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MOLECULAR TOXICOLOGY

The involvement of TRP channels in sensory irritation: a mechanistic approach toward a better understanding of the biological effects of local irritants

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Abstract Peripheral nerves innervating the mucosae of the nose, mouth, and throat protect the organism against chemical hazards. Upon their stimulation, characteristic perceptions (e.g., stinging and burning) and various reflexes are triggered (e.g., sneezing and cough). The potency of a chemical to cause sensory irritation can be estimated by a mouse bioassay assessing the concentration-dependent decrease in the respiratory rate (50 % decrease: RD_{50}). The involvement of the *N. trigeminus* and its sensory neurons in the irritant-induced decrease in respiratory rates are not well understood to date. In calcium imaging experiments, we tested which of eight different irritants (RD_{50} 5–730 ppm) could induce responses in primary mouse trigeminal ganglion neurons. The tested irritants acetophenone, 2-ethylhexanol, hexyl isocyanate, isophorone, and trimethylcyclohexanol stimulated responses in trigeminal neurons. Most of these responses depended on functional TRPA1 or TRPV1 channels. For crotyl alcohol, 3-methyl-1-butanol, and sodium metabisulfite, no activation could be observed. 2-ethylhexanol can activate both TRPA1 and TRPV1, and at low contractions (100 µM) G protein-coupled receptors (GPCRs) seem to be involved. GPCRs might also be involved in the mediation of the responses to trimethylcyclohexanol. By using neurobiological tools, we showed that sensory irritation in vivo could be based on the direct activation of TRP channels but also on yet unknown interactions with GPCRs present in trigeminal neurons. Our results showed that the potency suggested by the RD_{50} values was not reflected by direct nerve–compound interaction.

Keywords Respiratory rate · Trigeminal neurons · Calcium imaging · Chemosensation · TRP channels · Volatile organic compounds

Introduction

The human chemosensory system plays a primary role in the detection of and the protection against environmental pollutants. Among these pollutants are chemicals used in the working environment, for instance solvents, vapors as well as gases in urban environments or compounds evaporating from household or personal care products (Dinh et al. 2015; Fredriksson et al. 2003; Shusterman 2014). All these compounds share the ability to evoke adverse health effects in the upper airways and the mucous membranes of the eyes. In toxicology, these effects have been termed sensory irritation (Alarie 1973). The avoidance of this local effect is the most important endpoint in the regulation of workplace chemicals (Bruning et al. 2014). The chemosensory biology behind this effect is supposed to be the stimulation of trigeminal nerve endings that innervate the facial skin, the tongue, and the mucosae of mouth and nose. The trigeminal nerve is one important interface of the nervous system with the external environment. Many different

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receptors and ion channels like transient receptor potential (TRPs), acid-sensing ion channels (ASICs), purinergic receptors (e.g., P2X) or two-pore-domain potassium channel (KCNK) are present in terminals of trigeminal sensory neurons and can detect noxious chemicals.

To protect individuals from adverse health effects, Alarie proposed a bioassay (Alarie 1981) to describe the potency of volatile organic compounds (VOCs) causing sensory irritation during standardized inhalational exposure. This animal model, usually performed with mice, is based on the linear relationship between the decrease of respiratory rate and the exposure concentration of a particular chemical. Based on that relationship, the exposure concentration that evokes a 50 % respiratory rate decrease is obtained and termed RD_{50} (Alarie 1981). Nowadays, the RD_{50} is used to describe the potency of different chemicals to evoke sensory irritation (Schaper 1993). This respiratory depression is reported for a large number of VOCs. The involvement of the trigeminal nerve is biologically presumed, but the mechanisms behind this effect are not investigated suitably. Only a few compounds of a database of 150 chemicals have been investigated concerning their potential to activate receptors of the trigeminal nerve. For instance, for the industrial chemical toluene diisocyanate (TDI), the precise mechanism has been studied by in vitro and in vivo experiments (Taylor-Clark et al. 2009). Such descriptions of mechanisms are further reported for other industrial isocyanates and tear gases (Bessac et al. 2009). Nevertheless, these studies are often limited to investigations with single TRPs expressed in heterologous systems. To acquire further insight into biological interactions between irritants and receptors, an in vitro assay based on primary isolated neurons is important to study mechanisms of receptor activation in more detail. Using this in vitro model, which strongly resembles the in vivo situation, will provide the opportunity to investigate the whole receptor repertoire and the interaction among receptors present in the trigeminal ganglia neurons.

The family of multimodal TRP cation channels plays the most important role in trigeminal chemosensation (Caterina et al. 1997; Patapoutian et al. 2003; Story et al. 2003). It has been suggested that irritants can elicit similar sensations as the natural compounds that activate TRP channels expressed in sensory neurons. The TRPA1 receptor is targeted by naturally occurring agonists like isocyanates of wasabi, most notably allyl isothiocyanate (AITC) and by various noxious stimuli implicating a functional role in pain (Jordt et al. 2004; Uchida et al. 2012). Furthermore, Ca^{2+} directly gates TRPA1 or serves as co-agonist (Doerner et al. 2007). The TRPV1 receptor is activated by a variety of stimuli, among them capsaicin, the pungent ingredient of chili, as well as noxious heat (Caterina et al. 1997) or by ethanol (Trevisani et al. 2002). It is also described that different TRP channels are specifically activated by various odorants like vanillin, HTPA, helional, and geraniol (Lubbert et al. 2013).

Stimulation of multimodal trigeminal nerve fibers triggers reflexes like mucus secretion and respiratory depression and initiates the sensation of irritation (Baraniuk and Kim 2007). The involvement of TRP channels in the reduction of respiratory rate has been reported before (Lanosa et al. 2010). This study shows that in $TRPA1^{-/-}$ knockout mice, the reduction of respiratory rate could no longer be observed as described for wild-type mice in response to the electrophilic compounds styrene (75 ppm) and naphthalene (7.6 ppm).

Although it is documented that the RD_{50} is related to the activation of TRP channels, this limited evidence is in strong contrast to the widespread use of this parameter to describe the potency of chemicals to evoke sensory irritation. Especially for the setting of occupational exposure limits (OELs), sensory irritation is an important endpoint for the avoidance of adverse health effects (Bruning et al. 2014). Based on the RD_{50} values, the potency of an irritant to evoke sensory irritation can be described and a tentative OEL may be proposed (Arts et al. 2006; Kuwabara et al. 2007). This prediction is only based on the correlation between RD_{50} values and established OELs (Schaper 1993) without the exact knowledge of cellular mechanisms (e.g., stimulation of TRP channels) underlying the effect of sensory irritation.

In order to identify these activating mechanisms of several chemically diverse irritants with known RD_{50} values, we employed calcium imaging on trigeminal ganglia neurons (TG neurons) of mice (P5) as an in vitro assay. This in vitro assay allows a higher throughput for substance screening in comparison with the in vivo assay. Furthermore, this technique allows the direct investigation of trigeminal activation. By using diverse pharmacological tools, the activated receptors can be identified and examined. For our experiments, test compounds were selected according to published RD_{50} values (Schaper 1993). Often, substances that are structurally related are activating the same receptor (Bautista et al. 2006). Therefore, we also tested whether a structurally related substance with unknown RD_{50} value would have similar effects in our test system.

Understanding the underlying cellular mechanisms of receptor activation and signal transduction pathways of sensory irritation in more detail is very important for the risk assessment of irritants. Therefore, the aims of this study were: (1) to test whether chemicals with a low RD_{50} have a higher potency to activate TG neurons and (2) to understand whether and to which extent TRP channels and/or non-TRP receptors are involved in these responses.

Materials and methods

Animals

Swiss CD1 mice were obtained from Charles River (Cologne, Germany) and JANVIER LABS (Saint Berthevin Cedex, France). Animals were kept in a 12/12 light/dark cycle and offered regular laboratory chow and water ad libitum.

Primary cell culture of trigeminal ganglia neurons of mice

All experiments involving animals were carried out in accordance with the European Union Community Council guidelines. For primary cell cultures, trigeminal ganglia neurons of CD1 mice were dissected. At postnatal day 5 (P5), mice were killed by decapitation. The scull was opened, the brain was removed, and both trigeminal ganglia (TG) were excised. After short washing in PBS+/+ (Invitrogen), TG were collected in Leibovitz medium (L15, Invitrogen) on ice. After preparation, ganglia were transferred to Dulbecco's modified essential medium (DMEM) containing 0.025 % collagenase (type IA, Sigma-Aldrich) and incubated for 45 min in a humidified atmosphere (37 °C, 95 % air humidity, 6 % CO_2). TG were triturated using fire-polished pipettes, and the resulting cell suspension was centrifuged at 800 rpm for 4 min at room temperature. The cell pellet was resuspended in F-12 medium (Invitrogen; 31331-D-MEM/F-12, GlutaMax™) supplemented with 10 % fetal bovine serum (FBS; Invitrogen), 1 % penicillin and 1 % streptomycin. Afterward, 50 µl of cell suspension was plated on poly L-lysine-coated glass coverslips (14 mm, Thermo Scientific) and placed in 24-well cell culture plates. Cells were kept in humidified atmosphere (37 °C, 95 % air humidity, 6 % CO_2) for 1 h. Subsequently, 500 µl of F-12 medium supplemented with 10 % FBS, 1 % penicillin and 1 % streptomycin was added. Until use, cells were kept in an incubator (37 °C, 95 % air humidity, 6 % CO_2).

