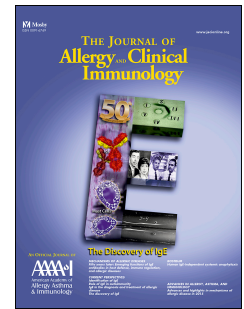


Accepted Manuscript

Enhanced Chemosensory Sensitivity in Idiopathic Rhinitis Patients and its Reversal by Nasal Capsaicin Treatment

L. Van Gerven, Y.A. Alpizar, B. Steelant, I. Callebaut, I. Kortekaas Krohn, M. Wouters, F. Vermeulen, G. Boeckxstaens, K. Talavera, P.W. Hellings



PII: S0091-6749(17)30517-1

DOI: [10.1016/j.jaci.2017.03.014](https://doi.org/10.1016/j.jaci.2017.03.014)

Reference: YMAI 12726

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 12 June 2016

Revised Date: 21 February 2017

Accepted Date: 2 March 2017

Please cite this article as: Van Gerven L, Alpizar YA, Steelant B, Callebaut I, Kortekaas Krohn I, Wouters M, Vermeulen F, Boeckxstaens G, Talavera K, Hellings PW, Enhanced Chemosensory Sensitivity in Idiopathic Rhinitis Patients and its Reversal by Nasal Capsaicin Treatment, *Journal of Allergy and Clinical Immunology* (2017), doi: 10.1016/j.jaci.2017.03.014.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Enhanced chemosensory sensitivity in idiopathic rhinitis patients and its reversal by nasal capsaicin treatment.

Van Gerven L.^{1,2}, Alpizar Y. A.³, Steelant B.², Callebaut I.¹, Kortekaas Krohn I.², Wouters M.⁴, Vermeulen F.⁵, Boeckxstaens G.⁴, Talavera K.³, Hellings P. W.^{1,2,6,7}

¹Clinical division of Otorhinolaryngology, Head & Neck Surgery, University Hospitals Leuven, Leuven, Belgium

²Laboratory of Clinical Immunology, Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium

³Laboratory for Ion Channel Research and TRP Research Platform Leuven (TRPL), Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium

⁴Translational Research Center for Gastrointestinal Disorders (TARGID), KU Leuven, Leuven, Belgium

⁵Cystic Fibrosis Reference Centre, University Hospitals Leuven, Leuven, Belgium

⁶Department of Otorhinolaryngology, University of Ghent, Belgium

⁷Department of Otorhinolaryngology, Academic Medical Center, Amsterdam, The Netherlands

Address correspondence to: Prof Dr P.W. Hellings, Univ. Hospitals Leuven, Kapucijnenvoer 33, 3000 Leuven, e-mail: Peter.Hellings@uzleuven.be; Tel: +32 16332342

Sources of funding:

Laura Van Gerven is supported by a grant of the Agency for Innovation by Science and Technology in Flanders, Belgium (IWT). Yeranddy A. Alpizar was supported by a Postdoctoral Mandate of the KU Leuven and is currently a postdoctoral researcher of the Fund for Scientific Research (FWO) Flanders, Belgium. Mira Wouters is a postdoctoral researcher of the Fund for Scientific Research (FWO) Flanders, Belgium. Guy Boeckxstaens is supported by a governmental grant (Odysseus program, G.0905.07, FWO). Peter Hellings is a recipient of a senior researcher fellowship from the Fund for Scientific Research Flanders, Belgium (FWO). This work was also supported by grants from the Belgian Federal Government (IUAP P6/28), the FWO (G076513N), and the Research Council of the KU Leuven (GOA/14/011 and PF-TRPL).

Word count: 4727

Key words: capsaicin, idiopathic rhinitis, non-allergic rhinitis, nasal hyperreactivity, nasal mucosal potential, TRPV1, TRPA1, treatment

Clinical implications/ key messages:

- Idiopathic rhinitis patients are more sensitive to the nociceptor stimulant allyl isothiocyanate than healthy controls, thus reflecting an altered state of the nasal afferent innervation
- After nasal capsaicin treatment the chemical sensitivity in idiopathic rhinitis patients is reduced in parallel with nasal symptoms
- Self-reported nasal hyperreactivity is a potential predictor for capsaicin treatment efficacy

Capsule summary (35 w):

We show electrophysiological evidence for an increased chemical sensitivity in idiopathic rhinitis patients compared to healthy controls. This abnormality is decreased after capsaicin treatment, along with a reduction of symptoms.

Abbreviations:

AITC: allyl isothiocyanate
 CDA: cold dry air
 CGRP: calcitonin gene-related peptide
 CSERP: chemosensory event-related potential
 HC: healthy controls
 IR: idiopathic rhinitis
 mRNA: messenger ribonucleic acid
 NANIR: non-allergic non-infectious rhinitis
 NGF : nerve growth factor
 NHR: nasal hyperreactivity
 NKA: neurokinin A
 NKB: neurokinin B
 NMP: nasal mucosal potential
 NPY: neuropeptide Y
 PNIF: peak nasal inspiratory flow
 RT-PCR: real time - polymerase chain reaction
 SP: substance P
 TRE: therapeutic response evaluation
 TRPA1: transient receptor potential cation channel subfamily A, receptor 1
 TRPV1: transient receptor potential cation channel subfamily V, receptor 1
 VAS: visual analogue scale

Introduction

Patients suffering from non-allergic non-infectious rhinitis (NANIR) represent a heterogeneous group, experiencing rhinitis symptoms not caused by allergy or infection (1). The term idiopathic rhinitis (IR) is used to describe a subgroup of NANIR patients in which the underlying etiology cannot be identified. After exclusion of occupational rhinitis, senile rhinitis (> 65 years old), gustatory rhinitis, hormonal rhinitis and drug-induced rhinitis, it is estimated that 50% of the NANIR group is suffering from IR, with an estimated total population incidence of 5% (1). Nasal hyperreactivity (NHR) is the key clinical feature in the majority of IR patients, meaning that nasal symptoms like nasal obstruction, sneezing, itch and/or rhinorrhea can be triggered by environmental factors such as temperature changes, strong odors, irritants, emotions and/or physical stress (2)(3)(4). The pathophysiology of NHR is largely unknown, but it is assumed that the local release of neuropeptides such as calcitonin gene-related peptide (CGRP), substance P (SP), neurokinin A and B (NKA/B), neuropeptide Y (NPY) and nerve growth factor (NGF) from afferent nerves upon activation of Transient Receptor Potential (TRP) channels induce nasal symptoms (5)(6). We have recently added evidence to the pathophysiologic role of sensory fibers in the pathophysiology of IR and NHR, and more specifically of the thermo-sensitive channels TRPV1 and TRPM8, present on these nerves (7). We found an over-expression of TRPV1 in IR patients compared to healthy control (HC) and our data on the combined reduction of nasal symptoms, NHR, and expression of SP, PGP 9.5, TRPV1 and TRPM8 by nasal capsaicin treatment strongly suggests the involvement of the TRPV1-SP nociceptive signaling pathway in IR (7).

