# TRP'ING ON QPATCH IN MULTI-HOLE MODE


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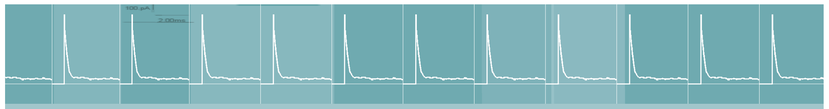
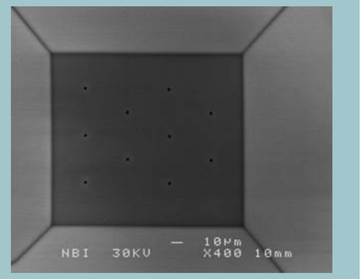


## INTRODUCTION

True giga seal patch clamping can be performed in parallel with QPatch multi-hole technology. This new multi-hole functionality has been tested on three different transient receptor potential ion channels (TRPA1, TRPV1 & TRPM8). All three targets were tested in both single-hole mode and in multi-hole mode with different agonists and antagonists.

The advantages of testing compounds in multi-hole mode will be a minimization of biological variance and an increase in current amplitude, since the current response from several cells are summarized. The disadvantage is lack of serial resistance compensation and in some cases large leak currents.




## MATERIALS AND METHODS

**Cells:** HEK - rTRPV1, HEK - hTRPA1, HEK - hTRPM8

**Ringer's solutions:**
**TRPA1:** IC (in mM): KF 120, KCl, 20, HEPES 10, EGTA 10, pH 7. 2, 300 mOsm EC (in mM): NaCl 155, KCl 5, $MgCl_2$ 1.6, HEPES 10, $BaCl_2$ 2, EGTA 5, glucose 10, pH 7.4, 314 Osm

**TRPV1:** IC (in mM): KF 120, KCl, 20, HEPES 10, EGTA 10, pH 7. 2, 300 mOsm EC (in mM): NaCl 145, KCl 5, $MgCl_2$ 1.75, HEPES 10, $CaCl_2$ 2, EGTA 10, glucose 10, pH 7.4, 310Osm

**TRPM8:** IC (in mM): NaCl 4, KCl 130, $MgCl_2$ 1, HEPES 10, BAPTA (or EGTA) 10, pH 7.4 EC NaCl 150, KCl 4, $CaCl_2$ 0.5, $MgCl_2$ 1, HEPES 10, pH 7.4

**Compounds:**
**A1:** Antagonist: 4-(4-Chlorophenyl)-3-methylbut-3-en-2-oxime (AP-18), supercinnamaldehyde (CA), Ruthenium Red. All compounds from Sigma. Each compound was dissolved in DMSO. The final DMSO concentration did not exceed 0.1%.

**V1:** Agonist: Capsaicin (sigma M2028). Antagonist: Capsazepine (Sigma C191) and Tetrabutylammonium chloride (86870 Aldrich)

**M8:** Agonist: Menthol (Sigma-Aldrich product # M2780. Antagonist: Capsazepine, Cayman Chemical (Cat.no. 10007518).

**Electrophysiology:** Whole-cell patch-clamp experiments were performed on QPatch. Standard and experimental voltage protocols are presented in the figures.

**Data analysis:** Recorded ion channel whole-cell currents were stored in an integrated database (Oracle). Drug effects were analyzed as function of time (I-t plot) and concentration (dose-response relationship). Data analysis was accomplished with the QPatch Assay Software.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## REFERENCE LIST


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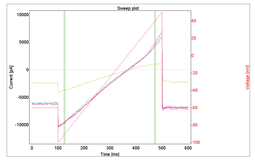
## SUMMARY

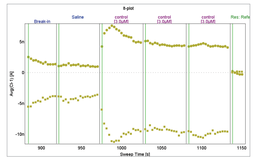

Transient receptor potential channels are activated and regulated by a wide range of stimuli and several different physiological pathways are involved. Automated patch clamping on TRP channels is therefore not trivial. To obtain high quality data assays need to be specially designed to test these targets. For most voltage gated targets an online leak protocol will be sufficient for running experiments in multi-hole mode. However, this is not possible for the TRP channels and other methods need to be applied. Recording of TRP channels in multi-hole mode shows a relative large leak component. By adding a high concentration of reference compound at the end of the experiment this large leak current can automatically be subtracted. This method requires a specific blocker which blocks the TRP current completely.