Calcium imaging experiments

Thirty to 45 min before starting an experiment, cells were incubated with the Ca^{2+}-sensitive fluorescence dye fura-2AM (Tocris, final concentration: 3 mM) (Grynkiewicz et al. 1985). Coverslips were then transferred into an inert measuring chamber and placed under an inverted microscope (Leica DMI 6000 B microscope) equipped with a fluorescence-optimized 20-fold Leica objective. Loaded cells were excited alternately at wavelengths of 340 nm (80 ms) and 380 nm (20 ms). Emitted light (510 nm) was detected via a monochrome charge-coupled device (CCD) camera (DFC 360 FX). Changes of intracellular calcium levels were calculated as the ratio of emission at 510 nm for both excitation wavelengths (f340/f380). During each experiment, cells were constantly superfused with standard extracellular buffer (NaCl 140 mM, KCl 5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM, HEPES 10 mM; pH 7.4), which could instantaneously be exchanged by different test solutions using a custom-made 8-in-1 miniaturized gravity-driven application system. Viability and neuronal character of the cells were verified by a short pulse (5 s) of buffer containing 45 mM potassium (NaCl 100 mM, KCl 45 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM, HEPES 10 mM; pH 7.4) at the end of each experiment.

Irritants were applied for 10 s with an interstimulus interval of 3 min. For blocker experiments, the antagonist was washed in 20 s prior to application of the irritant.

Each experiment is based on a data set from a minimum of three independent biological replicates including at least three technical replicates and was increased (e.g., 100 µM 2-EH) up to 13 biological replicates if necessary.

Chemicals

Irritants were prepared in a concentrated stock solution in dimethyl sulfoxide (DMSO), *aqua dest*., or polyethylene glycol and were diluted to their final concentration in standard extracellular buffer with a maximum final solvent concentration of 1 % DMSO. In experiments, 1 % DMSO was used as solvent control. Chemicals were purchased from Sigma-Aldrich or Tocris.

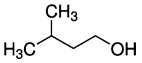
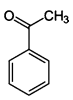
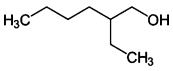
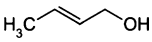
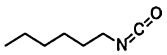
Data analysis and statistics

Calcium imaging data were analyzed with the Leica LAS AF 600 software (version: 4.0.0.11706). Baseline Ca^{2+} levels (mean of 10 measures prior stimulation) and response amplitudes were calculated using Excel 2011 (for Mac). The responses and their respective amplitudes were calculated from baseline. A threshold criterion of baseline Ca^{2+} level plus 4 times the baselines standard deviation (4× σ criterion) was used to distinguish responses from unspecific baseline fluctuation.

Efficacy of the unspecific and specific TRP channel blockers was calculated by comparing the number of TG neurons responding to the 1st and 2nd stimulation with and without blocker. Since tachyphylaxis was observed in the control experiments, the calculation of the blocker efficacies was corrected for this systematic variation of the response frequencies after repeated stimulation.

The statistical data analyses were performed using SPSS (version 22 IBM). A repeated-measures analysis of variance (rANOVA) was used to analyze the effect of different treatments on the response amplitude. Application of a

Table 1 List of all test compounds sorted according to their published RD_{50} values

Substance	CAS no	Structure	RD_{50} ppm	Vapor pressure at 25 °C (mmHg)
3-Methyl-1-butanol	123-51-3		729.0	3.5
Acetophenone	98-86-2		102.0	0.9
Sodium metabisulfite	7681-57-4	$Na_2S_2O_5$	49.8	Solid
2-Ethylhexanol	104-76-7		44.0	<7
Isophorone	78-59-1		27.8	0.4
Crotyl alcohol	6117-91-5		8.9	1.8
Hexyl isocyanate	2525-62-4		4.8	0.7
Trimethylcyclohexanol	116-02-9		–	0.1 (20 °C)

stimulus was the repeated-measures factor and treatment the between-subjects factor. The response amplitude was treated as dependent variable, while the treatment groups were handled as the independent variable. Amplitudes were expressed as % of first response amplitude. According to the levels of measurement, F values were used to determine the significance of the treatment effect. Statistical significance was set at $p \leq 0.05$, and multiple comparisons were adjusted by Bonferroni correction.

A generalized linear model (GENLIN) was used to analyze the effect of different treatments on the number of responding neurons. Wald χ^2 values were used to determine the significance of the treatment effect. By using a Wald-type 95 % confidence interval, a pairwise comparison of the number of responsive neurons (binary variable) to the control condition was performed. In order to evaluate differences between variances of the treatment and the control groups, the least significant difference (LSD) test was used as post hoc comparison method.

Figure design was performed with CorelDRAW Essentials X5 and Prism GraphPad (version 5 for Mac).

Results

Do RD_{50} values predict the activation of trigeminal neurons in vitro?

To select compounds for the investigation of sensory irritation via the activation of TG neurons in vitro, we used a database of 150 volatile organic chemicals (VOCs) for which sensory irritation potency (RD_{50}) has been reported in vivo in mice (Schaper 1993). Based on their published RD_{50} values, 7 compounds (Table 1) were selected and tested with respect to their potential to activate TG neurons. As a structurally related compound to isophorone (trimethylcyclohexanone), the alcohol trimethylcyclohexanol (no RD_{50} available) was also selected (last line Table 1).

To avoid evaporation of the chemicals during experiments, only substances with moderate vapor pressure were tested. For these test substances, neither the activation of the *N. trigeminus* nor the activation of receptors expressed in TG neurons has been described to date.

We examined concentration–response relationships by testing all substances in concentrations of 100 µM, 1 mM and 10 mM and applied them thrice for 10 s with an interstimulus interval of 3 min to confirm the robustness and validity of the elicited responses (see exemplary traces in Fig. 1). According to this stimulation protocol, five of the eight tested irritants reproducibly and repetitively activated TG neurons while the others did not elicit any Ca^{2+} influx (Fig. 1). Displayed are representative traces of cellular responses elicited by stimulation with concentrations that produced robust responses (≥20 % responsive TG neurons). For the substances that failed to activate TG neurons, the representative traces for the highest tested concentration are shown (Fig. 1).

We observed reproducible and concentration-dependent Ca^{2+} responses upon stimulation with acetophenone (RD_{50} 102.0 ppm), isophorone (RD_{50} 27.8 ppm), hexyl isocyanate (RD_{50} 4.8 ppm), 2-ethylhexanol (RD_{50} 44.0 ppm), and trimethylcyclohexanol (RD_{50} untested). Sodium metabisulfite (49.8 ppm), crotyl alcohol (8.9 ppm), and methylbutanol (729.0 ppm) did not activate TG neurons in our experiments (Table 2).

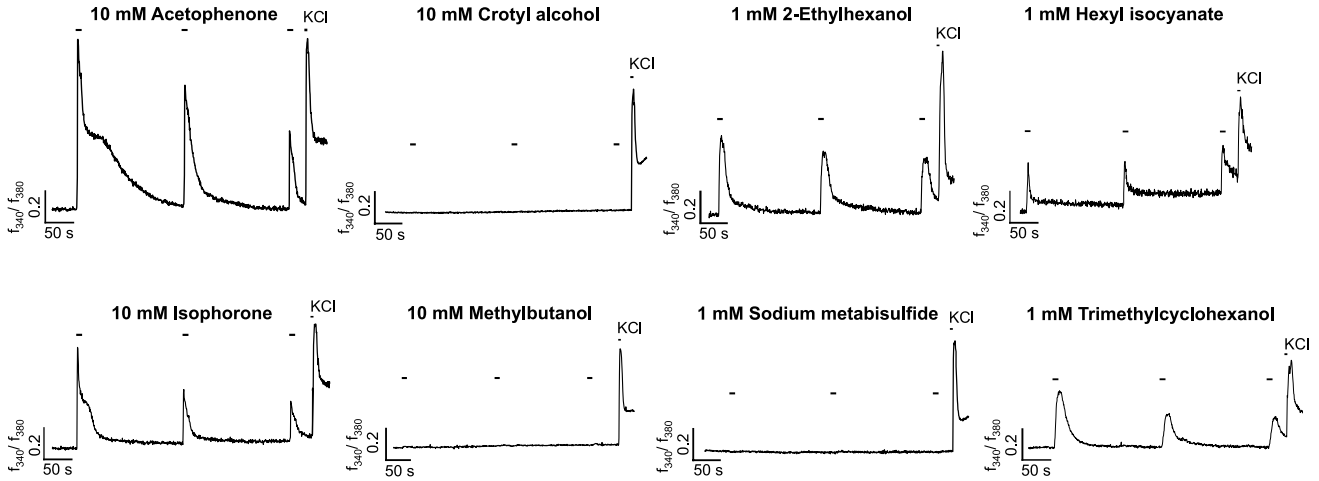


Fig. 1 Activation of TG neurons of mice (P5) by irritants. Ca^{2+} levels were measured by fura-2AM fluorometry. Shown are representative traces of Ca^{2+} imaging experiments from TGNs responding to 10 mM acetophenone, 1 mM 2-ethylhexanol, 1 mM hexyl isocyanate, 10 mM isophorone and 1 mM trimethylcyclohexanol. In contrast, 1 mM sodium metabisulfite, 10 mM crotyl alcohol and 10 mM methylbutanol failed to activate the TG neurons

Table 2 Concentration-dependent activation of TG neurons of mice (P5) by putative irritants