Although capsaicin, the pungent agent in chili peppers, is recommended for the treatment of IR (8), large-scale double-blinded placebo-controlled trials have not been performed. Furthermore, little is known about prognostic factors influencing the therapeutic outcome of capsaicin nasal treatment. These shortcomings need to be addressed as IR patients do not respond well to available anti-inflammatory treatment (9)(10)(11). An in-depth investigation of the neural pathways is an essential step forward towards a better understanding of the mechanisms of action of capsaicin. In this study, we evaluated the functional effects of capsaicin on the innervation of the nasal mucosa by measuring nasal mucosal potentials (NMP) (12)(13) evoked by chemical stimuli in IR patients before and after capsaicin treatment.

Hummel et al. reported on measurements of electrical responses from the nasal respiratory epithelium after painful stimuli with carbon dioxide in human volunteers (13). NMP responses were interpreted as a summated receptor potential and were restricted to the site of stimulation, i.e., the area of activated nociceptors. Further studies served to obtain a topographical map of the sensitivity of the nasal mucosa to trigeminal chemosensory stimuli (13)(14)(15). Besides NMP, trigeminal function can be measured by chemosensory event-related potentials (CSERPs), determining the combined function of the trigeminal and the olfactory system (16)(17). However, due to poor signal-to-noise ratio of elicited responses, CSERPs are difficult to assess, and advanced optimizing signal-processing methods are required (17). In the present study we used NMP measurements (18) for the evaluation of the functional (neural) consequences of capsaicin therapy in IR, providing functional insight in the pathophysiology of IR. As primary aim we investigated if the previous findings of over-expression of TRPV1 in nasal biopsies of IR patients compared to HC (7) had clinical consequences. More specifically, if IR patients were more sensitive to chemical stimuli compared to HC and if

we could find changes in nasal sensitivity after capsaicin treatment, reflecting the state of the nasal innervation. This was measured by recording NMP's upon AITC exposure before and at 4, 12 and 26 weeks after capsaicin treatment. As secondary aim we investigated if age, gender, major symptom, presence of NHR or nasal chemical sensitivity had any influence on the outcome.

Patients and Methods

Patients and Healthy controls

Thirty-three IR patients and 12 HC were recruited via the outpatient clinic of the Otorhinolaryngology Department of the University Hospitals of Leuven, Belgium.

IR patients were defined as non-smoking patients suffering from at least 2 nasal complaints being nasal obstruction, rhinorrhea and/or sneezing for more than 1 h a day and for more than 1 year, with negative skin prick test (SPT) results, no clinical signs of infection (discolored secretions) and without anatomical nasal abnormalities responsible for nasal symptoms. Inclusion criteria were the following: IR patients between 18 and 65 years old with reported lack of efficacy of nasal corticosteroid treatment at recommended dose (mometasone furoate 50µg/spray: 2x2 daily) for at least 4 w who signed the informed consent. A thorough workup to exclude patients with local allergic rhinitis (LAR) in the IR patients included in this double-blinded, randomized, placebo-controlled trial was performed in the pilot study of this trial and published before (7). In this latter study, total IgE was measured in the nasal fluid of IR patients at baseline (7). IR patients were only included if the total IgE levels in the nasal fluid were less than 5,745 kU/l (P90 in HC). The detection limit of total IgE was 2 kU/l. In this pilot study none of the IR patients included had to be excluded after IgE measurements, because levels did not differ from HC (7). In our experience, patients with LAR do benefit from INS since the underlying pathophysiology is mainly IgE-mediated and thus responsive to the classic anti-inflammatory treatment. By the exclusive inclusion of non-allergic rhinitis patient that are “non-responsive to INS”, we believe to have indirectly excluded patients with LAR.

Exclusion criteria were a positive SPT for the 18 most frequent inhalant allergens in Belgium (house dust mite, pollen of timothy grass, smooth meadow grass, orchard grass, nettle, plantago, oxeye daisy, mugwort, alder, birch, hazel, horse, cat, dog, rabbit, spores of *Alternaria*, *Aspergillus* and *Cladosporium*) (HAL Allergy, Leiden, The Netherlands), pregnancy or lactation, systemic disorders or malignancies, use of medication affecting nasal function such as β -blockers, use of local and/or systemic corticosteroids 4 w prior to the study, history of prolonged use or abuse of decongestant nasal spray such as xylomethazoline. Patients with colored secretions and/or inflammation at the level of the osteomeatal complex were excluded after nasal endoscopy.

HC were age- and sex- matched, non-smoking individuals, without nasal symptoms and with negative SPT results. HC did not have anatomic nasal deformities or signs of infection as evaluated by nasal endoscopy.

During the entire study duration nasal medication was prohibited.

Study design

The study was registered at ClinicalTrials.gov (NCT 01862523) and approved by the Medical Ethical Committee on Clinical Investigations at the University Hospitals of Leuven. The IR patients and HC were invited for an outpatient visit to the Department of Otorhinolaryngology of the University Hospitals of Leuven at 6 occasions (table S1).

This study was performed in a double-blinded, randomized, placebo-controlled way. Patients were randomized in a 2 to 1 ratio for either treatment with capsaicin or placebo. In HC individuals all examinations were performed in the same way as in IR patients, but HC did not receive any treatment.

The capsaicin and placebo solutions were prepared, using the formula previously reported by Van Rijswijk et al. (7,19). In brief, the solution contained capsaicin at 0.1 mM with 30.3 mg pelargonic acid vanillylamide (capsaicin powder, Sigma-Aldrich, Bornem, Belgium) dissolved in 3 ml ethanol (96%) and diluted in 1 l NaCl 0.9% solution. The placebo solution contained the same buffer, but lacked pelargonic acid vanillylamide. At the treatment visit, all IR patients were treated with a blinded nasal spray 5 times on a single day with 1 hour intervals (2 puffs in each nostril, 0.4 ml/puff). The healthy control group was not treated with capsaicin since previous reporting (7) did not show any differences before and after treatment. The nasal mucosa was anaesthetized prior to the first 2 applications by cocaine 5% nasal spray (same volume/spray as mentioned above). To ensure effective local anesthesia, an interval of 15 min was maintained between the cocaine and blinded nasal spray application.