By testing the three TRP targets in multi-hole mode the total current level increases by a factor of 10. It is therefore an advantage for targets with low expression on this platform. The agonists and antagonists tested in this study have comparable effects on the three targets using multi-hole and single-hole technology. Setting up the optimal assay the QPatch patch clamp technologies are suitable for getting insight to the behaviour of TRP channels.


## TRPA1 MULTI-HOLE

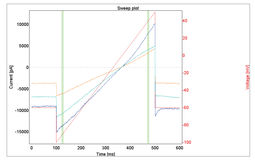
### Supercinnamaldehyde

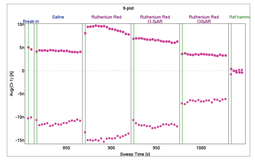



A voltage ramp from -100 mV to +50 mV (duration 400 ms) was executed every three seconds from a holding potential of -60 mV. Supercinnamaldehyde (30 µM) were added through out and the outward current was measured at +40 mV and inward current at -90 or -100 mV. Data were sweep subtracted (the fourth last recorded sweep was subtracted from the others) before further data handling. The data were sampled with a frequency at 2000 Hz and filtered at 800 Hz.

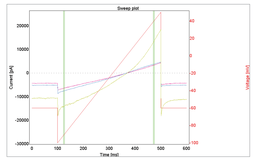
### Ruthenium Red

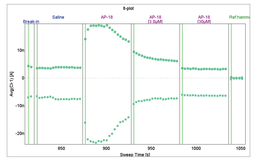



The current was activated by 30 µM of supercinnamaldehyde through out the experiment and a voltage ramp from -100 mV to +50 mV (duration 400 ms) was executed every three seconds from a holding potential of -60 mV and increasing concentrations of Ruthenium Red (3 & 30 µM) were added. Data were sweep subtracted (the fourth last recorded sweep was subtracted from the others) before further data handling. The data were sampled with a frequency at 2000 Hz and filtered at 800 Hz.

### AP-18

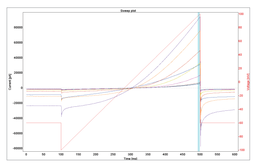


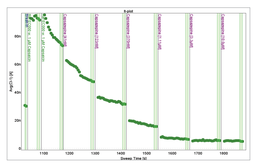



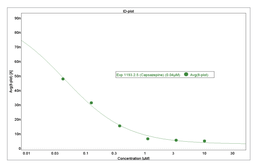

The current was activated by 30 µM of supercinnamaldehyde through out the experiment and a voltage ramp from -100 mV to +50 mV (duration 400 ms) was executed every three second from a holding potential of -60 mV and increasing concentrations of AP-18 (3 & 30 µM) were added. Data were sweep subtracted (the fourth last recorded sweep was subtracted from the others) before further data handling. The data were sampled with a frequency at 2000 Hz and filtered at 800 Hz.

## TRPV1 MULTI-HOLE

### Capsazepine

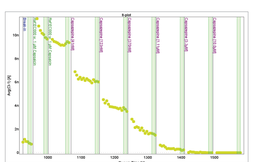


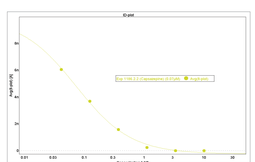




## TRPV1 SINGLE-HOLE

### Capsazepine

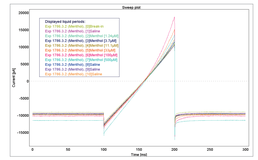



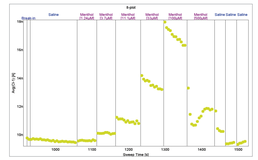



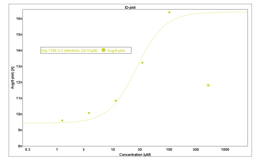

The current was activated with 1 µM capsaicin through out the experiment and a voltage ramp from -100 mV to +100 mV (duration 600 ms) was executed every fifth second from a holding potential of -60 mV. Increasing concentrations of capsazepine were added and the outward current measured at +90. The data was sampled with a frequency at 5 kHz and filtered at 1 kHz.

