Supplementary Methods

Culture of DRG neurons and intracellular Ca^{2+} imaging

Cultures from DRG neurons were prepared from 10 to 12 week-old wild-type (C57Bl/6J), TREK-1$^{-/-}$, TRAAK$^{-/-}$ and TREK-1$^{-/-}$-TRAAK$^{-/-}$ male mice. After dissection, enzymatic digestion and mechanical trituration, DRG neurons were plated on glass coverslips in 35 mm Petri dishes previously coated with poly-(L)-lysine and cultured for 2-7 days in Ham F12 complemented with 10 % inactivated foetal bovine serum, 1% penicillin/streptomycin and 50 ng/ml nerve growth factor-7S (Sigma). NGF was added to the culture medium to maintain thermo-sensitivity of DRG neurons over time in culture(Babes et al, 2004 ; Munns et al, 2007). DRG cultures from control C56Bl/6j and TREK-1$^{-/-}$, TRAAK$^{-/-}$ or TREK-1$^{-/-}$-TRAAK$^{-/-}$ mice were prepared and cultured in parallel and tested alternatively. For cell imaging, extracellular milieu was (mM) NaCl 135, KCl 5, $MgCl_2$ 1.2, $CaCl_2$ 2, Glucose 10, $NaHCO_3$ 1, Hepes 10, pH adjusted at 7.4 at 30°C. For experiments without external Ca^{2+}, Ca^{2+} was omitted, Mg^{2+} raised to 3 mM and EGTA 50 µM added to the solution. Cells were incubated 30 min at 30°C with Fluo-3-AM 10 µM in the imaging milieu with 1% BSA, 0.2% pluronic acid F-127 then left to recover 30 min at 30°C. Dye loading was calibrated at the end of each experiment with ionomycin 1µM/EGTA procedure. Bath was superfused at 30-31°C and temperature ramps were applied through local perfusion pipes of a CL-100 bipolar temperature controller (Warner instrument, USA) with the thermal-probe positioned near the observation field. Temperature rose from 30 to ~48.2 ± 0.7°C in 40 sec and cooling from 30 to 12.4 ± 0.4°C in 40 sec. Images were captured every 2-5 sec with an EM-CCD camera (Roper Scientific) mounted on a Observer Z.1 microscope (Zeiss, Germany), and Metafluor acquisition software (Molecular Devices). Image analysis was done with Metafluor and purpose built macros for ImageJ analysis software (Rasband W.S., U.S. National Institutes of Health, http://rsb.info.nih.gov/ij/). Based on previous electrophysiological characterization of cultured DRG, neurons were clearly identified as globular shaped cell bodies with a single unipolar protrusion. Because capsaicin 10 µM at 30°C is a potent stimulator of nociceptive neurons, it was applied last before dye calibration to avoid interference with other agonists' responses. A region of interest was drawn around the cell body of small diameter (< 20 µm) neurons and pixel values above background were summed. Fluorescence is expressed as the ratio F/F_0, where F_0 is the average pixels value from the first 10 frames. The threshold for sensitivity was set to 0.2 variation of fluorescence which was always superior to three times the standard deviation of F_0.

Single C-fibres recordings

The isolated skin-saphenous nerve preparation and single-fibre recording technique was used (Reeh, 1986). The skin of the right hind paw with the saphenous nerve of 3 to 4 month old male mice was dissected and kept under laminar superfusion of warm (~30°C) synthetic interstitial fluid (SIF). SIF composition mM : NaCl 107, KCl 3.48, $NaHCO_3$ 26.2, NaH_2PO_4 1.67, $MgSO_4$ 0.69, NaGluconate 9.64, glucose 5.5, sucrose 7.6, $CaCl_2$ 1.53, pH 7.4 with O_2/CO_2 – 95%/5%. Less than ~10% of C-fibres in the skin are mechano-insensitive fibres(Wetzel et al, 2007), therefore we search for the receptive field of an identified C-fibre by mechanical probing of the skin with a glass rod and further characterized the mechano-sensitivity with calibrated von Frey filaments. The search protocol for receptive fields implies that all C-fibres were mechano-sensitive. Conduction velocity was <1.3 ms. Receptive fields were isolated from the surrounding fluid with a thick-walled elrin ring (volume 600-800µl). Heated and cooled SIF was locally perfused inside the elrin ring through custom-made flow-through heater with sets of roller pumps. A stainless steel canula was equipped with a resistive wire (2.5 Ω/m) ending just before the application tip. The insulated wire was connected to a computer-controlled power supply. Heat ramps rose from bath temperature of 30°C to reach 50°C within 20 seconds. The criterion for assigning heat responsiveness to a fibre was a discharge of at least 2 spikes, reproduced during a second test 5 min later. The noxious heat-threshold was considered as the temperature at which the first spike was discharged. In all three mice populations the nerve fibre with the greatest heat response was excluded, because these occasional fibres distorted the population response. The cold stimulus lasted 60s and consisted of two phases, a first linear cooling phase from 30°C to 12°C with an initial cooling rate of ~1.5 °C/s and a second static phase at temperature between 12-10°C. The criterion for assigning cold responsiveness to a fibre was a discharge of at least 3 spikes, whereby the cold threshold was considered as the temperature at first spike during cooling. All results are given as mean ± s.e.m..