The power analysis (power of 80%) to determine the sample size, conducted before the start of the trial, revealed a number needed to include of at least 33 IR patients. This calculation was based on a two-sided chi-square test (with alpha 5%), with unequal group size (1/3 placebo, 2/3 capsaicin). The success rate of capsaicin was assumed to be 75%, based on the results of the previous trial conducted at our institution (7). Success was defined as a decrease of at least 30% on the visual analogue scale for the major nasal symptom. The estimated placebo effect was 25%.

Evaluation of nasal symptoms

All participants were asked to mark the typical nasal symptoms of IR, i.e. rhinorrhea, nasal obstruction, itch and sneezing on a visual analogue scale (VAS 0-10) at the screening visit (visit 1), baseline visit (visit 2) and follow-up (FU) visits: FU1 (visit 4 at w 4), FU2 (visit 5 at w 12) and FU3 (visit 6 at w 26). Only if the VAS score was more than 2 for one of the nasal symptoms, the symptom was considered relevant. The major nasal symptom was selected based on the highest VAS score at screening. The total symptom score (TSS) was the sum of the VAS scores for rhinorrhea, nasal obstruction, sneezing and itch. At visit 4, 5 and 6 a therapeutic response evaluation (TRE) was performed. IR patients were asked to score the overall improvement of their symptoms compared to baseline (0 = total reduction or symptom-free, 1 = major reduction, 2 = moderate reduction, 3 = minor reduction, 4 = no reduction of symptoms).

Subjective nasal hyperreactivity (NHR)

At the screening visit, each IR patient and HC was asked to evaluate their NHR. Subjects had to answer the question: "Can your nasal symptoms be triggered by temperature changes and/or strong odors". If 'YES', they had to indicate how long the symptoms lasted. If the induced symptoms lasted for > 5 min, we defined these patients as having NHR. Short-term (≤ 5 min) induction of nasal symptoms like rhinorrhea after sudden temperature changes were not considered as NHR.

Measurements of nasal mucosal potentials (NMPs) during nasal chemical stimulations

To investigate the role of afferent fibers in the human nasal mucosa in a functional way, trigeminal nerve monitoring was performed while the nasal mucosa was exposed to TRP channel agonists. We used capsaicin as specific TRPV1 agonist and allyl isothiocyanate (AITC) also known as mustard oil as agonist of both

TRPA1 and TRPV1 (20) During exposure of the nasal mucosa to the different substances, nasal mucosal potentials (NMPs) were recorded on the nasal floor of the left nostril at a depth of 5 cm, as described previously (12)(13). In brief, NMP recordings were performed using an 8 Fr nasogastric tube filled with 3% Agar in Ringers solution connected to a calomel electrode through a saturated KCl bath. The position of the catheter was controlled by anterior rhinoscopy and the catheter was taped to the nose to obtain a stable position during recording. The skin reference electrode was attached to the bridge of the nose. Both electrodes were connected to a bioamplifier (BMA-200, AD Instruments, Colorado Springs, CO) through a headstage (ISO-Z isolation headstage, ADInstruments). Data was acquired at a frequency of 50 Hz using a PowerLab device (Powerlab 4/30, ADInstruments) and LabChart software (v7.0., ADInstruments). For stimulation, the TRP channel agonists were administered via intranasal aerosol in increasing concentrations (5, 10 and 20 mM, dissolved in Ringer solution at room temperature). Concomitant mechanical or thermal stimulation was absent by this application form and the flow used (1.5 L/min) corresponded to physiological conditions. The sequence of testing the different stimuli was fixed, starting with AITC and finalizing with capsaicin. An interval of 2 to 3 minutes was left between stimuli. Maximal stimulus duration was 30 s, unless shorter if not tolerated (pain) by the subject. In between the different stimulations with TRP channel agonists a continuous aerosol with control solution (Ringer) was delivered. The multichannel aerosol device was placed behind the patient, so the patient could not anticipate on the stimulus. The stimulus threshold was determined by the lowest concentration at which there was a clear and fast (<10 s) deflection in the NMP recording. The deflection was considered positive when the negative deflection of the trace surpassed 5 times the standard deviation of the baseline measurement.. This threshold was determined for every subject at baseline and each FU visit. Both IR patients and HC underwent NMP measurements at different time-points (table S1).

A change in threshold was defined according to a decrease or increase in threshold concentration compared to baseline. In Figure 5A and B, this change (increase of 1 or 2 concentrations compared to baseline concentration for each individual) is used to visualize the relation with VAS major symptom and total symptom score.

Collection of nasal biopsies

Before treatment and at 4 and 26 w after treatment, a nasal biopsy was taken from the inferior turbinate with a Fokkens forceps, as reported earlier (7). The biopsy was snap frozen in liquid nitrogen and stored at -80 °C for real-time (RT)-qPCR.

RT-PCR for TRPV1, TRPA1, TRPV4, TRPM8, PGP 9.5 and NGF on nasal biopsies

The nasal biopsies were homogenized in lysis buffer from the Qiagen Mini RNeasy kit (Maryland, USA). RNA was isolated with the Qiagen Mini RNeasy kit (Maryland, USA) and first-strand cDNA was reverse transcribed by SuperScriptII RT (Invitrogen, Carlsbad, CA, USA) using 1 µg of total RNA according to manufacturer's instructions. RT-qPCR was performed for *TRPV1*, *TRPA1*, *TRPM8*, *PGP 9.5*, *NGF* and the housekeeping genes *β-actin* (*ACT2B*) and *TRPM7* in the ABI prism 7700 Sequence Detector System (Applied Biosystems, Foster City, CA, USA). cDNA plasmid standards, consisting of purified plasmid DNA specific for each

individual target, were used to quantify the target gene in the unknown samples, as described before (7). The primer and probe sequences were designed with Primer Express (Applied Biosystems).

Statistical Methodology

The results of the VAS measurements, TRE, AITC thresholds and RT-PCR data were compared using a Mann-Whitney U test. To correct for multiple comparisons the Bonferonni-Holm method was applied. All analyses were performed on available cases; hence it was assumed that data were missing completely at random (MCAR). This assumption was plausible since all missing values were purely due to logistic problems. To verify if the effect of capaisin differed between groups (NHR versus no NHR patients; AITC $\leq$ 5mM and 10 $\leq$ AITC $\leq$ 20mM) the Zelen's exact test was used. A difference was considered to be significant when $P < 0.05$. Values displayed represent mean $\pm$ standard error of the mean. For the RT-PCR data, a geometric mean of the 2 reference genes was calculated to have an accurate normalization. Analyses were performed using GRAPH PAD PRISM program (Prism 4, version 4.03, 1992–2005 Graph-Pad Software Inc., San Diego, CA, USA).

