

Characterization of CreaCell's hNa_v1.5 (A-0822) on Nanion's Patchliner®

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Introduction

In this Application Note we present data characterizing hNa_v1.5 overexpressing HEK293 cells. The data were collected with Nanion's Patchliner®.

The performance of the cells was very good on the Patchliner (Table 1).

Current responses of an individual cell expressing the hNa_v1.5 channel to an IV voltage protocol are shown in Figure 1. The corresponding current-voltage relationship is shown below.

The average mean peak current density of the cells was -215.1 ± 63 pA/pF at 0 mV (n=9).

R _{seal}	C _{slow}	R _s
2015 +/- 428	7.9 +/- 1.6	2.4 +/- 0.8

Table 1:

Cell's performance on the Patchliner. The mean seal resistance was 2015 +/- 428 MOhm, the size of the cells was 7.9 +/- 1.6 pF and the series resistance R_s was 2.4 +/- 0.8 MOhm, n=9, values are depicted +/- S.E.M.

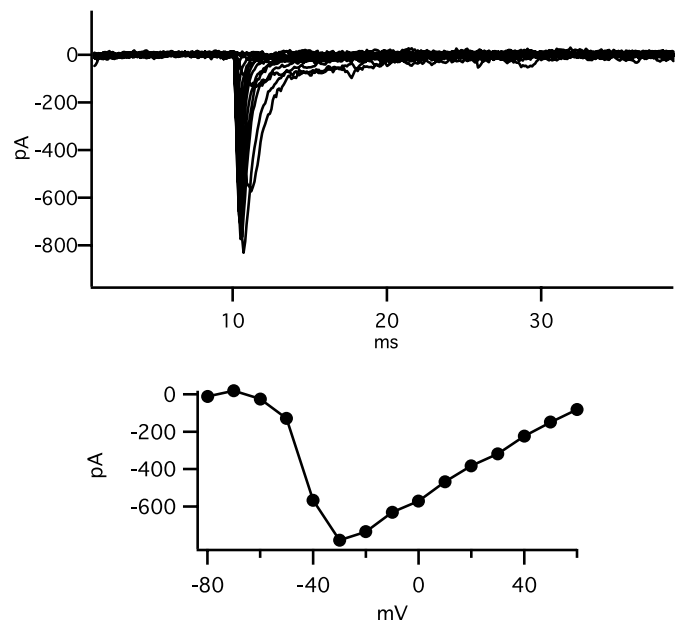


Figure 1:

Typical Na_v1.5 raw current responses to an IV protocol. Holding potential was -80 mV. Test potentials ranged from -80 mV to +60 mV. Below, corresponding current-voltage relationship is shown.

The mean current density was 215.1 ± 63 pA/pF (n=9; +/- S.E.M.).

Application Note

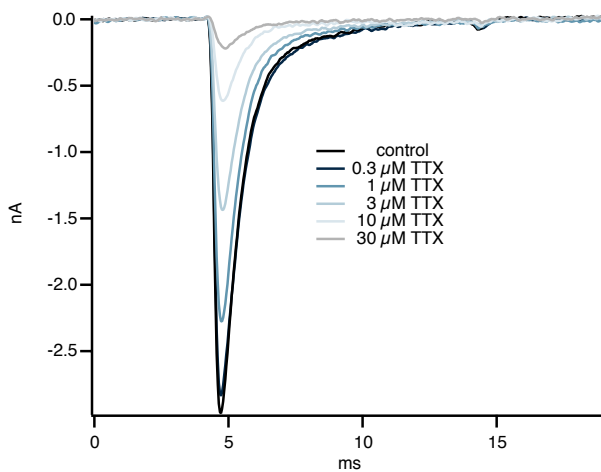
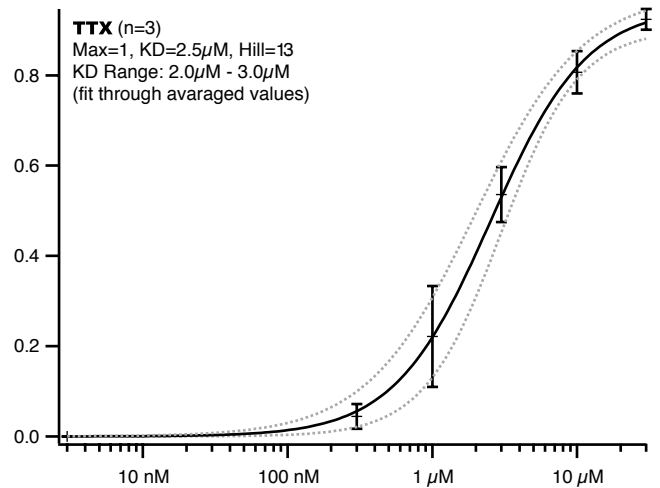


Figure 3:
Exemplary raw current traces from an individual cell expressing hNa_v1.5 under control conditions (black) and at increasing concentrations of TTX (as indicated).

Figure 3 shows recordings of a single cell in the absence and presence of increasing concentrations of TTX.

Figure 4 shows the average TTX dose response curve. IC₅₀ was determined as indicated in the graph.



TTX Single Point: Block @ 3e-05M = 0.92 ± 0.028 (n=3)
TTX IC₅₀: Max=0.94±0.06, KD=2.4μM±509.3nM, Hill=1.5±0.45 (3 fits averaged)

Figure 4:
Dose-response curve for TTX. The estimated IC₅₀ was 2.4 +/- 0.5 μM (n=3).

Methods

Cells

HEK293 cells stably expressing hNa_v1.5 were supplied by CreaCell. Cells were cultured and harvested according to Nanion's standard cell culture protocol.

Cell Culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner®. Currents were elicited using voltage step from a holding potential of -80 mV to 0 mV for 20 ms every 2 seconds.