Substance	100 µM	1 mM	10 mM
3-Methyl-1-butanol	×	×	×
Acetophenone	×	3.2 %	42.6 %
Sodium metabisulfite	×	×	–[a]
2-Ethylhexanol	8.8 %	25.3 %	85.6 %
Isophorone	2.7 %	11.8 %	51.2 %
Crotyl alcohol	×	×	×
Hexyl isocyanate	13.8 %	27.4 %	–[b]
Trimethylcyclohexanol	5.2 %	24.8 %	–[a]

Shown are percentages of neurons responding to application of irritants in calcium imaging experiments

[a] Not tested due to insolubility at this concentration

[b] Not tested due to loss of cell viability

Interestingly, we could not observe the expected association between the RD_{50} of a given substance and its potential to activate TG neurons. When comparing the different compounds with respect to their RD_{50} values, we could show that methylbutanol, with the highest RD_{50} of the tested substances (729.0 ppm), did not activate TG neurons at all. In contrast, acetophenone, which also has a relatively high RD_{50} of 102.0 ppm, showed a robust concentration-dependent activation of TG neurons (Table 2). The application of 10 mM acetophenone activated 42.6 % (388/910) of the TG neurons with a mean amplitude of $f_{340}/f_{380} = 0.29 \pm 0.02$. In contrast, sodium metabisulfite, with an intermediate RD_{50} value of 49.8 ppm, did not activate TG neurons (0/95). Moreover, 2-ethylhexanol with a RD_{50} of 44.0 ppm, which is comparable to that of sodium metabisulfite, was the most potent activator of all tested irritants in this study. The number of responding cells increased concentration-dependently from 8.8 % (59/672) by application of 100 µM to 25.3 % (91/360) by application of 1 mM and to 85.6 % (125/246) by application of 10 mM (Table 2). The substance isophorone, which has a relatively low RD_{50} (27.8 ppm), showed a concentration-dependent activation of TG neurons even at the lowest concentration. Application of 100 µM evoked responses in 2.7 % of TG neurons, 1 mM elicited responses in 11.8 % of the cells, and application of 10 mM isophorone elicited robust activation of 51.2 % (316/617) of the TG neurons with $f_{340}/f_{380} = 0.65 \pm 0.03$. In contrast to these robust responses to the test compound with intermediate RD_{50} value, 10 mM crotyl alcohol with an RD_{50} of 8.9 ppm completely failed to activate the TG neurons of mice (0/213).

The substance hexyl isocyanate (HIC) showed a low RD_{50} value in in vivo experiments (4.8 ppm). In our in vitro experiments, it activated TG neurons concentration-dependently. Upon application of 100 µM HIC, we observed responses in 13.8 % (24/174) of the cells, which increased to 27.4 % (90/328) by the application of 1 mM. The mean response amplitude significantly increased from $f_{340}/f_{380} = 0.18 \pm 0.02$ to $f_{340}/f_{380} = 0.26 \pm 0.02$ ($p \leq 0.05$) upon application of the higher concentration.

Our results strongly indicate that some but not all of the chemicals predicted as sensory irritants may be detected by neurons of the trigeminal nerve. Interestingly, we did not find a clear association between the activation of TG neurons by a given substance and its RD_{50} value.

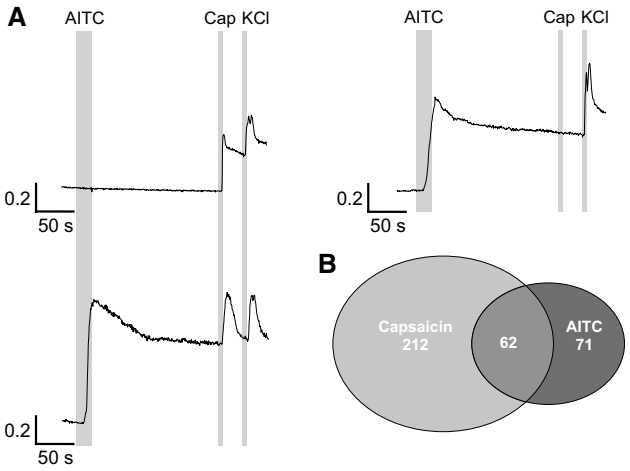


Fig. 2 Responses to the TRPA1 agonist AITC (50 μM) and the TRPV1 agonist capsaicin (1 μM). **a** Traces show typical Ca^{2+} imaging recordings. **b** *Venn plot* showing the overlap of TG neurons responding to both stimuli

Typically, chemoreceptors display a receptive field that is an array of substances by which they are activated (Bautista et al. 2006). Often, the substances of the receptive field are structurally related. In order to investigate the effect of a chemical structurally related to a test chemical used in our study, we tested the substance trimethylcyclohexanol (TMCH), which is the analogous alcohol of the ketone isophorone. Interestingly, TMCH concentration-dependently activated the trigeminal ganglia neurons. Upon the application of 100 μM TMCH, 5.2 % (11/210) of the cells responded with an increase of intracellular Ca^{2+}. At a concentration of 1 mM TMCH, 24.8 % (177/714) of the TG neurons responded to the stimulus. The application of 1 mM TMCH results in an activation of roughly twice as many TG neurons compared to the ketone isophorone.

TRP and non-TRP channels are involved in the detection of solvent irritants

TRP channels are known to detect many substances that elicit irritation by stimulation of trigeminal sensory fibers. Thus, we tested whether members of the TRP channel family would be involved in the detection of the five irritants activating TG neurons in vitro (Table 2). TRP channels typically involved in irritant detection encompass the capsaicin receptor TRPV1 and the TRPA1 receptor that is targeted by, e.g., mustard oil or allicin. First, we investigated the response pattern evoked by the specific agonists capsaicin (1 μM) and AITC (50 μM). We found that 14.9 % of all measured TG neurons of mice (133/892) were sensitive for AITC and 30.7 % were sensitive to capsaicin (274/892) (data not shown). In total, 345 AITC- and capsaicin-sensitive cells were analyzed. Upon AITC- and capsaicin-sensitive neurons, we identified three different classes of TG neurons. Namely, cell populations that only responded either to AITC only (20.6 % = 71/345) or to capsaicin only (61.4 % = 212/345) and a third class responding to both AITC and capsaicin (18 % = 62/345). Therefore, this third class of TG neurons likely expresses both receptors TRPA1 and TRPV1 (Fig. 2).

Both TRP channels can be inactivated by the unspecific TRP channel blocker ruthenium red (RR). We stimulated TG neurons with acetophenone, 2-ethylhexanol (2-EH), isophorone, hexyl isocyanate (HIC) and trimethylcyclohexanol (TMCH) in the presence of RR to test for an involvement of TRP channels in the detection of these substances. For these experiments, only the concentration of the compound, which yields a sufficient amount of responsive TG neurons (≥20 %), was applied. Upon repeated stimulation, a decrease from the 1st to the 2nd stimulation with respect to the percentage of responding neurons and the mean amplitudes was observed. This decrease was present in the control as well as in the presence of the blocker and consistently detected by stimulation with the different irritants. Thus, for statistical analysis the 2nd stimulation in the presence and absence of the blocker was compared, to investigate whether the blocker leads to a further reduction of the tested parameter. Additionally, a "blocker efficacy" was calculated by comparing the number of TG neurons responding to the 1st and 2nd stimulation to point out the cumulative reduction of responding cells (Table 3).

Table 3 Summary of blocker experiments in Ca^{2+} imaging measurements

Substance	10 μM RR (%)	5 μM CPZ (%)	30 μM HC (%)	Presumed receptor
Acetophenone	62	12	42	TRPA1
2-Ethylhexanol	43	26	35	TRPA1 and TRPV1
Hexyl isocyanate	100	10	94	TRPA1
Isophorone	55	58	13	TRPV1
Trimethylcyclohexanol	0	–	–	No TRP channel

Shown are efficacies of the tested unspecific and specific TRP channel blockers. The reduction of responding neurons from the 1st stimulation (100 %) to the 2nd stimulation was calculated and termed "blocker efficacy"

In the presence of RR (10 μM), the number of responsive TG neurons and mean response amplitudes from four of the five irritants were reduced from the 1st to the 2nd stimulation but recovered after blocker washout (3rd stimulation). In more detail, the number of responses to 10 mM acetophenone was diminished from 47 to 15 % ($p \leq 0.001$), and the mean response amplitude was significantly decreased to 29.3 ± 4.8 % of the first response amplitude ($p \leq 0.001$) (Fig. 3a, f, k). The number of responses to 1 mM 2-EH was decreased from 27 to 10 % ($p \leq 0.001$) 2-EH sensitive TG neurons, and the second response amplitude was reduced from 58.7 ± 5.1 % to 22.8 ± 6 % ($p \leq 0.001$) (Fig. 3b, g, l). The responses to 1 mM HIC were completely abolished in the presence of RR (Fig. 3c, h, m). The percentage of isophorone (10 mM) sensitive TG neurons was significantly decreased from 61 to 21 % ($p \leq 0.001$), and the response amplitude was reduced to 17.1 ± 3.5 % of the first response amplitude ($p \leq 0.001$) in the presence of RR (Fig. 3d, i, n). Trimethylcyclohexanol evoked responses were not sensitive to RR (Fig. 3e, j, o). Due to biological variations for this compound, the respective control experiments yielded a generally higher response frequency that was also subjected to a strong desensitization across the repeated stimulation (Fig. 3j). Due to the different time courses and responsiveness, a direct statistical comparison as calculated for the other compounds was not possible. Compared to the four other compounds given in Fig. 3, trimethylcyclohexanol did not show the characteristic "U-shaped" pattern, which indicates a complete or partial block due to the coapplication of RR. The number of responsive neurons and the mean amplitude were unaltered upon coapplication with RR (Fig. 3e, j, o).