Results

Baseline characteristics of IR patients and healthy controls

Patient characteristics

IR and HC subjects showed no major differences with respect to age distribution (IR group: 41 y (19-63 y), HC group: 29 y (19-57 y)) or race (all Caucasian) as shown in Table 1. Within the IR group, there were no significant differences between the capsaicin- and placebo-treated cohorts regarding age, gender or race. The female/male ratio was 18/15 in IR patients and 6/6 in HC. All subjects had negative SPT for the 18 most frequent inhalant allergens in Belgium and showed no endonasal anatomic or mucosal sign of rhinosinusitis. Forty-two per cent (N = 14) of IR patients had been operated in the past to improve their nasal symptoms. (e.g. septoplasty or reduction of the inferior turbinate) without self-reported significant effect on nasal symptom reduction. Fifty-seven per cent (N = 19) of all included IR patients had self-reported NHR.

VAS scores

At baseline, nasal obstruction was reported as major symptom by 21 patients, rhinorrhea by 10, nasal itch by 1 and sneezing by 1 (Table 1). The mean baseline VAS major symptom was 8.2 ± 1.9 cm. There were no major baseline differences in symptom scores between the capsaicin- and placebo-treated IR patients. HC were symptom-free at baseline and remained so throughout the study (data not shown).

mRNA expression of TRPV1, PGP 9.5, TRPA1, TRPV4, TRPM8 and NGF

Baseline mRNA expression of *TRPV1* was significantly higher in IR patients compared to HC ($P = 0.031$) as shown before (7) (Figure 6A). The *TRPV1* expression in IR patients with NHR showed a trend to be higher than in IR patients without NHR ($P = 0.2903$) (Figure 6A). Expression levels of the pan-neuronal marker *PGP 9.5*, the cation channels *TRPV4*, *TRPM8* and the neurotrophic factor *NGF* were not different between IR patients and HC at baseline ($P = 0.274$, $P = 0.497$, $P = 0.370$ and $P = 0.781$, respectively). For *TRPM8* two thirds of the samples had very low mRNA expression levels and had to be excluded. *TRPA1* mRNA could not be detected.

Lower baseline threshold for TRPV1/TRPA1-mediated activation of sensory nerves in IR patients

In order to objectively and functionally assess the baseline observation of *TRPV1* over-expression in IR patients compared to HC, we evaluated the chemical sensitivity of the afferent nerves in the nasal mucosa using NMP measurements during exposure to different TRP channel agonists in increasing concentrations. The threshold was determined for each individual. AITC (a *TRPV1* and *TRPA1* agonist) turned out to be the best stimulus. This was because capsaicin (a specific *TRPV1* agonist) often induced coughing, thereby disturbing the NMP measurement.

At baseline, the threshold for evoking changes in NMPs by AITC was significantly lower for IR patients compared to HC ($P = 0.0423$; Figure 1A-C). Consecutive NMP measurements in the HC group and the IR group treated with placebo at different time-points stayed stable (Figure 4), HC data not shown. Patients with self-reported NHR had lower AITC thresholds compared to IR patients without NHR ($P = 0.0335$; Figure 1D).

Clinical improvement of IR patients after capsaicin treatment

Compared to baseline, VAS scores for nasal obstruction, rhinorrhea and itch decreased significantly 4, 12 and 26 w after capsaicin treatment whereas no significant decrease was seen after placebo treatment

(Figure 2 C-F). For sneezing no significant changes were observed after treatment, however only few patients reported sneezing as symptom (N= 7/33). Sneezing is a more typical symptom in allergic rhinitis rather than in non-allergic rhinitis/ IR patients. VAS scores for major symptom were also significantly reduced at 4, 12 and 26 w after capsaicin treatment ($P = 0.2007$, $P = 0.0081$, $P = 0.0217$, respectively), as well as total symptom score ($P = 0.0064$, $P = 0.0167$, $P = 0.01468$, respectively). These changes were not observed in the placebo group (Figure 2 A and B).

The TRE yielded clear changes in the distribution of capsaicin-treated patients towards reduction of symptoms after 4, 12 and 26 w, which was not observed in the placebo-treated patients ($P = 0.0192$, $P = 0.0045$, $P = 0.0155$; Figure 3). Sixty-eight per cent, 81% and 72% of IR patients treated with capsaicin reported improvement (scored 0 to 3) at 4, 12 and 26 w, respectively. Major improvement (scored 0 to 2) was reported by 45%, 48% and 50%, respectively. Only 1 IR patient treated with placebo reported moderate improvement after 4 w and 2 patients after 12 w. At 26 w none of the patients in the placebo group reported improvement. The TSS in the HC group was 2.1 ± 0.5 at baseline.

Capsaicin treatment increased the threshold for sensitivity to AITC in correlation with an improvement in symptom scores

In the IR group treated with capsaicin the threshold for AITC shifted towards significantly higher concentrations at 4 and 12 w post-treatment compared to the placebo-treated IR group ($P = 0.0406$ at 4 w, $P = 0.0325$ at 12 w and $P = 0.0611$ at 26 w). The “degree” of shift in threshold, reflecting the reduction in nasal sensitivity to a chemical stimulus, correlated significantly with the change/improvement in total symptom score ($r = 0.604$, $P = 0.0018$ Figure 5A) and VAS major symptom ($r = 0.714$, $P = 0.0004$ Figure 5B) 4 w after capsaicin treatment. More specifically, patients with a threshold to AITC ≤ 5 mM at baseline that shifted after capsaicin treatment to threshold ≥ 20 mM, improved with +2 (no response on AITC 10 mM (+1) and 20 mM (+2)). IR patients with a shift from threshold 10 mM to 20 mM or 5 mM to 10 mM were considered as +1. Remarkably, all patients with an AITC threshold ≤ 5 mM shifted to a threshold of ≥ 20 mM ((Figure 4, +2 on Figure 5B). This demonstrates that patients with pronounced nasal sensitivity to AITC are very good responders to capsaicin (Figure 7B).

Capsaicin treatment induced no reduction in PGP 9.5, TRPV1, TRPV4, TRPM8 and NGF expression in IR patients 4 and 26 w after treatment

The expression levels of the pan-neuronal marker *PGP 9.5* were not different between IR patients and HC at baseline and did not change after treatment (data not shown). The same was observed for *TRPV4* and *TRPM8*. Although we found a significant over-expression of *TRPV1* in IR patients compared to HC at baseline ($P = 0.0313$; Figure 6A), no significant decrease was observed after capsaicin treatment. The mRNA levels of *TRPA1* were below the detection limit in the IR and HC group (data not shown). Nasal biopsies of IR patients and controls had similar expression of *NGF* mRNA before and after treatment (data not shown).

