

Characterization of ion channel currents endogenously expressed in Neuro2A cells using automated patch clamp

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Introduction

Neuro2A cells are a mouse neuroblastoma cell line used extensively to investigate neuronal differentiation, axonal growth and cell signalling pathways . They are also used as an expression system for studying ion channels. Neuro2A cells have been shown to endogenously express NaV channels, predominantly Na_v1.7 but also Na_v1.2, 1.3 and 1.4 (Lou et al., 2005), mechanosensitive channels Piezo 1 (Coste et al., 2010; Rotordam et al., 2019), purinergic receptors (Vetter & Lewis, 2010) and glutamate receptors (Van der Valk & Vijverberg, 1990). In this study, we used the medium and high throughput automated patch clamp devices, Patchliner and SyncroPatch 384, to record different ion channels endogenously expressed in Neuro2A cells.



Piezo1 activated by Yoda1 recorded on the SyncroPatch 384

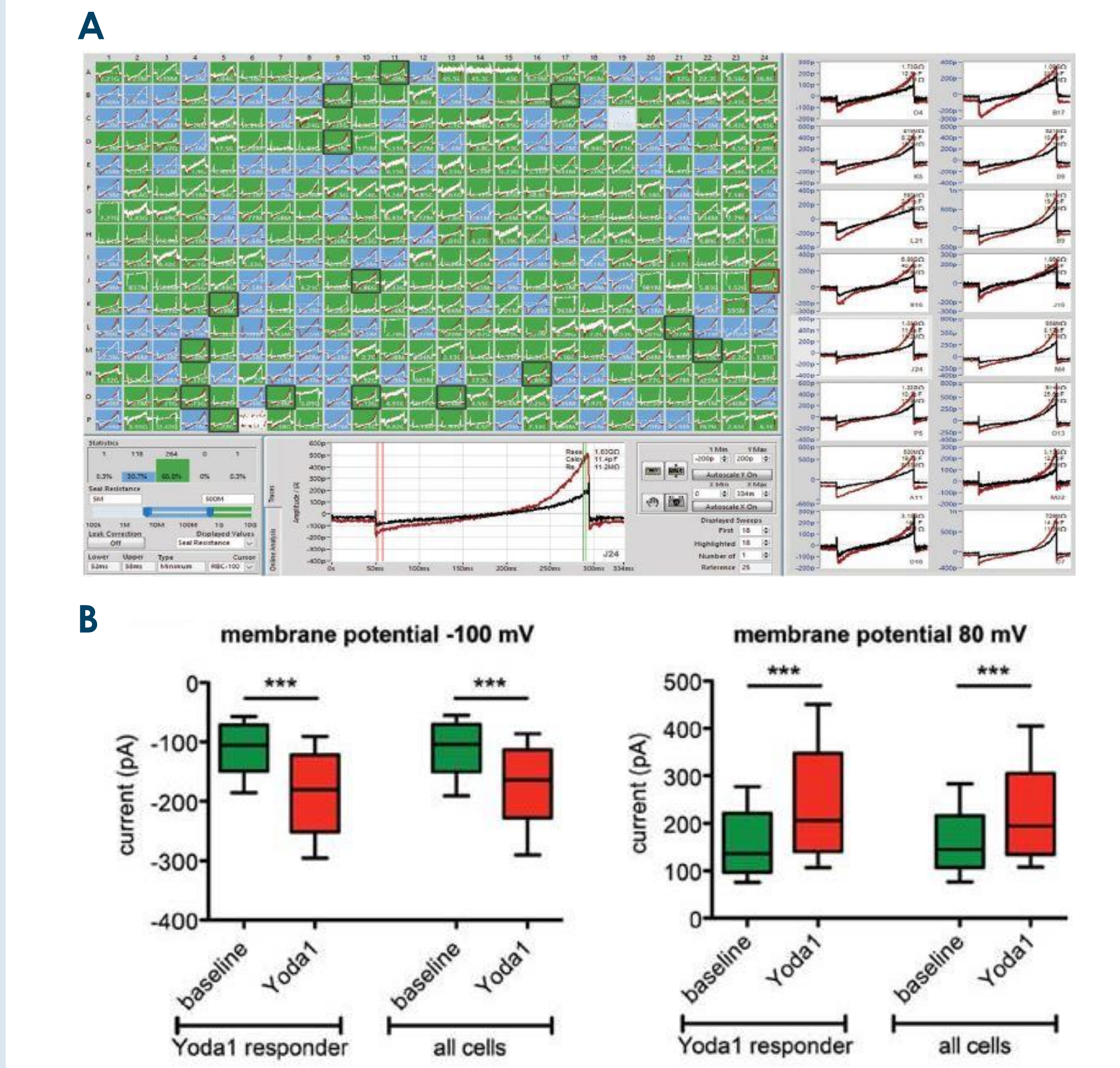


Figure 3: Piezo1 channels endogenously expressed in Neuro2A cells were recorded on the SyncroPatch 384. **A** Screenshot of PatchControl 384 software during an experiment. The wells are colour-coordinated based on seal resistance (green wells have $R_{seal} > 500$ M Ω , blue wells have R_{seal} 5 – 500 M Ω). Sixteen wells are shown enlarged on the right and one well is highlighted at the bottom of the screenshot. The black trace is control and red trace is in the presence of 10 μ M Yoda1. **B** Statistical analysis of the currents at -100mV (left) and at 80 mV (right). 140 out of 384 Neuro2A cells (37%) passed the quality criteria and 85 cells (60% of the valid cells) were considered as Yoda1 responders.

Data from Rotordam et al, 2019.

References

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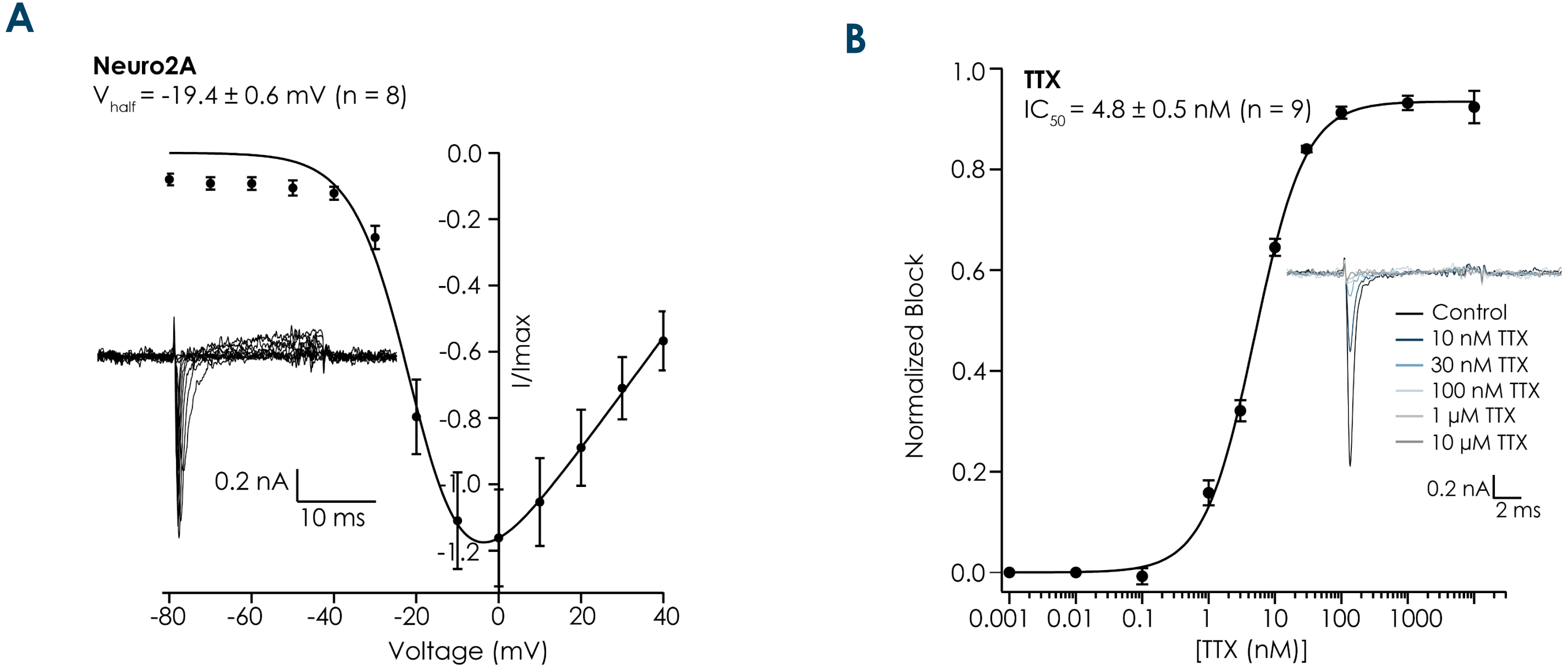
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NaV recorded from Neuro2A is TTX sensitive



