

Activation and inhibition of assay-ready TRPA1 and TRPV cells: an automated patch clamp study

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Introduction

Transient Receptor Potential (TRP) channels are widely distributed throughout the mammalian central and peripheral nervous systems. They can be directly activated by ligands, heat or cold and mechano-stimulation, and are important targets in drug discovery for the treatment of pain, respiratory diseases, cancer, immune disorders and others. Here, we studied the responses of TRPA1, TRPV1, TRPV3, TRPV4 and TRPM8 assay-ready and cultured cells activated using a variety of stimuli on automated patch clamp (APC) systems.



Port-a-Patch
One recording channel



Patchliner
4 to 8 recording channels



SyncroPatch 384i
384 recording channels

TRPM8 – activation by cold solution

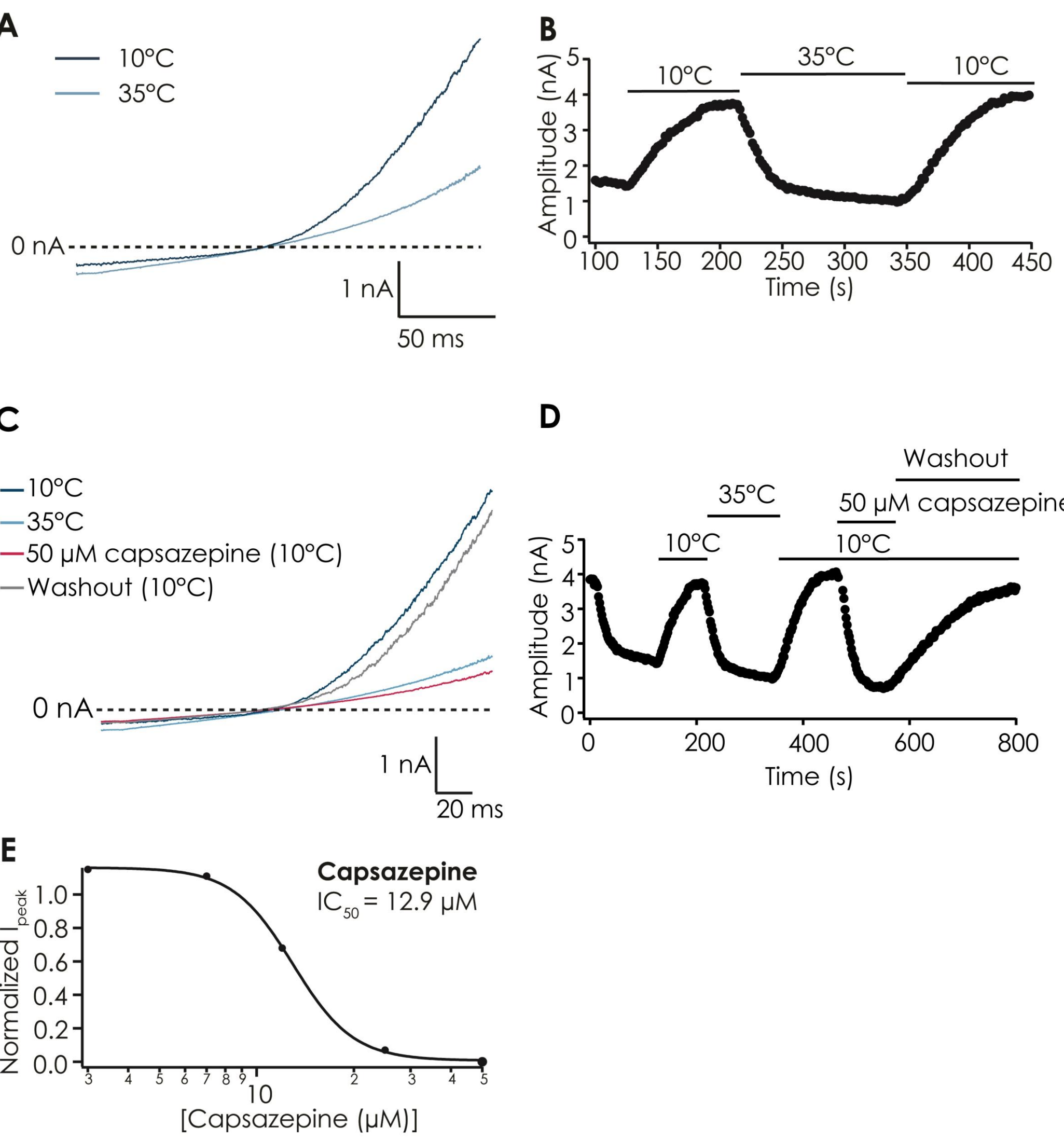


Figure 1: TRPM8 activation by cold solution and block by capsazepine recorded on the Port-a-Patch with temperature controlled external perfusion system. **A** Activation of TRPM8 expressed in CHO cells using a voltage ramp (-100 mV to +100 mV over 200 ms) by cold solution (10°C; dark blue) and subsequent block by warm solution (35°C; light blue). **B** Corresponding timecourse of the experiment. **C** Activation of TRPM8 by cold solution (10°C; dark blue) and subsequent block by either 50 µM capsazepine (red) or warm solution (35°C; light blue). TRPM8 was reactivated by cold solution following block by capsazepine (grey). **D** Corresponding timecourse of the experiment. **E** Concentration response curve (CRC) for capsazepine block of TRPM8 activated by cold solution. The curve was fit with a Hill equation and an $IC_{50} = 12.9 \mu M$ ($n = 1$) was calculated, in excellent agreement with the literature^{1,2}.

