Pharmacology of transient receptor potential cation (TRP) channels using different activation stimuli

Alison Obergrussberger[1], András Horváth[1], Tom A. Goetze[1], Ilka Rinke-Weiß[1], Nadine Becker[1], Lukas Focke[2], Oliver Wehmeier[2], Ralf Schwandner[3], Niels Fertig[1],

[1]Nanion Technologies, Munich, Germany, [2]acCELLerate, Hamburg, Germany, [3]Assay.Works, Regensburg, Germany

Contact: ali@nanion.de

nan]i[on

assay.works

1 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.

2 TRPM8 – activation by cold solution

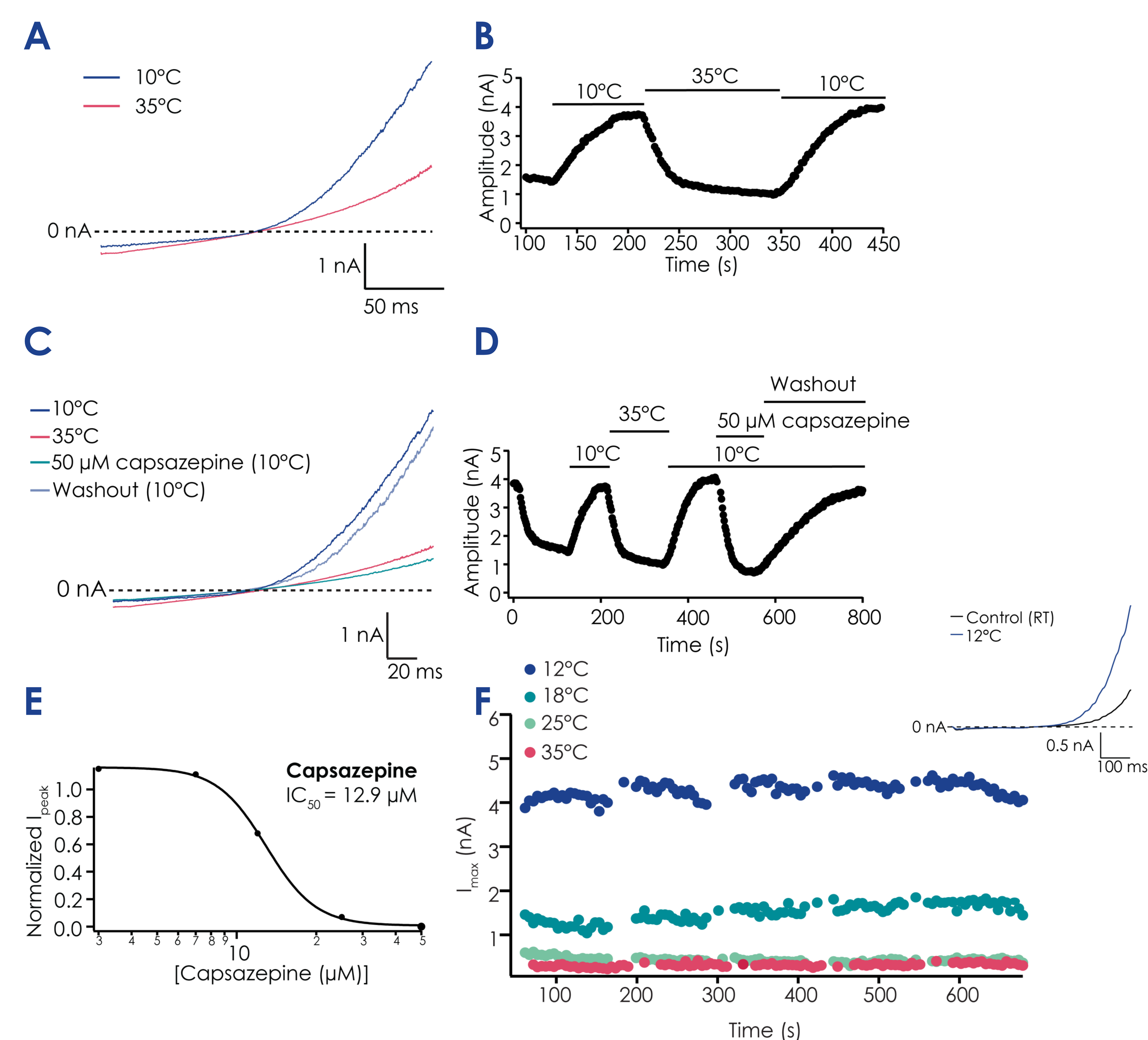
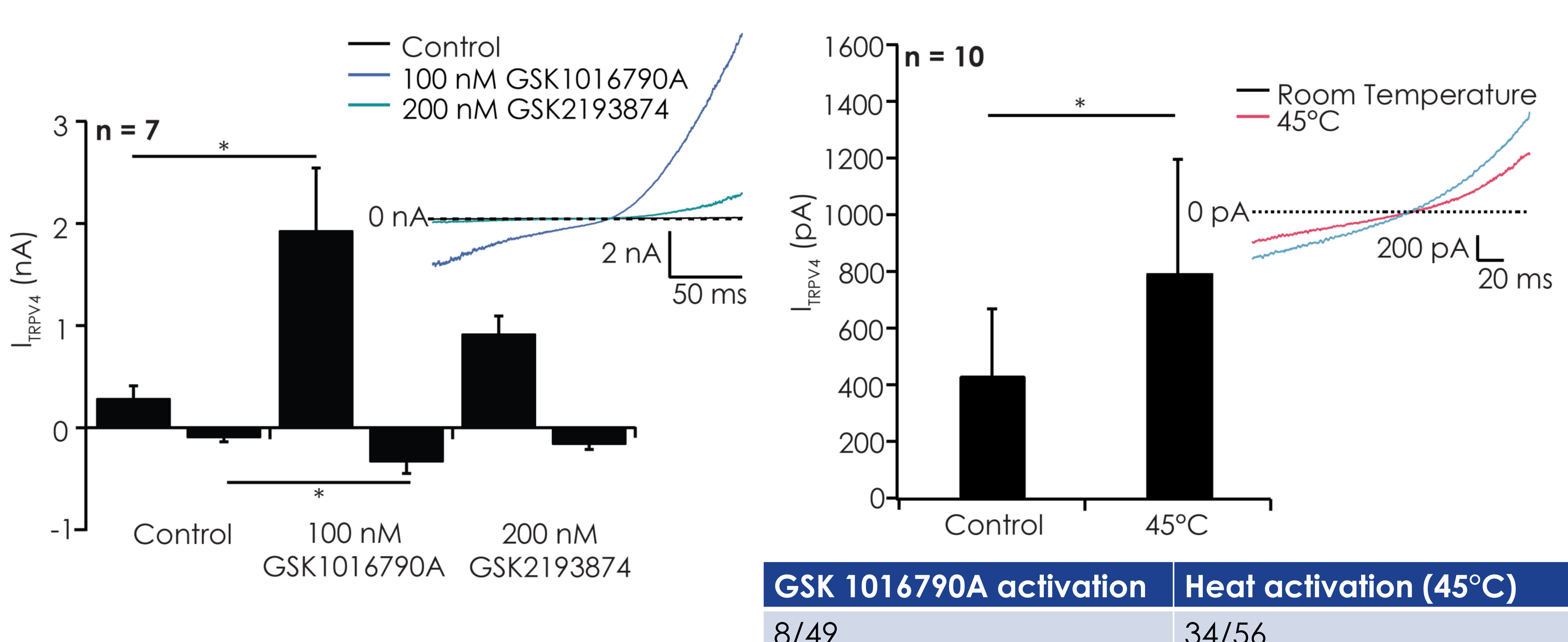


Figure 1: TRPM8 activation by cold solution and block by capsazepine recorded on the Port-a-Patch with temperature controlled external perfusion system. A Activation of TRPM8 expressed in CHO cells using a voltage ramp (-100 mV to +100 mV over 200 ms) by cold solution (10°C; dark blue) and subsequent block by warm solution (35°C; red). **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 (green) or warm solution (35°C; red). TRPM8 was reactivated by cold solution following block by capsazepine (light blue). **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 µM (n = 1) was calculated, in excellent agreement with the literature[1,2]. **F** TRPM8 was also activated by decreasing temperature on the SyncroPatch 384.