Electrophysiology

We used the whole-cell configuration of the patch clamp technique to measure membrane currents (voltage clamp) or membrane potentials (current clamp). Recordings were made at 30°C using a RK-400 amplifier (Bio-Logic Science Instruments) with a 3 kHz low-pass filter. Data were sampled at 10 kHz, digitized by a Digidata 1322A A-D/D-A converter (Axon Instruments) and recorded on computer with pClamp 9 software (Axon Instruments). Patch pipettes (1-4 MΩ) contained (in mM): 135 KCl, 2.5 Na_2-ATP, 2 $MgCl_2$, 2.1 $CaCl_2$, 5 EGTA, 10 HEPES (pH 7.25 with KOH). Solutions were applied to individual patch-clamped cells using a home-made microperfusion system driven by microsolenoid valves (Sirai, Italy) allowing rapid

solution changes through a temperature controller (CL-100, Warner Instruments). The bath solution contained (in mM): 145 NaCl, 5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, glucose 10 mM, 10 HEPES, pH 7.4 with NaOH.

Mice

All experiments were performed on 20–24 g male mice of the N10F2 backcross generation to C57Bl/6J congenic strain. The detailed experimental methodology used to generate and genotype TREK1 and TRAAK knock-out mice has been previously described (Heurteaux et al, 2004). TREK1/TRAAK double heterozygote mice were generated by crossing single knock-out mice to each other (N10F1 $^{-/-}$ TREK-1 males to N10F1 $^{-/-}$ TRAAK females). Then TREK-1-TRAAK double knock-outs were obtained by intercrossing double heterozygotes. All mice were acclimatized to the laboratory conditions for at least 1 week prior to testing. They were housed in grouped cages in a temperature-controlled environment with food and water *ad libitum*. The behavioural experiments were performed blind to the genotype, in a quiet room, by the same experimenter for a given test taking great care to minimize or avoid discomfort of the animals.

Real-Time quantitative RT-PCR analysis

Lumbar and thoracic DRGs from 4 male mice12 to 16 weeks old were prepared for RNAs extraction. Total RNAs from DRG of TREK-$1^{+/+}$-TRAAK$^{+/+}$ and TREK-$1^{-/-}$-TRAAK$^{-/-}$ mice were isolated with the NucleoSpin RNA II kit (Macherey Nagel). 2 μg of total RNAs were used for reverse transcription reaction carried out with the Superscript II reverse transcriptase (Invitrogen) according to the protocol of the supplier. Real-time PCR analysis (SYBR green mix Roche) was performed to estimate the level of expression of TRPA1, TRPM8 and TRPV1, the endogenous reference was the cyclophilin D (CycloD). Real-time PCR assays (triplicate for each target gene tested, n=3) were performed in 96-well plates on a light cycler 480SW apparatus (Roche). Data were analyzed using the comparative Ct method where the amount of target was normalized to the cycloD endogenous reference. Primers used for the different amplicons were as follows:

TRPA1-Forward AGGTGATTTTTAAAACATTGCTGAG ;
TRPA1- Reverse CTCGATAATTGATGTCTCCTAGCAT;
TRPM8- Forward GTGTCTTCTTTACCAGAGACTCCAAGGCCA ;
TRPM8- Reverse TGCCAATGGCCACGATGTTCTCTTCTGAGT;
TRPV1-Forward CGTGCACTCCTCCCTTTATGA;
TRPV1-Reverse CGATCACCTCCAGCACTGAA;
CycloD forward GGCTCTTGAAATGGACCCTTC;
CycloD reverse CAGCCAATGCTTGATCATATTCTT.