Our results indicate that functional TRP channels on dissected mouse TG neurons are essential for most of the responses evoked by different solvent irritants.

Acetophenone, hexyl isocyanate, isophorone, and 2-ethylhexanol are novel agonists for TRP channels

Our experiments with the unspecific open-channel blocker RR indicate an involvement of TRP channels in the detection of the test compounds. We next used the specific TRP channel antagonists HC-030031 (HC) and capsazepine (CPZ) to test for an involvement of TRPA1 and TRPV1 in the detection of acetophenone, hexyl isocyanate, isophorone and 2-ethylhexanol, by TG neurons, respectively. Exemplary traces and blocker efficacies are shown in Fig. 4.

In the presence of 30 μM HC-030031, acetophenone-induced responders were significantly diminished from 52 to 28 % ($p = 0.002$), and the mean response amplitude was significantly reduced to 46.4 ± 5.4 % of the first response amplitude ($p \leq 0.001$) (Fig. 4a, d, g). Coapplication of acetophenone with the TRPV1 antagonist CPZ had no effect on the number of responding TG neurons and a slight but significant effect on the resulting response amplitude (Supplementary Figure S1A, D and G). The size of this effect is comparable to the tachyphylaxis seen upon repetitive stimulation of TRP channels, which we observed in our control experiments. These results indicate that most of the acetophenone-induced responses are mediated via the TRPA1 channel. The responses to the substance hexyl isocyanate were abolished by HC-030031 as well. The percentage of responding cells was diminished from 33 to 2 % ($p \leq 0.001$), and mean response amplitude was significantly reduced to 2.8 % ($p \leq 0.001$) of the first response (Fig. 4b, e, h). As shown for many other isocyanates, HIC-induced responses appear to be mediated by TRPA1 as well. The TRPV1 receptor antagonist CPZ had no effect on HIC-induced responses (Supplementary Figure S1B, E and H). In the presence of CPZ, the percentage of TG neurons responding to isophorone decreased from 46 to 15 % ($p < 0.001$). Accordingly, the mean amplitude was decreased to 12.5 ± 3.0 % ($p \leq 0.001$) (Fig. 4c, f, i). In contrast, the TRPA1 channel antagonist HC had no effect on the tested parameters (Supplementary Figure S1C, F and I). These results indicate that isophorone-induced responses are mediated via TRPV1.

Table 3 summarizes the results of the unspecific and specific blocker experiments (Figs. 3, 4, 5; Supplementary Figure S1) for the five compounds that evoked a robust influx of Ca^{2+} in TG neurons (Fig. 1). The reduction by the percentage of responding neurons from the 1st to the 2nd stimulation in the presence of the blocker was calculated as "blocker efficacy." For this calculation, the 1st stimulation was set to 100 %. The "blocker efficacy" includes the decrease caused upon repeated stimulation.

The 2-ethylhexanol-induced responses were decreased in the presence of TRPA1 antagonist as well as in the presence of TRPV1 antagonist (Fig. 5). Because of this difference in the detection of the 2-EH-induced responses, we performed additional experiments to investigate differences in receptor activation compared to the other test compounds.

Special characteristics of responses to 2-ethylhexanol

In human volunteer studies, acute exposure to 2-ethylhexanol provokes *very strong* annoyance (van Thriel et al. 2007), defense mechanisms evoked by the stimulation of the trigeminal nerve (increased blinking frequency) (Kiesswetter et al. 2005), and neurogenic inflammation indicated by increased concentration of the neuropeptide substance P in nasal lavage fluid of the volunteers (van Thriel et al. 2003).

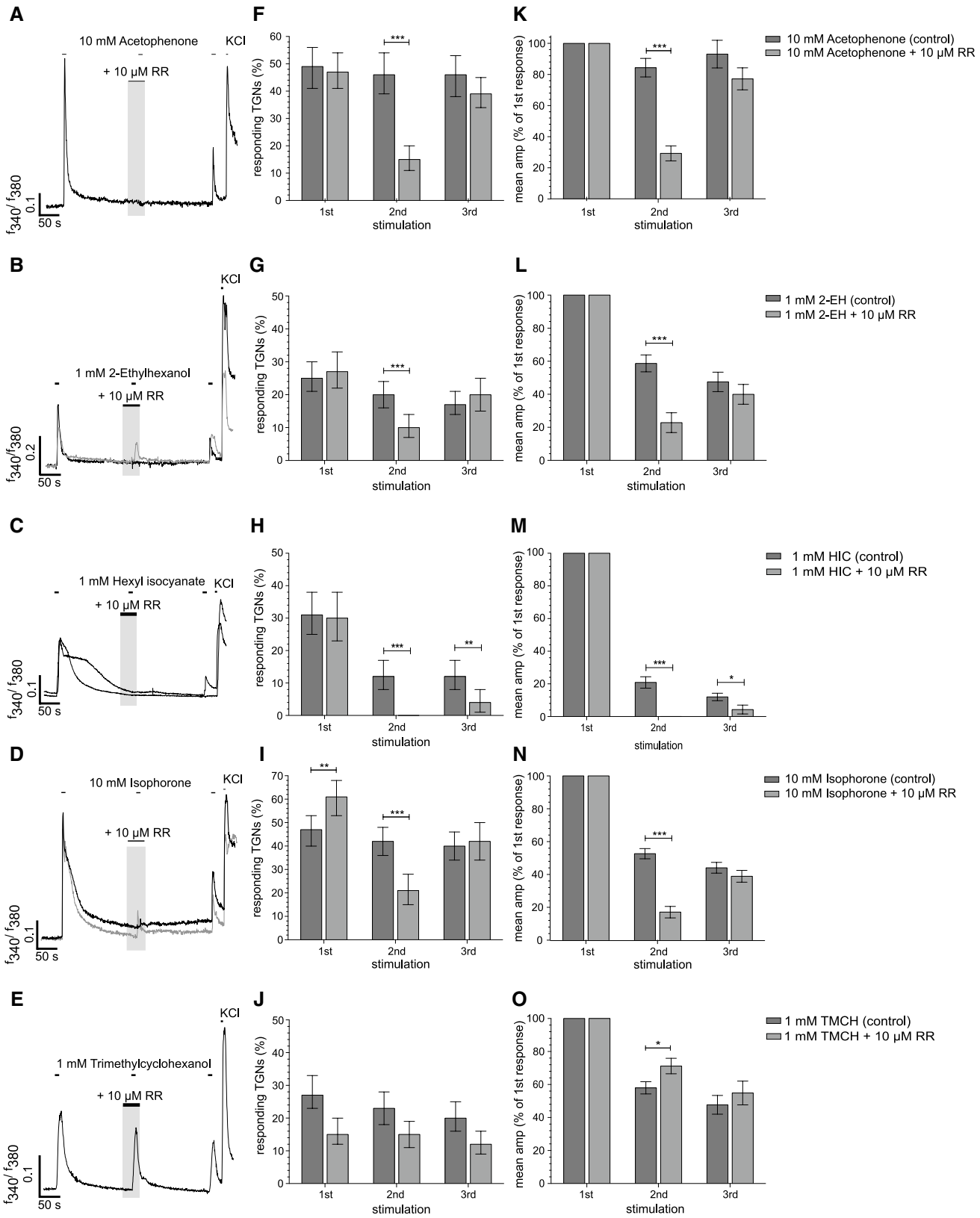


Fig. 3 Involvement of TRP channels in the detection of solvent irritants by TG neurons of mice. **a–e** Representative Ca^{2+} imaging recording showing responses to 10 mM acetophenone, 1 mM 2EH, 1 mM HIC, 10 mM isophorone and 1 mM TMCH in the presence and absence of 10 µM RR. **f–j** Percentage of responding TG neurons and **k–o** mean response amplitudes normalized to the first response. *Bars* in **f–j** show mean ± confidence interval. *Bars* in **k–o** show mean ± SEM. Results of multiple comparisons are indicated as $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$