Activation and inhibition of TRPA1 using high throughput APC

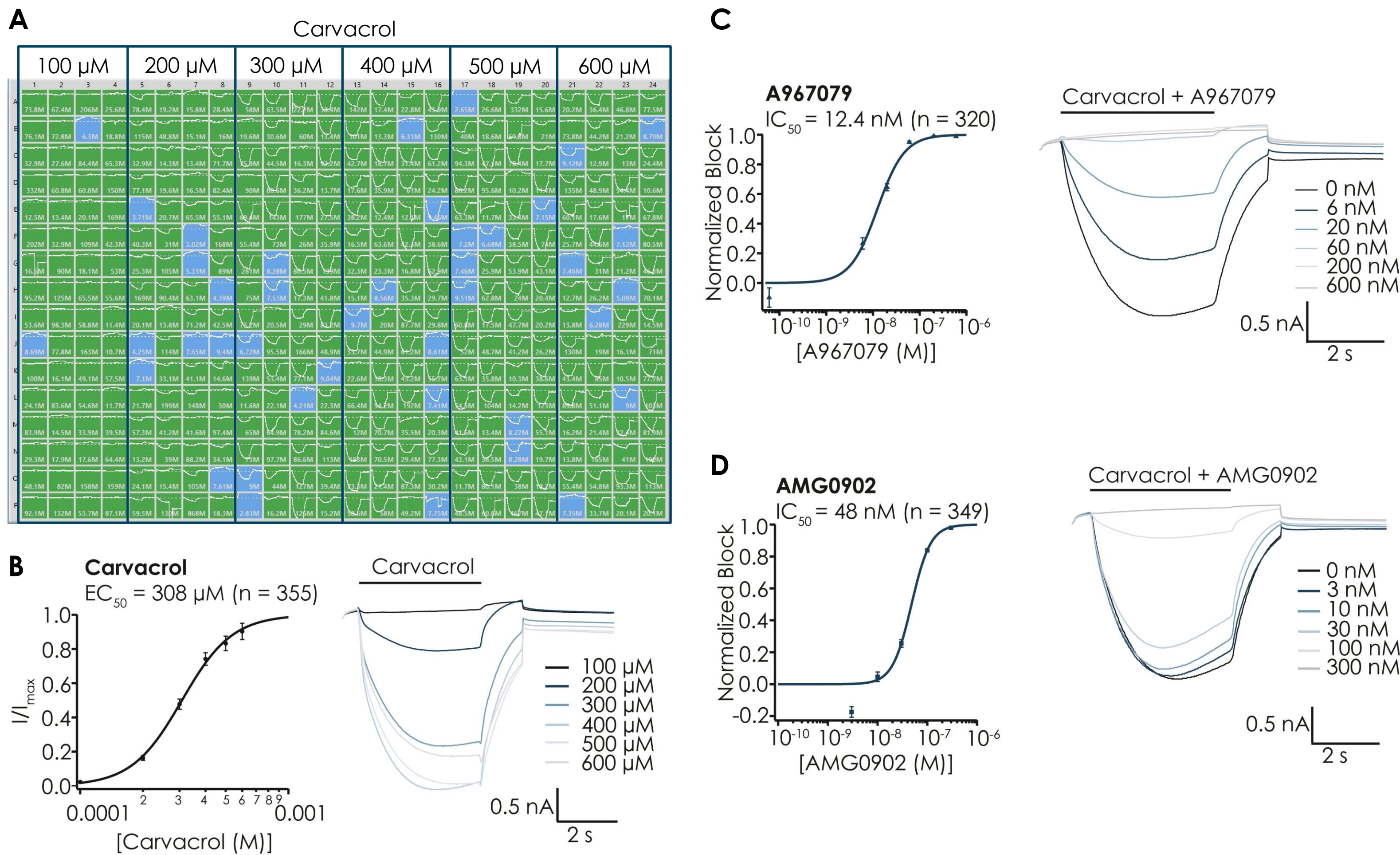


Figure 2: Activation of TRPA1 expressed in CHO cell on the SyncroPatch 384i. Top panel shows graphical user interface of the screening and data analysis software used on the SyncroPatch 384i. A Screenshot depicts raw data traces of TRPA1-expressing CHO cells as recorded on one NPC-384 patch clamp chip (4 holes). A single concentration of carvacrol was added to each well and the concentration response curve calculated across the plate. B Average CRC for carvacrol for $n = 355$ wells (left) and average traces. TRPA1 was robustly activated by carvacrol as expected^{3,4}. At higher concentrations (above 400 μM) desensitization of the channel was observed (tachyphylaxis) as previously reported³ which resulted in smaller amplitudes upon repeated application. C Inhibition of TRPA1 by A967079 and AMG0902. C TRPA1-mediated responses were blocked by A967079 in a concentration-dependent manner with an IC_{50} value (12.4 nM) in good agreement with the literature value of 50 nM⁵. D TRPA1-mediated responses were blocked by AMG0902 in a concentration-dependent manner with an IC_{50} value of 48 nM ($n = 349$) in good agreement with the literature value of 68 nM⁵.

All experiments were performed in perforated patch mode using multi-hole (4 holes per well) NPC-384 chips.