Baseline hTRPV1 expression correlated with the TRE 12 w after capsaicin treatment

IR patients with high baseline *TRPV1* expression showed a better response to capsaicin treatment (TRE moderate improvement until symptom-free) compared to IR patients with median or low *TRPV1* expression, which showed only a minor improvement or no improvement, respectively ($P = 0.024$; Figure 6B).

Self-reported nasal hyperreactivity can be a promising predictor of capsaicin treatment efficacy in IR

In the capsaicin-treated patient cohort, 63% reported subjective NHR. IR patients with self-reported NHR showed a clear trend to be better responders to capsaicin treatment compared to IR patients without self-reported NHR ($P = 0.10$, Zelen's exact test), 12 w post-treatment; Figure 7A). Capsaicin-treated IR patients with low baseline AITC thresholds had better TREs compared to IR patients with high baseline AITC thresholds (Figure 7B), however without reaching significance. Age, gender and baseline major symptom had no significant influence on TRE (data not shown).

Discussion

We here provide novel insight into the pathophysiology of IR by functional measurements of neural responses before and after capsaicin treatment. Our data strongly support the involvement of the TRPV1 and TRPA1 nociceptors in the pathophysiology of IR. First, we found an increased nasal chemical sensitivity to the TRPA1 and TRPV1 agonist AITC in IR patients and second, both the sensitivity to AITC and the IR symptoms decreased after capsaicin treatment. The baseline over-expression of *TRPV1* (RT-PCR) in combination with the increased chemical sensitivity in IR patients illustrates the crucial role of the TRPV1-Substance P nociceptive signaling pathway in IR pathophysiology, both structurally and functionally, as previously suggested (7). The correlation between the reduction of symptoms (VAS major symptom and TSS) and the degree of decrease in chemical sensitivity to AITC underlines the reliability and relevance of the functional data.

The therapeutic response to capsaicin was not associated with a clear decrease in *TRPV1* expression as previously reported (7). In contrast to the previous study (7), NHR was not an inclusion criterion and the IR cohort reported here included IR patients with and without NHR, with no significant “overall” decrease of *TRPV1* after capsaicin treatment. Of note, self-reported NHR seems to be a potential prognostic factor for a good therapeutic response to nasal capsaicin treatment. In addition, the moment of harvesting the nasal biopsies after treatment was different (at 4 and 26 w post-treatment compared to 12 w in the previous report). At 26 w, re-innervation might be present, which may explain the recurrence of symptoms at this time point in some patients, and which also corresponds to the return of the threshold for AITC detection to baseline levels (Figure 4). At 4 w the maximal therapeutic effect is not yet reached, as shown by the individual symptom reduction and TRE (Figure 3).

The strength of this study lies within the functional neuronal readout. Based on our previous findings (7), we wanted to investigate the role of the chemosensory TRP channels more in depth (20–22). For this we built a multichannel aerosol device with the possibility to apply several compounds in sequence. This, in combination with measurements of changes in nasal mucosal potential allowed us to estimate the concentration thresholds of the responses to the TRPA1 and TRPV1 agonist AITC. A comparable technique to measure mucosal potentials was already used and validated in 1993 by Thürauf et al (12). Our method of intranasal application by the multichannel aerosol is more patient-friendly than the nasal lavages with TRP agonists described before by Alenmyr et al (5). Our study demonstrates an increased chemical sensitivity in IR patients compared to HC at baseline and “normalization” after capsaicin treatment. At present, we can only speculate on the mechanisms underlying the differences in baseline chemical sensitivity between IR patients and HC. Retrospective analysis of our IR patient files revealed a previous nasal fracture in almost one third of them, suggesting a potential aberrant re-innervation after a trauma of the nasal mucosa. This hypothesis needs further study.

Interestingly, our data suggest a better capsaicin treatment response in patients with self-reported NHR compared to IR patients without history of NHR (Figure 7A). Unfortunately, due to the small number of patients in the subgroup analysis only a clear trend could be observed, but this observation is of tremendous

clinical value since a history of NHR is a clinical parameter during history taking, with a seemingly high predictive value for a therapeutic effect of capsaicin. In addition, this observation matches with the clinical finding that patients with more pronounced nasal hypersensitivity (threshold of AITC 5 mM) respond better to capsaicin therapy compared to IR patients with a higher threshold (AITC 20 mM)(Figure 7 B). These data suggest that although our inclusion criteria for IR patients were very strict, the cohort is still heterogeneous regarding functional properties and features of the nasal afferent innervation. The broad *TRPV1* baseline expression in IR patients (range: 22-749) are in line with this assumption. Nevertheless, the correlation between increased baseline *TRPV1* expression and good TRE strongly support the role of TRP channels (Figure 6) in capsaicin treatment effects. As for future clinical practice, baseline measurements of NHR by cold dry air (CDA)-provocation or by chemical stimulation could be recommended before capsaicin treatment to predict the therapeutic outcome. Recent findings showed that objective NHR measurements (e.g. Cold-dry air challenge) were more sensitive compared to self-reported NHR (unpublished, Van Gerven et al.) and would therefore be superior in the pre-treatment setting.

Since our first experience with capsaicin treatment more than 10 years ago, we repeated the nasal treatment in the majority of responders several times with a variable interval between capsaicin treatments, ranging from 6 months to several years. This phenomenon of recurrent symptoms prompted us to study the mechanisms of recurrence of symptoms in a functional way by recording NMPs. Lacroix et al. (25) reported on the beneficial effects up to 6 months after capsaicin treatment and Van Rijswijk et al.(19) up to 9 months. However, no studies were specifically designed to investigate the exact duration of the therapeutic response, or to investigate the mechanisms underlying this phenomenon. We found a decreased sensitivity to AITC early after capsaicin treatment and restoration of the sensitivity at 6 months. In contrast to NMP recordings at 6 months after nasal capsaicin treatment, symptom severity was still lower at 6 months compared to baseline. The discrepancy between symptom recurrence and re-innervation may be linked to the fact that full neural functional recovery may take longer than 6 months.

In conclusion, we show for the first time functional evidence for increased chemical sensitivity in IR patients compared to HC, reflecting the state of the nasal afferent innervation and the important role of the nociceptors *TRPV1* and *TRPA1* in the pathophysiology of IR. Moreover, we demonstrate a clear decrease in sensitivity and symptoms after capsaicin treatment, revealing at least part of the working mechanism of this therapeutic agent. Finally, we found self-reported NHR being a potential predictor for capsaicin treatment efficacy. In the future, NHR might be assessed prior to capsaicin treatment in order to select patients more likely to respond.