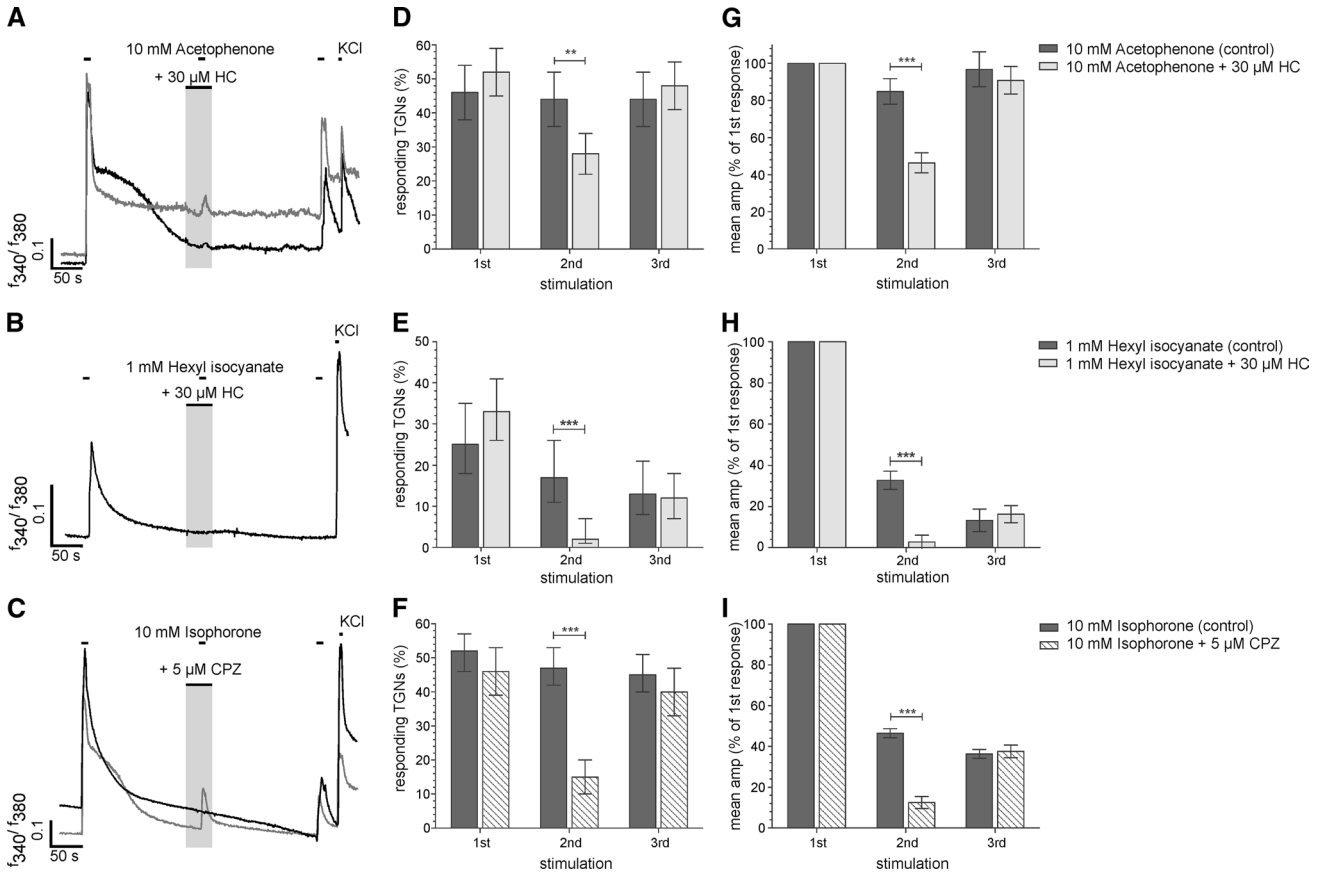


Fig. 4 Identification of TRP channels activated by acetophenone, HIC, and isophorone. **a–c** Representative Ca^{2+} imaging recording from TG neurons responding to 10 mM acetophenone, 1 mM HIC and 10 mM isophorone in the presence of the TRPA1 and TRPV1 blocker. **d–f** responding TG neurons and **g–i** mean response amplitudes as percentage of the first response upon repeated stimulation and in the presence of blocker (2nd stimulation). *Bars* in **d–f** show mean ± confidence interval. *Bars* in **g–i** show mean ± SEM. Results of multiple comparisons are indicated as $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$

With the help of our in vitro studies, we could now show for the first time that 2-EH dose-dependently activates the trigeminal nerve directly. With increasing concentration, more cells responded to the stimulus whereas the mean response amplitude was not affected.

As documented in Table 3, both specific TRP channel blockers markedly reduced the number of responding neurons. Therefore, we suspected the involvement of both TRPA1 and TRPV1 in the mediation of the responses to 2-EH. This is in contrast to the other compounds that appeared to only activate one of the tested TRP channels.

Our results show that the number of responders was reduced from 22 to 10 % in the presence of the TRPA1 antagonist HC and from 24 to 13 % in the presence of the TRPV1 antagonist CPZ. Similarly the mean amplitudes were reduced to 23.8 % ($p \leq 0.001$) and to 38.9 % ($p = 0.021$) in the presence of HC and CPZ, respectively (Fig. 5). These results led us to the question of whether these two TRP channels were the only TRP channels involved in the mediation of the response. By combining the TRPA1 and TRPV1 antagonists, we tested whether there is an additive effect of these blockers. Through the coapplication of TRPA1 and TRPV1 channel antagonists together with 1 mM 2-EH, the number of responsive neurons was reduced to 8 % and the mean response amplitude was diminished to 10 ± 2.9 % of the first response amplitude ($p \leq 0.001$) (Fig. 5d, e). This block of responsive neurons by 68 % is comparable to the observed effect of the unspecific TRP channel block with RR (63 %). The reduction of the mean amplitude by 87.5 % was stronger compared to the reduction by about 81.3 % by RR (Fig. 5e). This finding indicates that no other TRP channel is involved in mediating the 2-EH-induced responses.

According to these results, we suggest that the stimulation with 1 mM 2-EH activates both TRPA1 and TRPV1.

2-EH is activating non-TRP channels at low concentrations

By testing different concentrations of 2-EH, we observed substantial responses of TG neurons even at the lowest tested concentration of 100 µM. Surprisingly, the substance RR

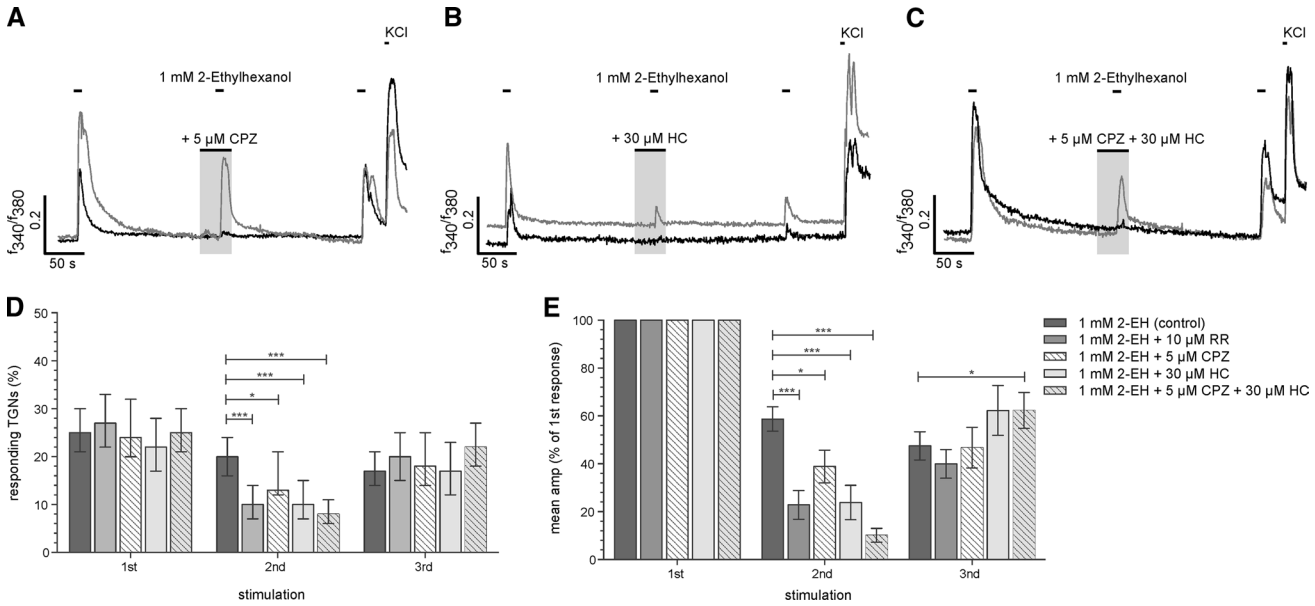


Fig. 5 Involvement of TRPA1 and TRPV1 in the mediation of 2-EH-induced responses. **a**–**c** Representative recordings of Ca^{2+} imaging of TG neurons showing the effect of 5 µM CPZ and 30 µM HC-030031 to 2-EH responses. **d** 2-EH responders and **e** mean response amplitude as percentage of first response by repeated stimulation and by treatment with TRP antagonists (2nd stimulation). *Bars* in **d** show mean ± confidence interval. *Bars* in **e** show mean ± SEM. Results of multiple comparisons are indicated as $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$

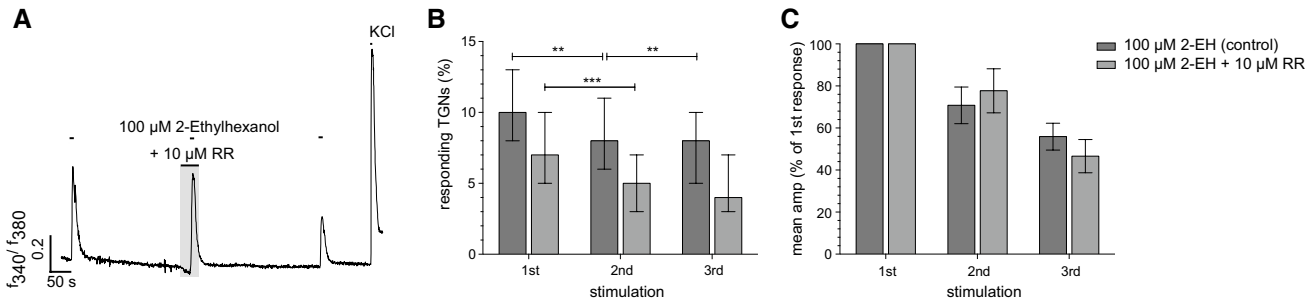


Fig. 6 Involvement of TRP channels in the responses evoked by 100 µM 2-EH. **a** Representative recordings of Ca^{2+} imaging from TG neurons showing the effect of 10 µM RR to responses to 100 µM 2-EH. **b** 2-EH responders and **c** mean response amplitude as percentage of first response by repeated stimulation and by treatment with RR (2nd stimulation). *Bars* in **b** show mean ± confidence interval. *Bars* in **e** show mean ± SEM. Results of multiple comparisons are indicated as $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$

does not block these responses. In contrast, the responses elicited by 1 mM of 2-EH were blocked by RR indicating the involvement of TRP channels (Fig. 6). Thus, at lower concentrations the responses seem not to be mediated by TRP channels. However, we observed a decrease of responding cells and mean amplitude upon repeated stimulation. But this decrease was independently from the RR-treatment as it was also observed for the untreated control neurons (Fig. 6b). This effect is well documented as tachyphylaxis of TRPA1 and TRPV1 receptors (Akopian et al. 2007; Koplas et al. 1997) and is not an effect of RR (Fig. 6b, c).