Activation and inhibition of TRPV4

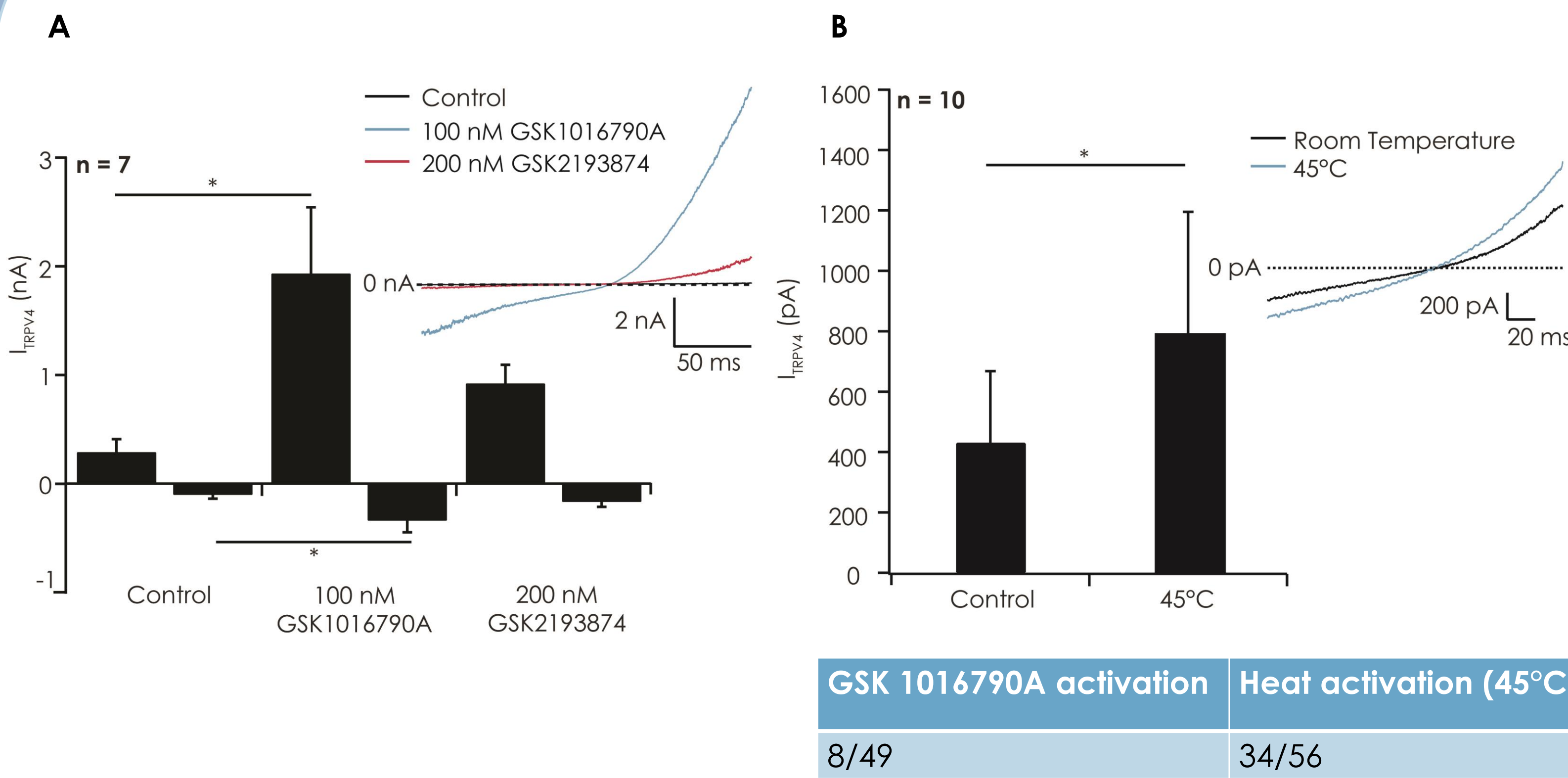


Figure 3: TRPV4 activation and inhibition on the Patchliner. A TRPV4 expressed in CHO cells were activated by the specific and potent TRPV4 agonist GSK1016790A^{7,8} and this effect could be blocked by GSK2193874, a specific antagonist of TRPV4⁷. B TRPV4 expressed in CHO cells was also moderately activated by heated solution (45°C). External solution was heated inside the pipette of the Patchliner and rapidly applied to the cells. In 34/56 cells tested, activation by heated solution could be observed.