Acknowledgments

The authors would like to thank Mr. Luc Grammet for his technical assistance with the development and the construction of the multichannel aerosol and for his careful maintenance of the device. We also thank Mrs. Ellen Dilissen and Mrs. Emily Dekimpe for their help with the blinding process, guidance through the RT-

406 PCR data and the help during the NMP recordings. We thank Prof. Steffen Fieus for his help with the
407 statistical analysis.

408

409

Legends:

Figure 1: Baseline nasal mucosal potential (NMP) measurements in healthy controls (HC) and idiopathic rhinitis (IR) patients.

A and **B**, Representative experiments of NMP recordings during exposure to allyl isothiocyanate (AITC) in a HC (**A**) and an IR patient (**B**). **C**, Distribution of HC and IR patients according to the concentration threshold for NMP responses to AITC. **D**, Distribution of IR patients with and without nasal hyperreactivity (NHR) according to the concentration threshold for NMP responses to AITC.

NMP= nasal mucosal potential, HC= healthy control, IR= idiopathic rhinitis, AITC= allyl isothiocyanate, NHR= nasal hyperreactivity

Figure 2: Response of IR patients to capsaicin and placebo treatments.

A and **B**, Average total symptom score (TSS, **A**) and average visual analogue scale (VAS) for major symptom at baseline and at 4, 12 and 26 w after capsaicin or placebo treatment in IR patients (n = 24 and n = 9, respectively). **C-F** Average VAS scores for the individual symptoms at 4, 12 and 26 w after capsaicin or placebo treatment in IR patients. The maximum of the scale for TSS and VAS for major symptom/individual symptoms were 40 cm and 10 cm, respectively. *, p < 0.05 and **, p < 0.005.

TSS= total symptom score, VAS= visual analogue scale, IR= idiopathic rhinitis

Figure 3: Distribution of therapeutic response evaluation at 4, 12 and 26 w after capsaicin or placebo treatment in relation to baseline. *, p < 0.05 and **, p < 0.005

Figure 4: Distribution of the capsaicin- and placebo-treated patients according to their threshold for the NMP responses to AITC, determined a baseline and at 4, 12 and 26 weeks after treatment. *, p < 0.05

NMP= nasal mucosal potential, AITC= allyl isothiocyanate

Figure 5: Capsaicin treatment decreases the sensitivity to AITC in IR patients in parallel with the nasal symptoms.

A and **B**, Correlation between the change in threshold for NMP responses to AITC and changes in total symptom score (TSS)(**A**) and visual analogue scale (VAS) for major symptom (**B**), determined 4 w after capsaicin treatment. **C** and **D**, Examples of NMP responses to AITC recorded in an IR patient, before (**C**) and 4 w after capsaicin treatment (**D**). Note the sharp negative deflection of the trace in response to application of 5 mM AITC before the treatment, and the lack of such response 4 w after treatment even at 20 mM AITC.

AITC= allyl isothiocyanate, IR= idiopathic rhinitis, NMP= nasal mucosal potential, TSS= total symptom score, VAS= visual analogue scale

Figure 6: TRPV1 expression in HC and IR patients at baseline and in relation with the therapeutic response.

A, *TRPV1* mRNA expression (RT-PCR) in homogenates of nasal biopsies in HC and IR patients at baseline. The IR group is subdivided in IR patient with and without NHR. **B**, Relation between baseline *TRPV1* mRNA expression in IR patients and the therapeutic response 12 w after capsaicin treatment. *, p < 0.05, ***, p < 0.0005

HC= healthy control, IR= idiopathic rhinitis, RT-PCR= real-time PCR, NHR= nasal hyperreactivity

Figure 7: Therapeutic response of IR patients 12 weeks after capsaicin/placebo treatment

A and **B**, Distribution of the therapeutic response of patients when classified according to the presence of nasal hyperreactivity (NHR, **A**) or to the concentration threshold for the NMP response to AITC determined at baseline (**B**).

IR= idiopathic rhinitis, NHR= nasal hyperreactivity , AITC= allyl isothiocyanate

Figure E :

Illustration of the multichannel aerosol

Table 1: Patient characteristics.

Table S1: List of registrations and sample collections that were performed at each visit in the HC and IR group.