Irritant-induced responses that are not evoked by the activation of TRP channels could possibly be generated by the activation of G protein-coupled receptors (GPCRs). Signal transduction pathways involving GPCRs cause the activation of second messengers, e.g., phospholipase C (PLC). To investigate typical second messenger signaling cascades of GPCRs with pharmacological tools, diverse drugs that modulate GPCR targets can be used. Therefore, we investigated the involvement of PLC-induced signal transduction. An increase in intracellular calcium can be caused by an influx of calcium from the extracellular space or by the release of calcium from intracellular stores like the endoplasmic reticulum. Thapsigargin is a non-competitive inhibitor of the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) that causes the depletion of intracellular Ca^{2+} stores.

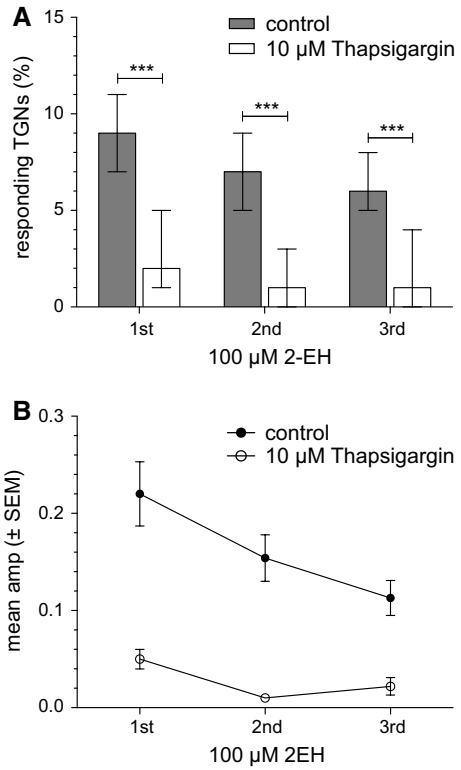


Fig. 7 Involvement of non-TRP channels in the responses evoked by 100 µM 2-EH. **a** TG neurons responding to 100 µM 2-EH. *Gray bars* 2-EH control; *white bars* pretreated with 10 µM thapsigargin. *Bars* show mean ± confidence interval by repeated stimulation. **b** Mean amplitudes ± SEM are shown after pretreatment with 10 µM thapsigargin in comparison with the control amplitudes. Results of multiple comparisons are indicated as $*p \leq 0.05$; $**p \leq 0.01$; $*** p \leq 0.001$

After incubation with 10 µM thapsigargin for 10 min, TG neurons showed almost no response to stimulation with 100 µM 2-EH. This indicates that Ca^{2+} signals evoked by 2-EH involve Ca^{2+} release from intracellular stores (Fig. 7).

We assumed that the cell population of 8.8 % responding to 100 µM 2-EH are the same cell population remaining in the blocker experiment with 1 mM 2-EH and RR (10 %). This cell population probably expresses the same non-TRP channel, which mediates the responses. In a next step, the depletion of intracellular Ca^{2+} stores by thapsigargin and the following application of 1 mM 2-EH in presence of RR led to a complete block of responses (Supplementary Figure S2).

TMCH-induced responses are mediated by non-TRP channels

As described above, the substance TMCH evoked responses that were also not blocked by the unspecific TRP channel blocker RR. Pretreatment with 10 µM thapsigargin showed a highly significant reduction of TMCH-responsive TG neurons to 5 % by the stimulation with 1 mM TMCH (Supplementary Figure S3). As shown for 100 µM 2-EH, the activation of neurons evoked by application of 1 mM TMCH could be mediated mainly by non-TRP channels as well. A possible signal transduction pathway along with the involvement of a PLC second messenger cascade could also play a role. The responses of structurally similar chemicals (TMCH and isophorone) are not mediated by the same receptor. We identified isophorone as TRPV1 agonist (Fig. 4), whereas TMCH is mediated by a yet not identified GPCR.

Discussion

In the present study, we gather convincing evidence that in vivo sensory irritation by well-known irritants is only partly based on the direct activation of trigeminal ganglion neurons. TRP channels as well as other chemical sensing receptors in the membranes of TG neurons are involved in these direct interactions. We could show robust and reproducible concentration-dependent responses to acetophenone, 2-ethylhexanol, hexyl isocyanate, isophorone, and trimethylcyclohexanol. The potency of activating TG neurons directly is not related to the in vivo RD_{50} values of the compounds, and indirect interactions with chemosensory pathways might be involved in the particular breathing response pattern observed in mice. We identified 2-ethylhexanol as a new agonist of TRPA1 and TRPV1. In addition, this compound as well as TMCH might also act via G protein-coupled receptors (GPCRs) located in the membranes of TG neurons. By using a cellular model based on primary chemosensory neurons, we were able to provide new insights into the modes of action underlying these local effects of chemicals in the mucous membranes of the upper respiratory system and the outer eye.

Direct activation of TG neurons by our test compounds

We started with the hypothesis that the potency of irritants to elicit sensory irritation in vivo is directly related to their ability to activate TG neurons of mice. Based on in vivo studies in which the 50 % respiratory rate depression (RD_{50}) of mice was calculated as a sign for sensory irritation, we chose 8 chemically diverse substances for our experiments. Although these chemicals are widely used in both working and home environments, the underlying cellular mechanisms of receptor activation and signal transduction pathways are unknown. TRP channels are highly expressed in sensory neurons and therefore most likely have a part in the detection of airborne chemicals (Caterina et al. 1997; Patapoutian et al. 2003; Story et al. 2003). It

has been suggested that airborne chemicals, which elicit sensory irritation, are detected by the same receptors that are activated by natural compounds that elicit similar sensations. The TRPA1 receptor is naturally targeted by several isocyanates of wasabi, most notably allyl isothiocyanate (AITC) and by various noxious stimuli (Jordt et al. 2004; Uchida et al. 2012). The TRPV1 receptor is activated by a variety of stimuli, among them capsaicin, the ingredient of chili, as well as noxious heat (Caterina et al. 1997) or ethanol (Trevisani et al. 2002). We demonstrated that most of our test compounds, mainly industrial chemicals used as solvents, glues or in the manufacturing of pesticides, target TRPA1 and/or TRPV1 receptors expressed in TG sensory neurons. Thereby our experiments with hexyl isocyanate (HIC) confirmed previous results, which demonstrated that industrial isocyanates specifically target the neuronal TRPA1 receptor (Bessac et al. 2009). HIC is a potent agonist compared to the other test substances and evokes robust responses even at a concentration of 100 µM. Moreover, we could show for the first time that acetophenone activates TRPA1. However, acetophenone emerged to be a weak agonist of TRPA1 because we only observed robust responses at a high concentration of 10 mM. In contrast, hexyl isocyanate in our study and the industrial isocyanates investigated by Bessac et al. (2009) elicited Ca^{2+} influx at micromolar concentrations. Furthermore, we discovered isophorone as a novel TRPV1 agonist. We could show a concentration-dependent activation of TG neurons by isophorone even at a low concentration of 100 µM. Depending on these results, we classify isophorone as a medium activator of the trigeminal nerve. These three irritants seem to specifically activate TRPA1 or TRPV1 expressed in TG neurons of mice. As another novel finding, we discovered that 2-ethylhexanol acts rather unspecifically on TG neuron receptors.