NaV pharmacology: Tetracaine and Lidocaine

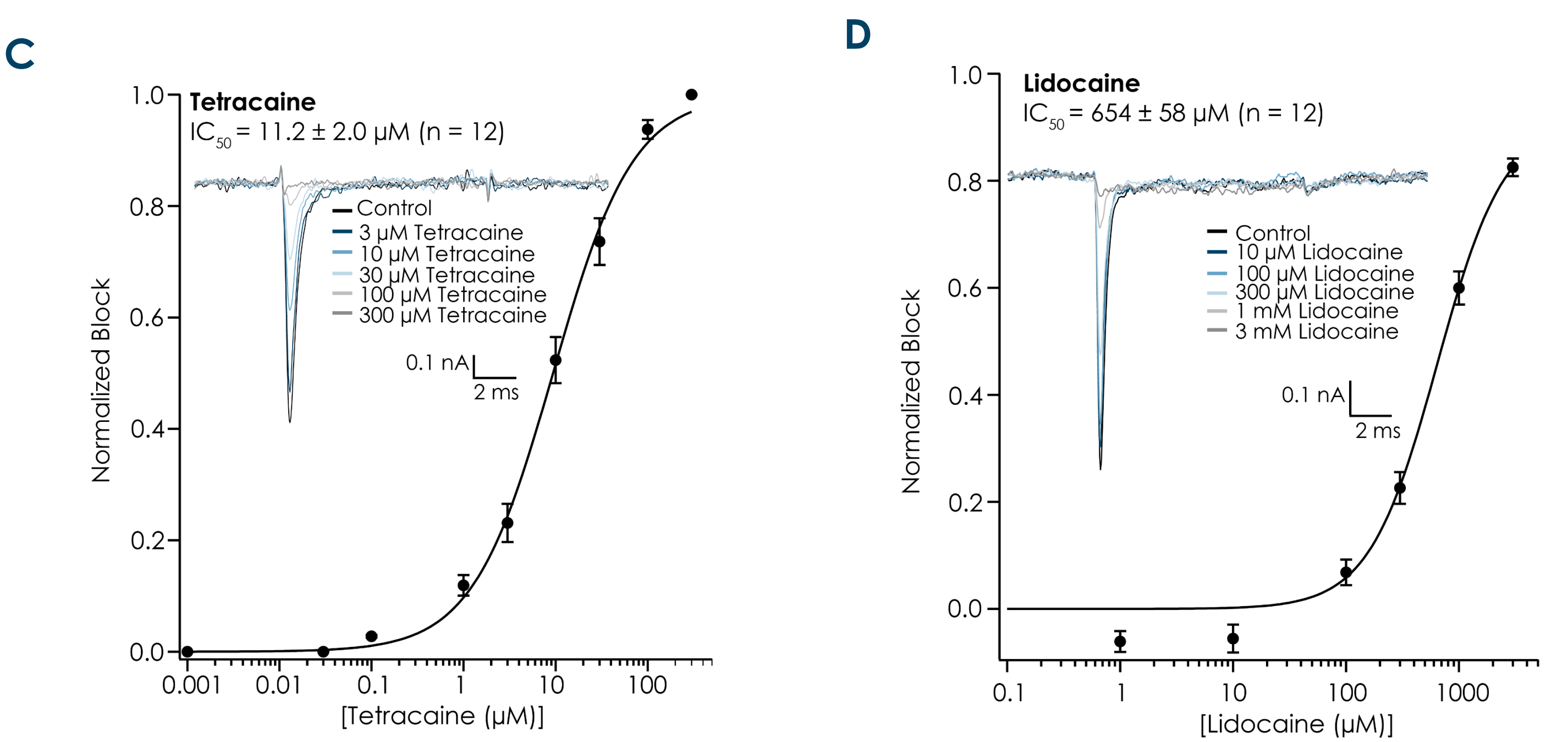


Figure 1: A voltage-gated sodium current was recorded from Neuro2A cells. **A** We calculated a V_{half} of activation of -19.4 $\pm$ 0.6 mV (n = 8 cells) using a Boltzmann equation. **B** The NaV current was completely blocked by nM concentrations of TTX with an IC_{50} of 4.8 $\pm$ 0.5 nM (n = 9 cells). Multiple concentrations of TTX were added to each well of the NPC-16 chip of the Patchliner and complete concentration response curves were constructed from 9 individual cells. **C** & **D** The NaV current was also blocked by Tetracaine (**C**) and Lidocaine (**D**) with an IC_{50} of 11.2 $\pm$ 2.0 μ M (n = 12) and 654 $\pm$ 58 μ M (n = 12), respectively. Holding potential was -100 mV.