## TRPM8 MULTI-HOLE

### Menthol

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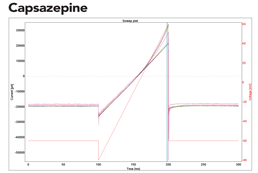
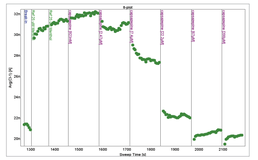
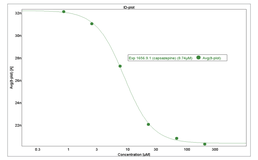
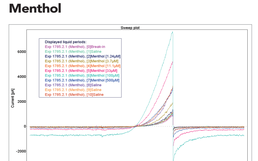


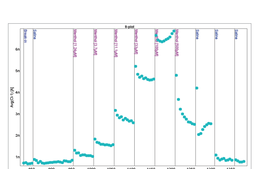



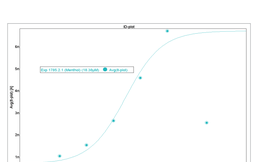




## TRPM8 SINGLE-HOLE

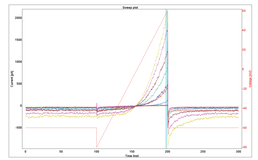
### Menthol

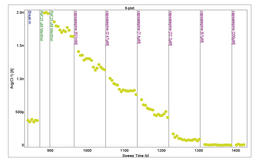


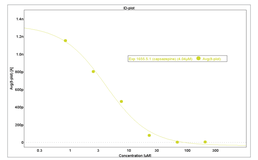




### Capsazepine



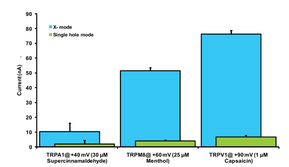

### Menthol

A voltage ramp from -80 mV to +60 mV (duration 100 ms) was executed every fifth second from a holding potential of -60 mV. Increasing concentrations of menthol were added and the outward current measured at +60 mV. The data were sampled with a frequency at 10 kHz and filtered at 3 kHz.

### Capsazepine

The current was activated with 25 µM Menthol through out the experiment and a voltage ramp from -80 mV to +60 mV (duration 100 ms) was executed every fifth second from a holding potential of -60 mV. Increasing concentrations of capsazepine were added and the outward current measured at +60 mV. The data were sampled with a frequency at 10 kHz and filtered at 3 kHz.

## COMPARISON OF SINGLE-HOLE AND MULTI-HOLE RESULTS




Average outward TRP current amplitude in single-hole mode (green) and in multi-hole mode (blue). The current was measured at +40, +60 and +90 mV for TRPA1, TRPM8 and TRPV1, respectively.

| | | Single-hole mode | Multi-hole mode |
|---|---|---|---|
| TRPA1 | Ruthenium Red | n.d. | 20.19±5.37 µM (n=7) |
| | AP-18 | n.d. | 4.53±1.76 µM (n=10) |
| TRPV1 | Capsaicin | n.d. | 7.59±6.15 nM (n=6) |
| | Capsazepine | 30.7±7.77 nM (n=4) | 43.3±12.6 nM (n=8) |
| TRPM8 | Menthol | 99.35±50.43 nM (n=6) | 21.47±1.12 nM (n=10) |
| | Capsazepine | 6.03±1.31 µM (n=3) | 15.56±3.04 µM (n=5) |

$EC_{50}$ and $IC_{50}$ values for different TRP agonists and antagonists were obtained for both multi-hole, and in single-hole mode recordings.