HC= healthy control, IR= idiopathic rhinitis

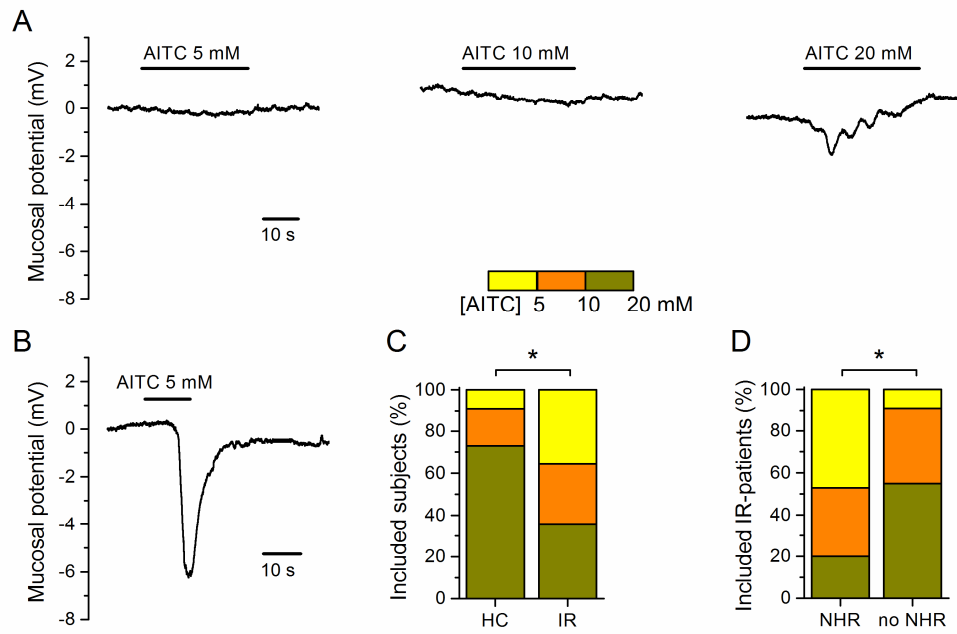
References

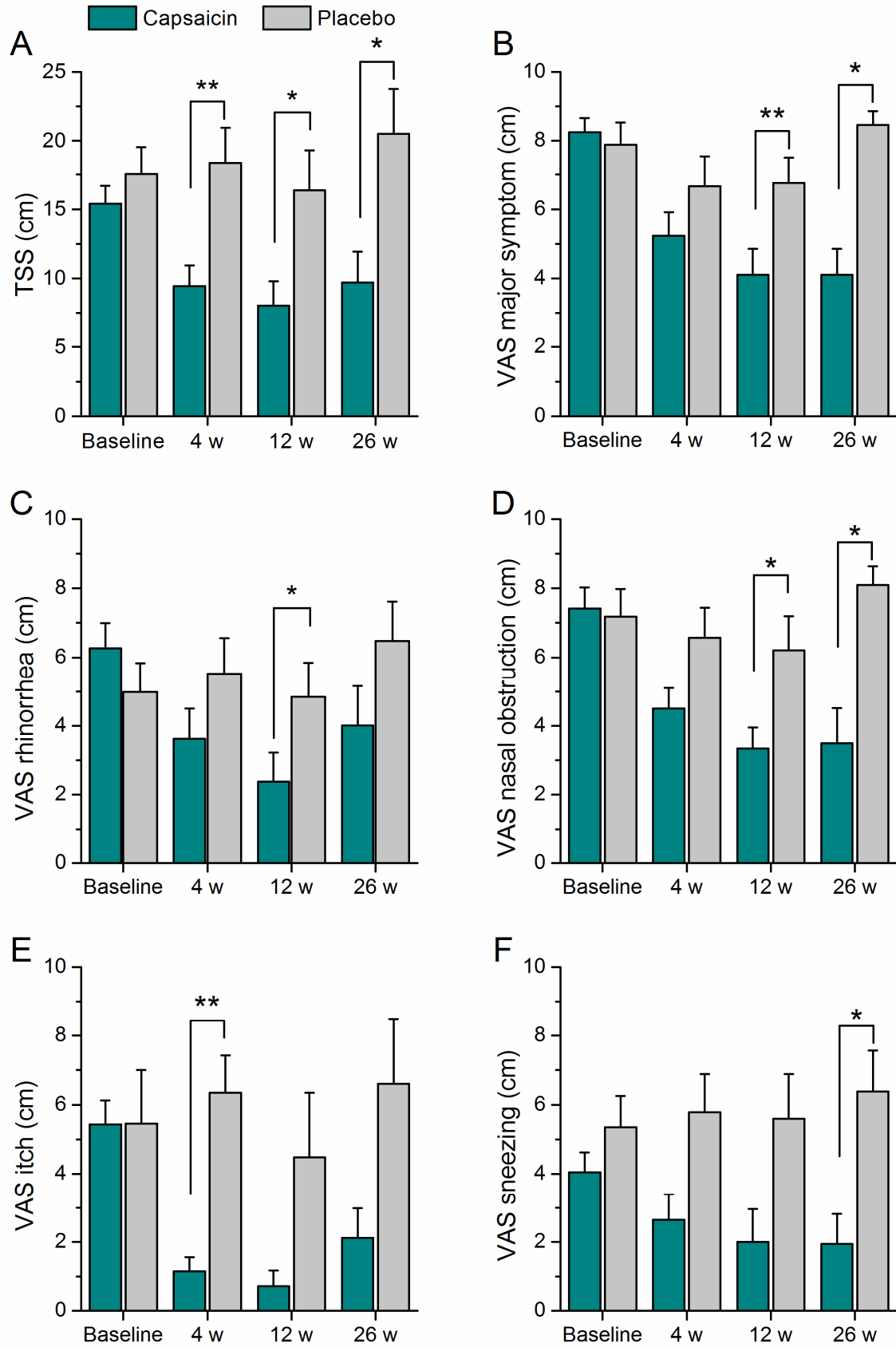
1. Bousquet J, Fokkens W, Burney P, Durham SR, Bachert C, Akdis CA, et al. Important research questions in allergy and related diseases: nonallergic rhinitis: a GA2LEN paper. *Allergy*. 2008 Jul;63(7):842–53.
2. Gerth van Wijk RG, de Graaf-in 't Veld C, Garrelds IM. Nasal hyperreactivity. *Rhinology*. 1999 Jun;37(2):50–5.
3. Van Gerven L, Boeckxstaens G, Hellings P. Up-date on neuro-immune mechanisms involved in allergic and non-allergic rhinitis. *Rhinology*. 2012 Sep;50(3):227–35.
4. Segboer CL, Holland CT, Reinartz SM, Terreehorst I, Gevorgyan A, Hellings PW, et al. Nasal hyper-reactivity is a common feature in both allergic and nonallergic rhinitis. *Allergy*. 2013 Nov;68(11):1427–34.
5. Alenmyr L, Herrmann A, Högestätt ED, Greiff L, Zygmunt PM. TRPV1 and TRPA1 stimulation induces MUC5B secretion in the human nasal airway in vivo. *Clin Physiol Funct Imaging*. 2011 Nov;31(6):435–44.
6. Baraniuk JN, Merck SJ. Neuroregulation of human nasal mucosa. *Ann N Y Acad Sci*. 2009 Jul;1170:604–9.
7. Van Gerven L, Alpizar YA, Wouters MM, Hox V, Hauben E, Jorissen M, et al. Capsaicin treatment reduces nasal hyperreactivity and transient receptor potential cation channel subfamily V, receptor 1 (TRPV1) overexpression in patients with idiopathic rhinitis. *J Allergy Clin Immunol*. 2013 Oct 15;
8. Gevorgyan A, Segboer C, Gorissen R, van Drunen CM, Fokkens W. Capsaicin for non-allergic rhinitis. *Cochrane Database Syst Rev*. 2015;7:CD010591.
9. Blom HM, Godthelp T, Fokkens WJ, KleinJan A, Mulder PG, Rijntjes E. The effect of nasal steroid aqueous spray on nasal complaint scores and cellular infiltrates in the nasal mucosa of patients with nonallergic, noninfectious perennial rhinitis. *J Allergy Clin Immunol*. 1997 Dec;100(6 Pt 1):739–47.
10. Jacobs R, Lieberman P, Kent E, Silvey M, Locantore N, Philpot EE. Weather/temperature-sensitive vasomotor rhinitis may be refractory to intranasal corticosteroid treatment. *Allergy Asthma Proc Off J Reg State Allergy Soc*. 2009 Apr;30(2):120–7.
11. Lundblad L, Sipilä P, Farstad T, Drozdiewicz D. Mometasone furoate nasal spray in the treatment of perennial non-allergic rhinitis: a nordic, multicenter, randomized, double-blind, placebo-controlled study. *Acta Otolaryngol (Stockh)*. 2001 Jun;121(4):505–9.
12. Thürauf N, Hummel T, Kettenmann B, Kobal G. Nociceptive and reflexive responses recorded from the human nasal mucosa. *Brain Res*. 1993 Dec 3;629(2):293–9.
13. Hummel T, Kraetsch HG, Pauli E, Kobal G. Responses to nasal irritation obtained from the human nasal mucosa. *Rhinology*. 1998 Dec;36(4):168–72.