Heat-activated response is mediated by TRPV3 and TRPV4

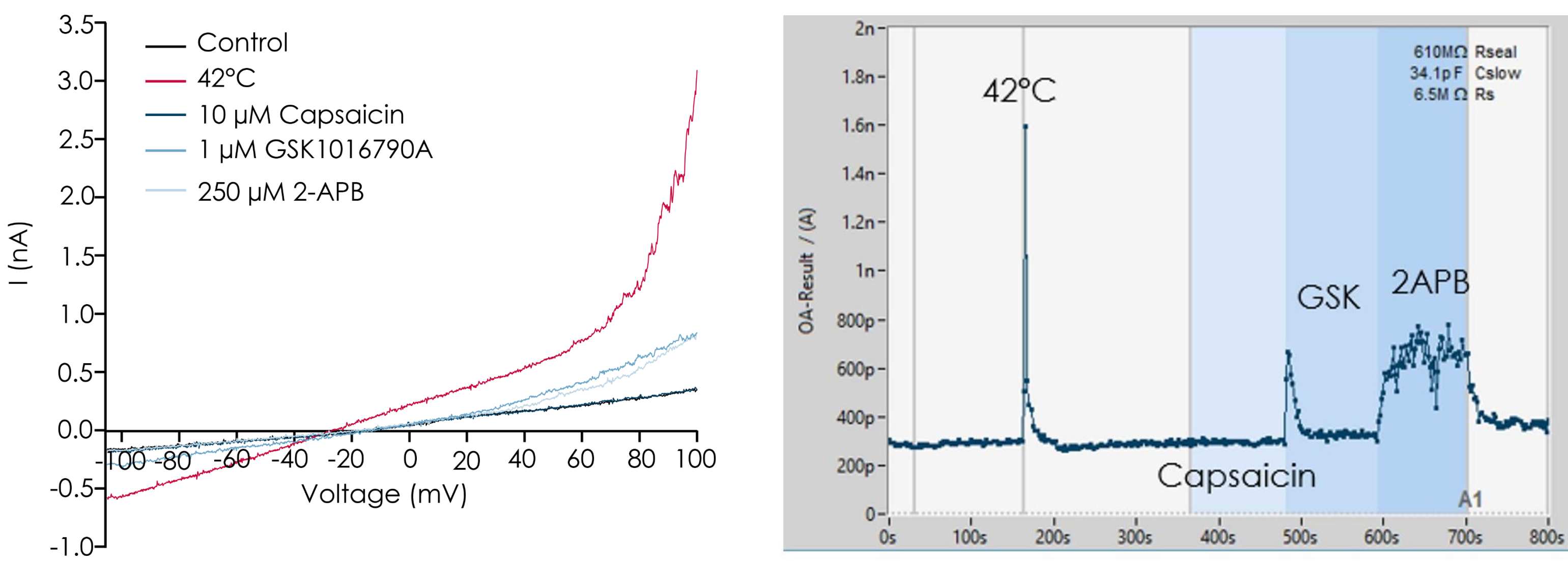


Figure 2: TRP channels are expressed in Neuro2A cells. External solution was heated in the pipette of the Patchliner and rapidly applied to the cells. A heat-activated response was seen in 8/8 cells at 42°C. Following this, different TRP channel activators were applied. No activation was seen with capsaicin in 8/8 cells, whereas all cells (8/8) responded to GSK1016790A and 4/8 cells responded to 2-APB. Shown are raw current traces from an example cell (left) and the timecourse of the experiment (right). This indicates that TRPV4 and in some cells, TRPV3, is expressed in Neuro2A cells but not TRPV1.

Conclusions

- Neuro2A cells can be used on automated patch clamp devices with success rates of 60-80% for >1 G Ω seals
- Voltage-gated Na currents were recorded with a V_{half} of activation of -19.4 $\pm$ 0.6 mV and blocked by nM concentrations of TTX
- The NaV current was also blocked by lidocaine and tetracaine at μ M concentrations
- A heat activated response was measured after application of external solution at 42°C. This was mediated by TRPV4 or TRPV3 but not TRPV1
- Piezo1 was recorded from Neuro2A cells on the SyncroPatch 384 following application of Yoda1
- Neuro2A cells are a suitable cell type for investigating endogenous NaV currents, as well as Piezo1 and some TRP channels.