Ligand activation of TRPV1 and TRPV3

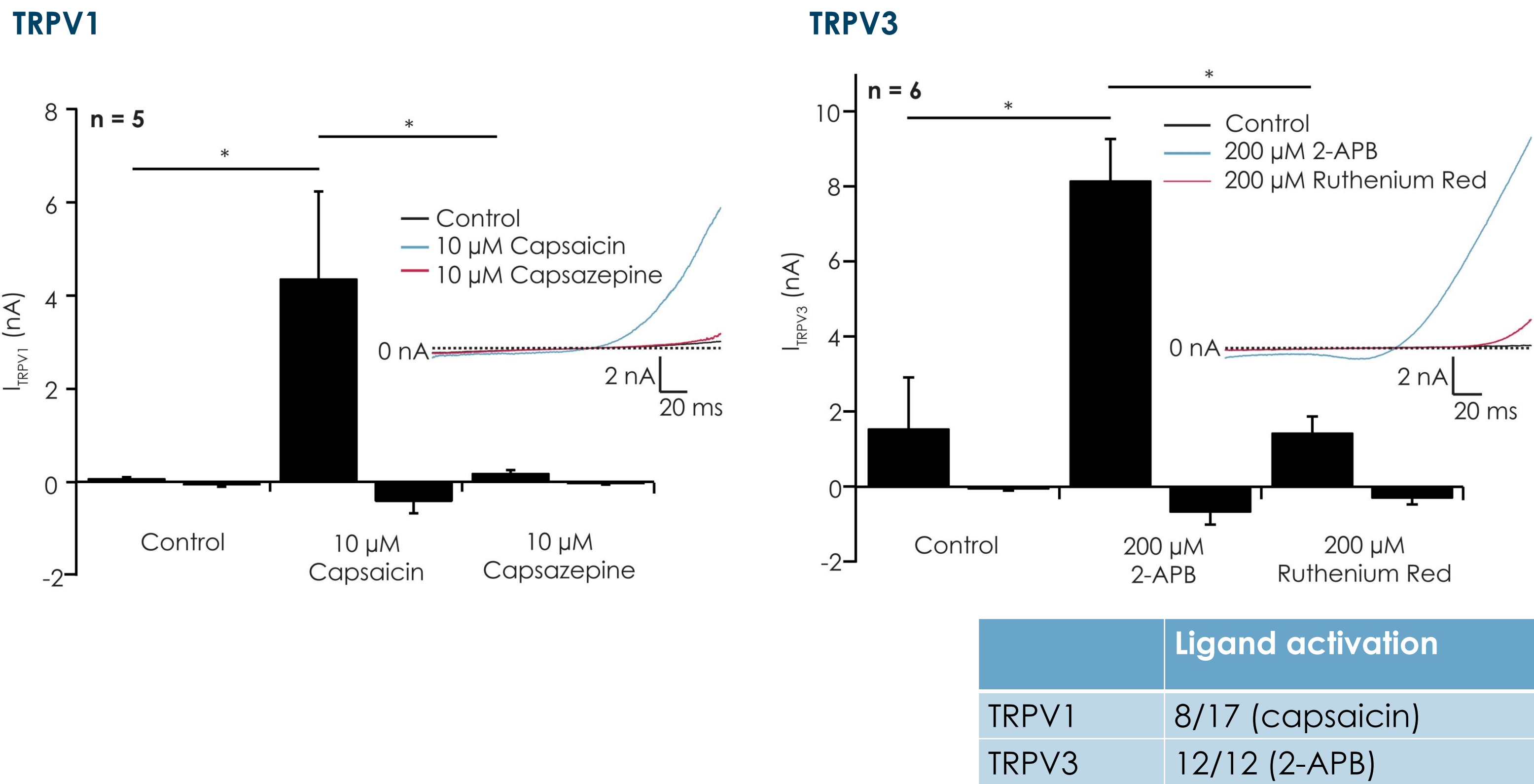


Figure 4: Activation and inhibition of TRPV1 and TRPV3 on the Patchliner. TRPV1 expressed in CHO cells was activated by capsaicin and blocked by capsazepine (left). TRPV3 was activated by 2-APB and blocked by ruthenium red on the Patchliner (right). TRPV1-mediated currents could be activated by capsaicin in 8/17 cells tested. TRPV3 was activated by 2-APB in all 12/12 cells tested.

