATXII concentration to activate late $Na_V1.5$ currents. The EC_{50} was determined to be 4.60 nM, Hill coefficient: 1.06. Based on these data a concentration of 30 nM ATXII was selected to activate late $Na_V1.5$ currents. After about 5 min of application the maximal current is reached and the current amplitude is stable. The first compound concentration is added after at least 500 s after the first application of ATXII.

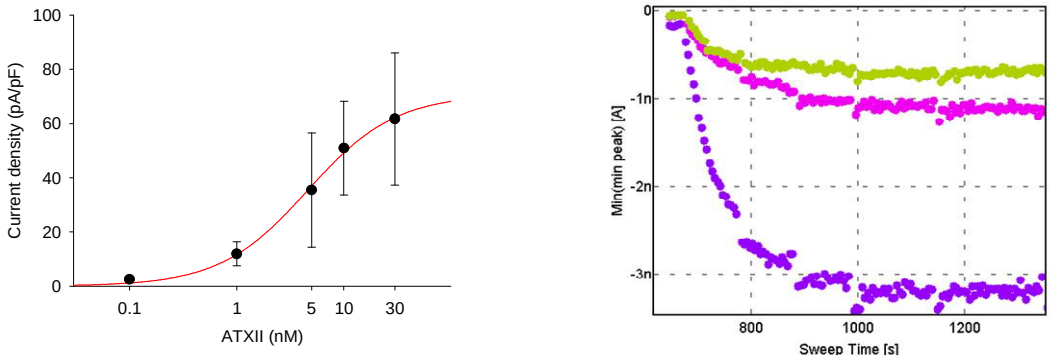

Fig. 1: Effect of ATXII on late $Na_V1.5$ currents. left: dose response curve of ATXII, right IT plot of onset of late $Na_V1.5$ current. (each color represents an individual cell)

To validate the assay to screen late $Na_V1.5$ currents, four reference compounds were tested for their effects on peak and late $Na_V1.5$ currents.

Tab. 1: Summary of IC_{50} values for peak and late $Na_V1.5$ currents.

Compound name	Peak current: IC_{50} / Hill coefficient	Late current: IC_{50} / Hill coefficient	Ratio IC_{50} late / IC_{50} peak
Flecainide	43.08 µM / 1.04	0.23 µM / 1.05	0.005
Lidocaine	446.01 µM / 1.12	16.88 µM / 1.08	0.037
Mexiletine	48.49 µM / 1.35	11.94 µM / 0.80	0.246
Ranolazine	93.85 µM / 10.7	18.51 µM / 0.82	0.197
TTX	2.34 µM / 0.75	1.09 µM / 1.07	0.466

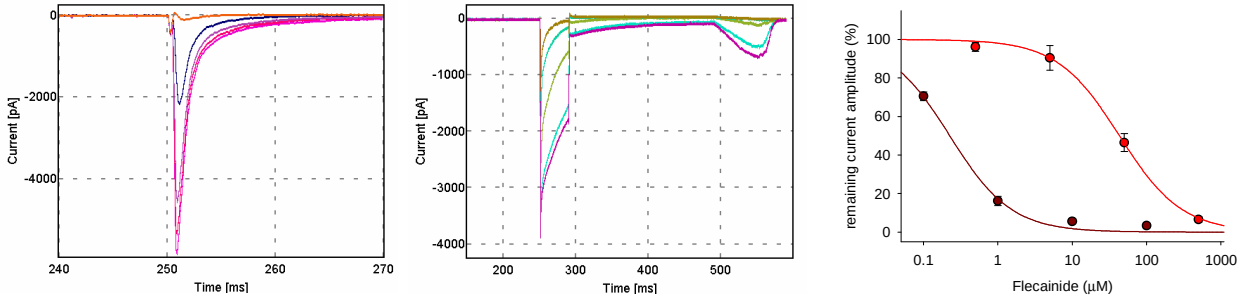

Fig. 2: Effect of Flecainide on peak and late $Na_V1.5$ currents. Left: $Na_V1.5$ peak current, middle: $Na_V1.5$ late current (stimulated by 30 nm ATXII), right: dose response curves

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