2-Ethylhexanol is a very potent irritant acting via various chemoreceptors

Surprisingly, 2-ethylhexanol, with an intermediate RD_{50} value, was the most potent activator of TG neurons among all irritants tested in this study. We could observe robust responses even by application of relatively low concentrations (100 µM). Furthermore, in contrast to the other trigeminal irritants, 1 mM 2-EH is activating both TRPA1 and TRPV1. Another specific finding was the activation of non-TRP channels at lower concentrations. The responses to 100 µM 2-EH were not blocked by RR in Ca^{2+} imaging experiments. Prior depletion of endoplasmic Ca^{2+} stores with thapsigargin showed the involvement of Ca^{2+} from intracellular stores upon stimulation of TG neurons with 100 µM 2-EH. We conclude that alternative signal transduction mechanisms seem to be involved in the detection of 2-EH at this concentration. Possible candidate receptors are G protein-coupled receptors (GPCRs) that are expressed in sensory nerves and are involved in the detection of noxious, irritant, and inflammatory stimuli (Veldhuis et al. 2015). GPCRs form the largest family of membrane proteins in vertebrates and invertebrates and are activated by a spectrum of structurally diverse substances. More than 800 GPCR sequences are expressed in the human genome, but the physiologic function of most of them is still unknown (Fredriksson et al. 2003). The size of this receptor family and the number of orphan GPCRs make the identification of the receptor activated by 2-EH very challenging. The chemosensory perceptions could also be elicited by olfactory receptors (ORs) that are involved in the detection of volatile compounds present in the environment. ORs are G protein-coupled receptors expressed beside the olfactory epithelium in various tissues including the trigeminal nerve (Busse et al. 2014; Feldmesser et al. 2006; Flegel et al. 2013; Manteniotis et al. 2013; Spehr et al. 2003). The expressions of some GPCRs, potentially involved in the detection of chemicals, like the vomeronasal organ (VNO), Mas-related G protein-coupled receptors (MRGPRs) or formyl peptide receptor-like proteins (FPRs) have been described in TG neurons. FPRs were additionally detected in lung tissue, which leads to the suggestion of an involvement in the trigeminally mediated reflex of respiratory rate depression (Flegel et al. 2015). Most of the test compounds used in this study have a strong and distinct odor, and thus, the involvement of olfactory receptors in the mediation of 2-EH evoked responses is obvious. For one of our test compounds, acetophenone, Jiang et al. reported the activation of 48 ORs (Jiang et al. 2015). Unfortunately, the specific ORs activated by 2-EH and TMCH and their possible expression on TG neurons are unknown. The activation of ORs by the substance TMCH is also possible because no TRP channels are activated and Ca^{2+} is recruited from intracellular stores. The results of this study show that GPCRs, most likely ORs, could be involved in the mediation of responses to volatile irritants as well.

Limitations of the in vitro system for some compounds

The substances methylbutanol, sodium metabisulfite, and crotyl alcohol produced respiratory depression in vivo in mice but failed to activate TG neurons in vitro. Other cellular target organs possibly mediate the sensory irritation induced by these airborne chemicals. TRP receptors are expressed in various other tissues potentially influencing the breathing pattern, e.g., primary sensory neurons innervating the upper and lower airways as well as in dorsal root ganglia which project C-fibers to the airways and lungs (Anand et al. 2008; Geppetti et al. 2014; Nassenstein et al. 2008). Another cell type important for the detection

of irritants and high concentrations of odors is solitary chemosensory cells (SCCs) present in the respiratory epithelium of the nasal cavity (Lin et al. 2008; Tizzano et al. 2010). These SCCs are innervated by trigeminal nerve fibers, and thus, activation can trigger trigeminal reflexes like respiratory depression, even without a direct interaction with trigeminal receptors in the first place. For the detection of chemicals, different elements of the bitter taste signaling cascade, for example bitter taste receptors (Tas2R family), Gα-gustducin, phospholipase Cβ2 (PLCβ2) and TRPM5, are expressed in SCCs (Finger et al. 2003; Kaske et al. 2007; Lin et al. 2008). The bitter taste signaling is typically associated with the detection of potentially harmful substances. It is possible that the activation of receptors expressed in those tissues/cells induces the depression of respiratory rate, which is described by Schaper (1993), whereas TG neurons are unaffected.

For some chemicals (e.g., styrene and naphthalene), local metabolism by cytochrome P450 enzymes in the nasal epithelium seems to be important to activate TRP channels (Lanosa et al. 2010). In vivo, crotyl alcohol is metabolized to crotonaldehyde (Gray and Barnsley 1971), and unsaturated aldehydes are usually strong irritants with low RD_{50} values (Steinhagen and Barrow 1984). Furthermore, acrolein and formaldehyde are known TRPA1 agonists (Macpherson et al. 2007). For some of our test compounds, only weak structural modifications by enzymes that are not present in our in vitro system might be necessary for their conversion into TRP channel agonists.

Low RD_{50} values might also be caused by tissue damage occurring during the assessment of this value in animals. RD_{50} assessments in mice have been reported to cover exposure periods ranging from 1 to 180 min (Kuwabara et al. 2007). During prolonged exposures, the irritants might directly target epithelial cells. Such necrotic cells will produce various signaling molecules (e.g., bradykinin, nerve growth factor, and prostaglandin E2), which can be detected by receptors expressed in the trigeminal nerve endings. Additionally, these signaling molecules are known to modulate the actions of TRP channels (Bhave et al. 2002; Diogenes et al. 2007; Ji et al. 2002; Lopshire and Nicol 1997; Wang et al. 2008; Zhang et al. 2008).

Relevance for occupational safety and health

TRPA1 and TRPV1 together may contribute to chemical hypersensitivity, chronic cough, and airway inflammation in asthma, COPD and reactive airway dysfunction syndrome (Bessac and Jordt 2008). As we could identify some of the tested irritants as TRPA1 and TRPV1 agonists for the first time, inhalational exposure in occupational fields may cause similar effects as described above. The activation of TRPA1 by industrial isocyanates is well documented and has the same effects as those of tear gases and elicits nocifensive responses (Bessac et al. 2009). Therefore, the identification of TRPA1 and TRPV1 agonists by in vitro assays might contribute to the avoidance of adverse health effects after prolonged exposure to these compounds.

Conclusions

In our work, we demonstrated in vitro approaches to characterize trigeminal activation evoked by airborne chemicals. However, the RD_{50} is an important parameter to investigate sensory irritation and has been established for a large number of environmental and industrial chemicals, and the activation of the trigeminal nerve is not proven. With our technique, we have the opportunity to directly measure the nerve–compound interaction of trigeminal ganglia neurons of mice. Additionally, we can investigate mechanisms of receptor activation and a variety of signal transduction pathways with pharmacological targets that are well described for a large number of receptors. Beside the higher throughput for substance screening compared to the in vivo assay, our in vitro assay is more precise with respect to classifying the potency of a given chemical to elicit sensory irritation. The trigeminal nerve innervates the facial skin, mucosae of eyes and nose, and also nerve endings lining the airways. Therefore, irritants can cause a variety of trigeminal reflexes and defense mechanisms like ocular pain, lacrimation, respiratory depression, cough, sneeze, and edema. Identifying activated receptors could help providing adverse health effects, and antagonists of TRP receptors might also provide a useful therapeutic option for cough and migraine (Szallasi et al. 2006). In addition, the results obtained in this work could help predicting adverse health effects and could possibly carry out risk assessment of solvent irritants.

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References

Akopian AN, Ruparel NB, Jeske NA, Hargreaves KM (2007) Transient receptor potential TRPA1 channel desensitization in sensory neurons is agonist dependent and regulated by TRPV1-directed internalization. J Physiol 583(Pt 1):175–193. doi:10.1113/jphysiol.2007.133231

Alarie Y (1973) Sensory irritation by airborne chemicals. CRC Critic Rev Toxicol 2(3):299–363. doi:10.3109/10408447309082020

Alarie Y (1981) Dose-response analysis in animal studies: prediction of human responses. Environ Health Perspect 42:9–13. doi:10.1289/ehp.81429

Anand U, Otto WR, Facer P et al (2008) TRPA1 receptor localisation in the human peripheral nervous system and functional studies in cultured human and rat sensory neurons. Neurosci Lett 438(2):221–227. doi:10.1016/j.neulet.2008.04.007

Arts JH, de Heer C, Woutersen RA (2006) Local effects in the respiratory tract: relevance of subjectively measured irritation for setting occupational exposure limits. Int Arch Occup Environ Health 79(4):283–298. doi:10.1007/s00420-005-0044-9

Baraniuk JN, Kim D (2007) Nasonasal reflexes, the nasal cycle, and sneeze. Curr Allergy Asthma Rep 7(2):105–111. doi:10.1007/s11882-007-0007-1

Bautista DM, Jordt SE, Nikai T et al (2006) TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 124(6):1269–1282. doi:10.1016/j.cell.2006.02.023

Bessac BF, Jordt SE (2008) Breathtaking TRP channels: TRPA1 and TRPV1 in airway chemosensation and reflex control. Physiology 23:360–370. doi:10.1152/physiol.00026.2008

Bessac BF, Sivula M, von Hehn CA, Caceres AI, Escalera J, Jordt SE (2009) Transient receptor potential ankyrin 1 antagonists block the noxious effects of toxic industrial isocyanates and tear gases. FASEB J Off Publ Fed Am Soc Exp Biol 23(4):1102–1114. doi:10.1096/fj.08-117812

Bhave G, Zhu W, Wang H, Brasier DJ, Oxford GS, Gereau RWT (2002) cAMP-dependent protein kinase regulates desensitization of the capsaicin receptor (VR1) by direct phosphorylation. Neuron 35(4):721–731. doi:10.1016/S0896-6273(02)00802-4

Bruning T, Bartsch R, Bolt HM et al (2014) Sensory irritation as a basis for setting occupational exposure limits. Arch Toxicol 88(10):1855–1879. doi:10.1007/s00204-014-1346-z

Busse D, Kudella P, Gruning NM et al (2014) A synthetic sandalwood odorant induces wound-healing processes in human keratinocytes via the olfactory receptor OR2AT4. J Invest Dermatol 134(11):2823–2832. doi:10.1038/jid.2014.273

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389(6653):816–824. doi:10.1038/39807

Dinh TV, Kim SY, Son YS et al (2015) Emission characteristics of VOCs emitted from consumer and commercial products and their ozone formation potential. Environ Sci Pollut Res Int 22(12):9345–9355. doi:10.1007/s11356-015-4092-8