Heat activation of TRPV1 and TRPV3

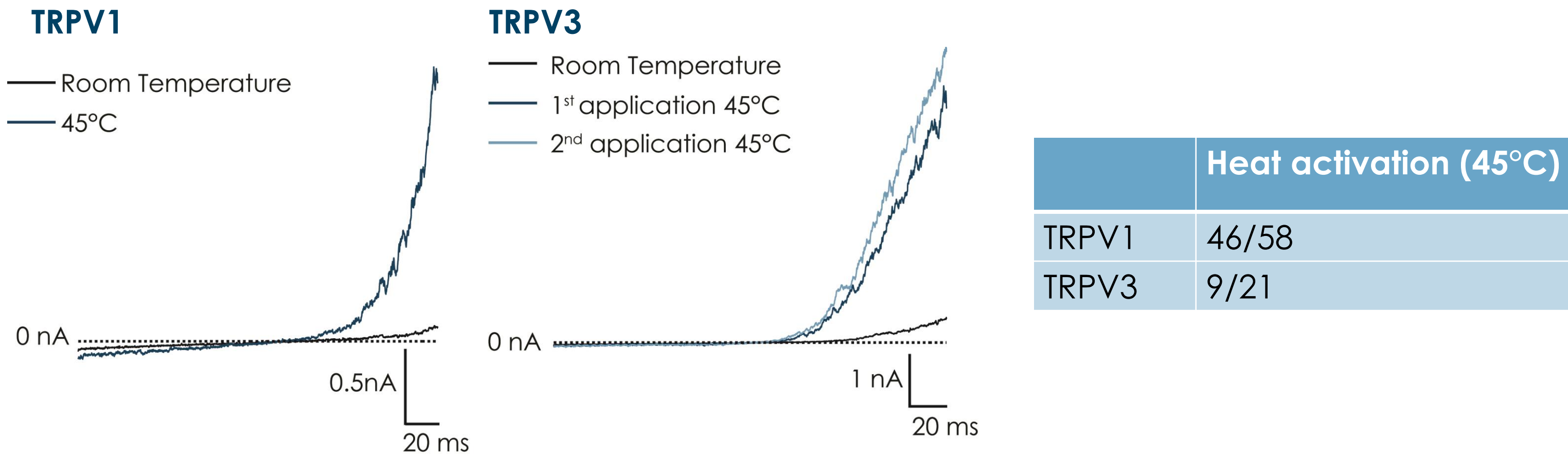


Figure 5: TRPV1 and TRPV3 were activated by heated solution. TRPV1 expressed in CHO cells was activated by heated solution (45°C) in 46/58 cells tested (left). TRPV3 expressed in CHO cells was activated by heated solution in 9/21 cells tested (right). Upon repeated activation with heated solution, currents became larger. This sensitization of TRPV3 to repeated ligand or temperature exposure has been reported previously^{10,11} and is a phenomenon we have observed in previous experiments¹².

Summary

- TRP channels can be reliably measured using automated patch clamp.
- TRPM8 was activated by cold solution and this was blocked by capsazepine on the Port-a-Patch.
- TRPA1 was robustly activated by carvacrol on a high throughput APC device (SyncroPatch 384i) with an EC_{50} in good agreement with the literature. TRPA1-mediated responses were blocked by A967079 and AMG0902 as expected.
- TRPV4 was activated by GSK1016790A and blocked by GSK2193874 on the Patchliner. TRPV4 was also moderately activated by heated solution (45°C).
- TRPV1 was activated by capsaicin and heated solution (45°C) and blocked by capsazepine.
- TRPV3 was activated by 2-APB and heated solution (45°C). The 2-APB activated response was blocked by ruthenium red.
- TRPV3 sensitized upon repeated activation by heated solution.

References

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¹² Heat activation of TRPV3 on Nanion's Patchliner
<https://www.nanion.de/en/products/patchliner/137-home/articles/473-trpv3-heat-activation-of-trpv3-on-nanion-s-patchliner.html> (accessed 21.12.2020)