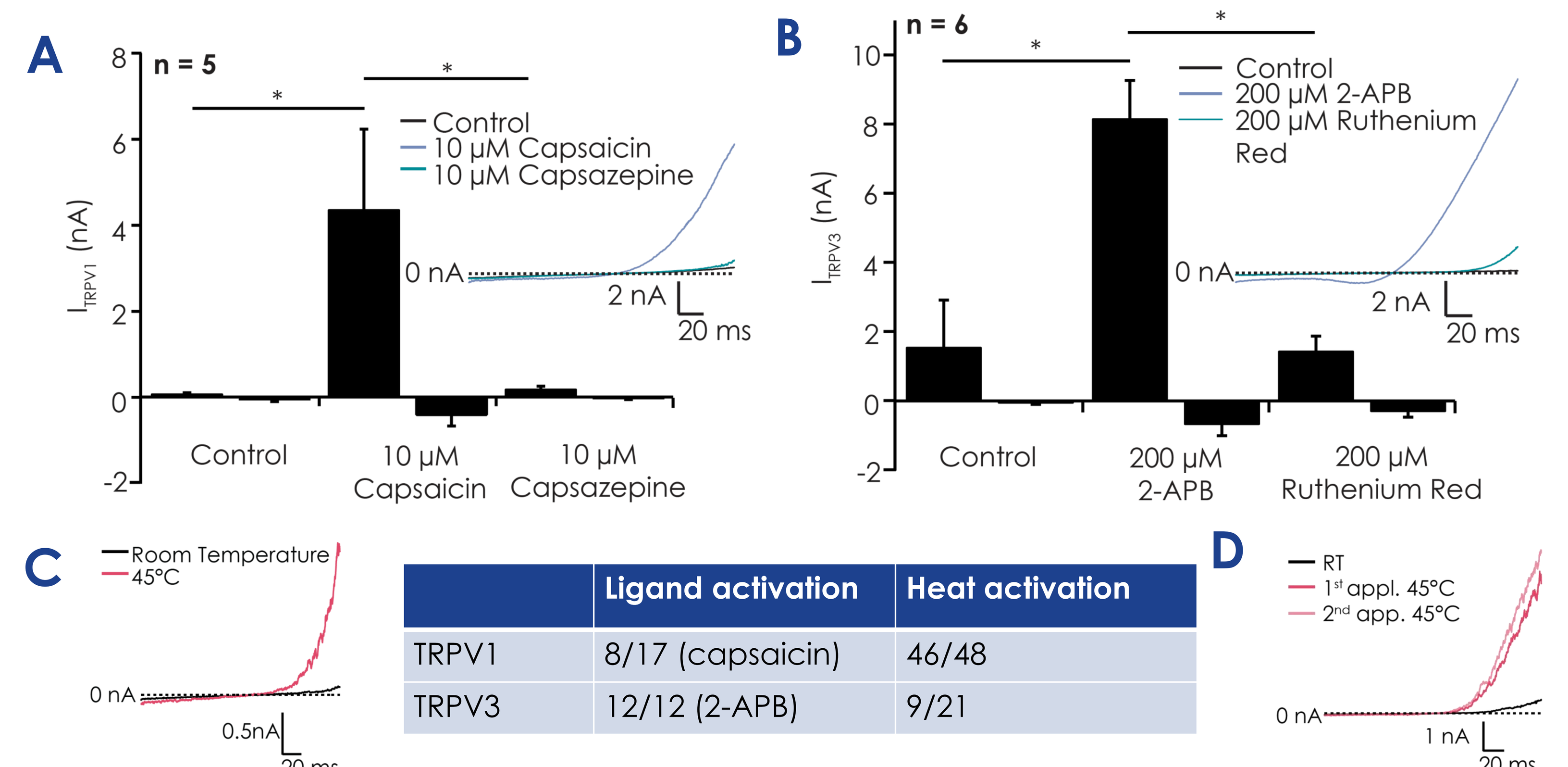
3 TRPV4 – activation and inhibition



GSK 1016790A activation	Heat activation (45°C)
8/49	34/56

Figure 2: TRPV4 activation and inhibition on the Patchliner. A TRPV4 expressed in CHO cells were activated by the specific and potent TRPV4 agonist GSK1016790A[3,4] and this effect could be blocked by GSK2193874, a specific antagonist of TRPV4[5]. **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.

4 TRPV1 and TRPV3 – ligand and heat



	Ligand activation	Heat activation
TRPV1	8/17 (capsaicin)	46/48
TRPV3	12/12 (2-APB)	9/21

Figure 3: Activation and inhibition of TRPV1 and TRPV3 on the Patchliner. A TRPV1 expressed in CHO cells was activated by capsaicin and blocked by capsazepine. **B** TRPV3 was activated by 2-APB and blocked by ruthenium red. TRPV1-mediated currents could be activated by capsaicin in 8/17 cells tested and activated by heated solution in 46/48 cells tested (**C**). TRPV3 was activated by 2-APB in all 12/12 cells tested and activated by heated solution in 9/21 cells tested (**D**).