Diogenes A, Akopian AN, Hargreaves KM (2007) NGF up-regulates TRPA1: implications for orofacial pain. J Dent Res 86(6):550–555. doi:10.1177/154405910708600612

Doerner JF, Gisselmann G, Hatt H, Wetzel CH (2007) Transient receptor potential channel A1 is directly gated by calcium ions. J Biol Chem 282(18):13180–13189. doi:10.1074/jbc.M607849200

Feldmesser E, Olender T, Khen M, Yanai I, Ophir R, Lancet D (2006) Widespread ectopic expression of olfactory receptor genes. BMC Genom 7:121. doi:10.1186/1471-2164-7-121

Finger TE, Bottger B, Hansen A, Anderson KT, Alimohammadi H, Silver WL (2003) Solitary chemoreceptor cells in the nasal cavity serve as sentinels of respiration. Proc Natl Acad Sci USA 100(15):8981–8986. doi:10.1073/pnas.1531172100

Flegel C, Manteniotis S, Osthold S, Hatt H, Gisselmann G (2013) Expression profile of ectopic olfactory receptors determined by deep sequencing. PLoS One 8(2):e55368. doi:10.1371/journal.pone.0055368

Flegel C, Schobel N, Altmuller J et al (2015) RNA-seq analysis of human trigeminal and dorsal root ganglia with a focus on chemoreceptors. PLoS One 10(6):e0128951. doi:10.1371/journal.pone.0128951

Fredriksson R, Lagerstrom MC, Lundin LG, Schioth HB (2003) The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Mol Pharmacol 63(6):1256–1272. doi:10.1124/mol.63.6.1256

Geppetti P, Patacchini R, Nassini R (2014) Transient receptor potential channels and occupational exposure. Curr Opin Allergy Clin Immunol 14(2):77–83. doi:10.1097/ACI.0000000000000040

Gray JM, Barnsley EA (1971) The metabolism of crotyl phosphate, crotyl alcohol and crotonaldehyde. Xenobiot Fate Foreign Compd Biol Syst 1(1):55–67. doi:10.3109/00498257109044379

Grynkiewicz G, Poenie M, Tsien RY (1985) A new generation of Ca2+ indicators with greatly improved fluorescence properties. J Biol Chem 260(6):3440–3450

Ji RR, Samad TA, Jin SX, Schmoll R, Woolf CJ (2002) p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. Neuron 36(1):57–68. doi:10.1016/S0896-6273(02)00908-X

Jiang Y, Gong NN, Hu XS, Ni MJ, Pasi R, Matsunami H (2015) Molecular profiling of activated olfactory neurons identifies odorant receptors for odors in vivo. Nat Neurosci. doi:10.1038/nn.4104

Jordt SE, Bautista DM, Chuang HH et al (2004) Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature 427(6971):260–265. doi:10.1038/nature02282

Kaske S, Krasteva G, Konig P et al (2007) TRPM5, a taste-signaling transient receptor potential ion-channel, is a ubiquitous signaling component in chemosensory cells. BMC Neurosci 8:49. doi:10.1186/1471-2202-8-49

Kiesswetter E, Thriel C, Schaper M, Blaszkewicz M, Seeber A (2005) Eye blinks as indicator for sensory irritation during constant and peak exposures to 2-ethylhexanol. Environ Toxicol Pharmacol 19(3):531–541. doi:10.1016/j.etap.2004.12.056

Koplas PA, Rosenberg RL, Oxford GS (1997) The role of calcium in the desensitization of capsaicin responses in rat dorsal root ganglion neurons. J Neurosci Off J Soc Neurosci 17(10):3525–3537

Kuwabara Y, Alexeeff GV, Broadwin R, Salmon AG (2007) Evaluation and application of the RD50 for determining acceptable exposure levels of airborne sensory irritants for the general public. Environ Health Perspect 115(11):1609–1616. doi:10.1289/ehp.9848

Lanosa MJ, Willis DN, Jordt S, Morris JB (2010) Role of metabolic activation and the TRPA1 receptor in the sensory irritation response to styrene and naphthalene. Toxicol Sci Off J Soc Toxicol 115(2):589–595. doi:10.1093/toxsci/kfq057

Lin W, Ogura T, Margolskee RF, Finger TE, Restrepo D (2008) TRPM5-expressing solitary chemosensory cells respond to odorous irritants. J Neurophysiol 99(3):1451–1460. doi:10.1152/jn.01195.2007

Lopshire JC, Nicol GD (1997) Activation and recovery of the PGE2-mediated sensitization of the capsaicin response in rat sensory neurons. J Neurophysiol 78(6):3154–3164

Lubbert M, Kyereme J, Schobel N, Beltran L, Wetzel CH, Hatt H (2013) Transient receptor potential channels encode volatile chemicals sensed by rat trigeminal ganglion neurons. PLoS One 8(10):e77998. doi:10.1371/journal.pone.0077998

Macpherson LJ, Xiao B, Kwan KY et al (2007) An ion channel essential for sensing chemical damage. J Neurosci Off J Soc Neurosci 27(42):11412–11415. doi:10.1523/JNEUROSCI.3600-07.2007

Manteniotis S, Lehmann R, Flegel C et al (2013) Comprehensive RNA-Seq expression analysis of sensory ganglia with a focus on ion channels and GPCRs in Trigeminal ganglia. PLoS One 8(11):e79523. doi:10.1371/journal.pone.0079523

Nassenstein C, Kwong K, Taylor-Clark T et al (2008) Expression and function of the ion channel TRPA1 in vagal afferent nerves innervating mouse lungs. J Physiol 586(6):1595–1604. doi:10.1113/jphysiol.2007.148379

Patapoutian A, Peier AM, Story GM, Viswanath V (2003) ThermoTRP channels and beyond: mechanisms of temperature sensation. Nat Rev Neurosci 4(7):529–539. doi:10.1038/nrn1141

Schaper M (1993) Development of a database for sensory irritants and its use in establishing occupational exposure limits. Am Ind Hyg Assoc J 54(9):488–544. doi:10.1080/15298669391355017

Shusterman D (2014) Occupational irritant and allergic rhinitis. Curr Allergy Asthma Rep 14(4):425. doi:10.1007/s11882-014-0425-9

Spehr M, Gisselmann G, Poplawski A et al (2003) Identification of a testicular odorant receptor mediating human sperm chemotaxis. Science 299(5615):2054–2058. doi:10.1126/science.1080376

Steinhagen WH, Barrow CS (1984) Sensory irritation structure-activity study of inhaled aldehydes in B6C3F1 and Swiss-Webster mice. Toxicol Appl Pharmacol 72(3):495–503. doi:10.1016/0041-008X(84)90126-1

Story GM, Peier AM, Reeve AJ et al (2003) ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 112(6):819–829. doi:10.1016/S0092-8674(03)00158-2

Szallasi A, Cruz F, Geppetti P (2006) TRPV1: a therapeutic target for novel analgesic drugs? Trends Mol Med 12(11):545–554. doi:10.1016/j.molmed.2006.09.001

Taylor-Clark TE, Kiros F, Carr MJ, McAlexander MA (2009) Transient receptor potential ankyrin 1 mediates toluene diisocyanate-evoked respiratory irritation. Am J Respir Cell Mol Biol 40(6):756–762. doi:10.1165/rcmb.2008-0292OC

Tizzano M, Gulbransen BD, Vandenbeuch A et al (2010) Nasal chemosensory cells use bitter taste signaling to detect irritants and bacterial signals. Proc Natl Acad Sci USA 107(7):3210–3215. doi:10.1073/pnas.0911934107

Trevisani M, Smart D, Gunthorpe MJ et al (2002) Ethanol elicits and potentiates nociceptor responses via the vanilloid receptor-1. Nat Neurosci 5(6):546–551. doi:10.1038/nn852

Uchida K, Miura Y, Nagai M, Tominaga M (2012) Isothiocyanates from Wasabia japonica activate transient receptor potential ankyrin 1 channel. Chem Senses 37(9):809–818. doi:10.1093/chemse/bjs065

van Thriel C, Seeber A, Kiesswetter E, Blaszkewicz M, Golka K, Wiesmuller GA (2003) Physiological and psychological approaches to chemosensory effects of solvents. Toxicol Lett 140–141:261–271. doi:10.1016/S0378-4274(03)00022-5

van Thriel C, Kiesswetter E, Schaper M et al (2007) From neurotoxic to chemosensory effects: new insights on acute solvent neurotoxicity exemplified by acute effects of 2-ethylhexanol. Neurotoxicology 28(2):347–355. doi:10.1016/j.neuro.2006.03.008

Veldhuis NA, Poole DP, Grace M, McIntyre P, Bunnett NW (2015) The G protein-coupled receptor-transient receptor potential channel axis: molecular insights for targeting disorders of sensation and inflammation. Pharmacol Rev 67(1):36–73. doi:10.1124/pr.114.009555

Wang S, Dai Y, Fukuoka T et al (2008) Phospholipase C and protein kinase A mediate bradykinin sensitization of TRPA1: a molecular mechanism of inflammatory pain. Brain J Neurol 131(Pt 5):1241–1251. doi:10.1093/brain/awn060

Zhang X, Li L, McNaughton PA (2008) Proinflammatory mediators modulate the heat-activated ion channel TRPV1 via the scaffolding protein AKAP79/150. Neuron 59(3):450–461. doi:10.1016/j.neuron.2008.05.015