5 High throughput activation and inhibition of TRPA1

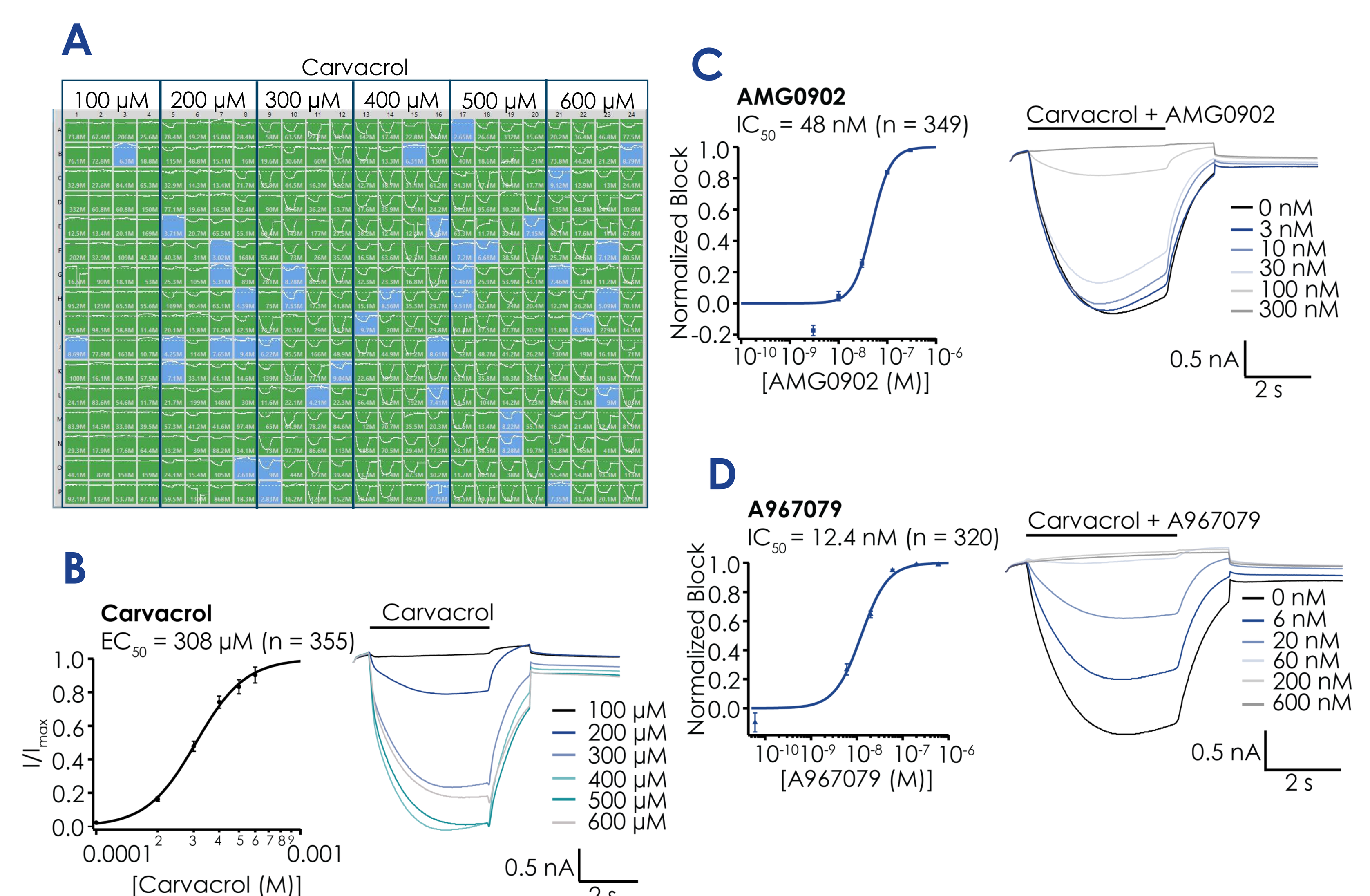


Figure 4: Activation and inhibition of TRPA1 expressed in CHO cell on the SyncroPatch 384. A Graphical user interface of the screening and data analysis software used on the SyncroPatch 384. Screenshot depicts raw data traces of TRPA1-expressing CHO cells as recorded on one NPC-384 patch clamp chip (4 holes). A single concentration of carvacrol was added to each well and the concentration response curve calculated across multiple wells. **B** Average CRC for carvacrol for n = 355 wells (left) and average traces (right). TRPA1 was robustly activated by carvacrol as expected[6,7]. At higher concentrations (above 400 µM) desensitization of the channel was observed (tachyphylaxis) as previously reported[6] which resulted in smaller amplitudes upon repeated application. **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 nM[8]. **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 nM[9]. All experiments were performed in perforated patch mode using multi-hole (4 holes per well) NPC-384 chips.

6 Conclusions

- 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. TRPM8 was also activated by decreasing temperature on the SyncroPatch 384.
- 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.
- TRPA1 was robustly activated by carvacrol on a high throughput APC device (SyncroPatch 384) with an EC_{50} in good agreement with the literature. TRPA1-mediated responses were blocked by A967079 and AMG0902 as expected.

References

[1] Behrendt, H.J., et al., 2004. Br. J. Pharmacol. 141, 737– 745
[2] Malkia, A., et al., 2009. Mol. Pain. 6: 62
[3] Thorneloe, K.S. et al, 2008. J. Pharmacol. Exp. Ther. 326 (2): 432–442
[4] Darby, W.G., et al. 2016. Int. J. Biochem. Cell. Biol. 78: 217.228
[5] Cheung, M., et al. 2017. ACS Med. Chem. Lett. 8: 549−554
[6] Xu, H., et al., 2006. Nat. Neurscci. 9(5): 628-635
[7] Saito, S., et al. 2012. JBC. 287(36): 30743-30754
[8] Chen, J., et al., 2011. PAIN 152: 1165–1172
[9] Lehto, S.G., et al., 2016. Mol. Pain. 12: doi: 10.1177/1744806916677761