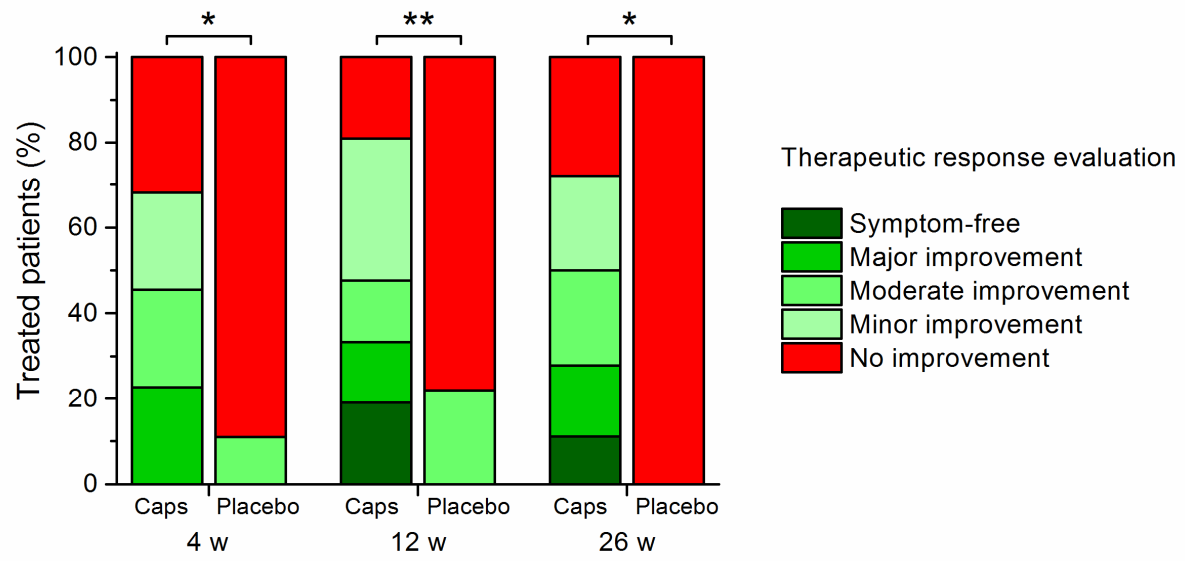
14. Meusel T, Negoias S, Scheibe M, Hummel T. Topographical differences in distribution and responsiveness of trigeminal sensitivity within the human nasal mucosa. *Pain*. 2010 Nov;151(2):516–21.
15. Scheibe M, van Thriel C, Hummel T. Responses to trigeminal irritants at different locations of the human nasal mucosa. *The Laryngoscope*. 2008 Jan;118(1):152–5.
16. Laudien JH, Wencker S, Ferstl R, Pause BM. Context effects on odor processing: an event-related potential study. *NeuroImage*. 2008 Jul 15;41(4):1426–36.
17. Rombaux P, Huart C, Mouraux A. Assessment of chemosensory function using electroencephalographic techniques. *Rhinology*. 2012 Mar;50(1):13–21.
18. Naehrlich L, Ballmann M, Davies J, Derichs N, Gonska T, Hjelte L, et al. Nasal potential difference measurements in diagnosis of cystic fibrosis: an international survey. *J Cyst Fibros Off J Eur Cyst Fibros Soc*. 2014 Jan;13(1):24–8.
19. Van Rijswijk JB, Boeke EL, Keizer JM, Mulder PGH, Blom HM, Fokkens WJ. Intranasal capsaicin reduces nasal hyperreactivity in idiopathic rhinitis: a double-blind randomized application regimen study. *Allergy*. 2003 Aug;58(8):754–61.
20. Everaerts W, Gees M, Alpizar YA, Farre R, Leten C, Apetrei A, et al. The capsaicin receptor TRPV1 is a crucial mediator of the noxious effects of mustard oil. *Curr Biol CB*. 2011 Feb 22;21(4):316–21.
21. Alpizar YA, Boonen B, Gees M, Sanchez A, Nilius B, Voets T, et al. Allyl isothiocyanate sensitizes TRPV1 to heat stimulation. *Pflüg Arch Eur J Physiol*. 2014 Mar;466(3):507–15.
22. Gees M, Alpizar YA, Boonen B, Sanchez A, Everaerts W, Segal A, et al. Mechanisms of transient receptor potential vanilloid 1 activation and sensitization by allyl isothiocyanate. *Mol Pharmacol*. 2013 Sep;84(3):325–34.

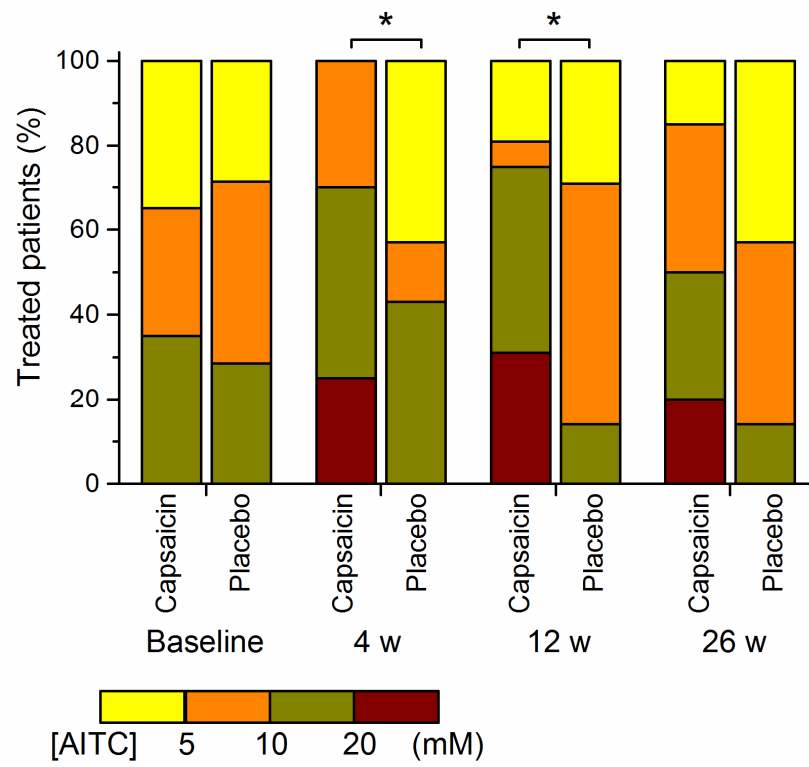
Table 1. Patient Characteristics.

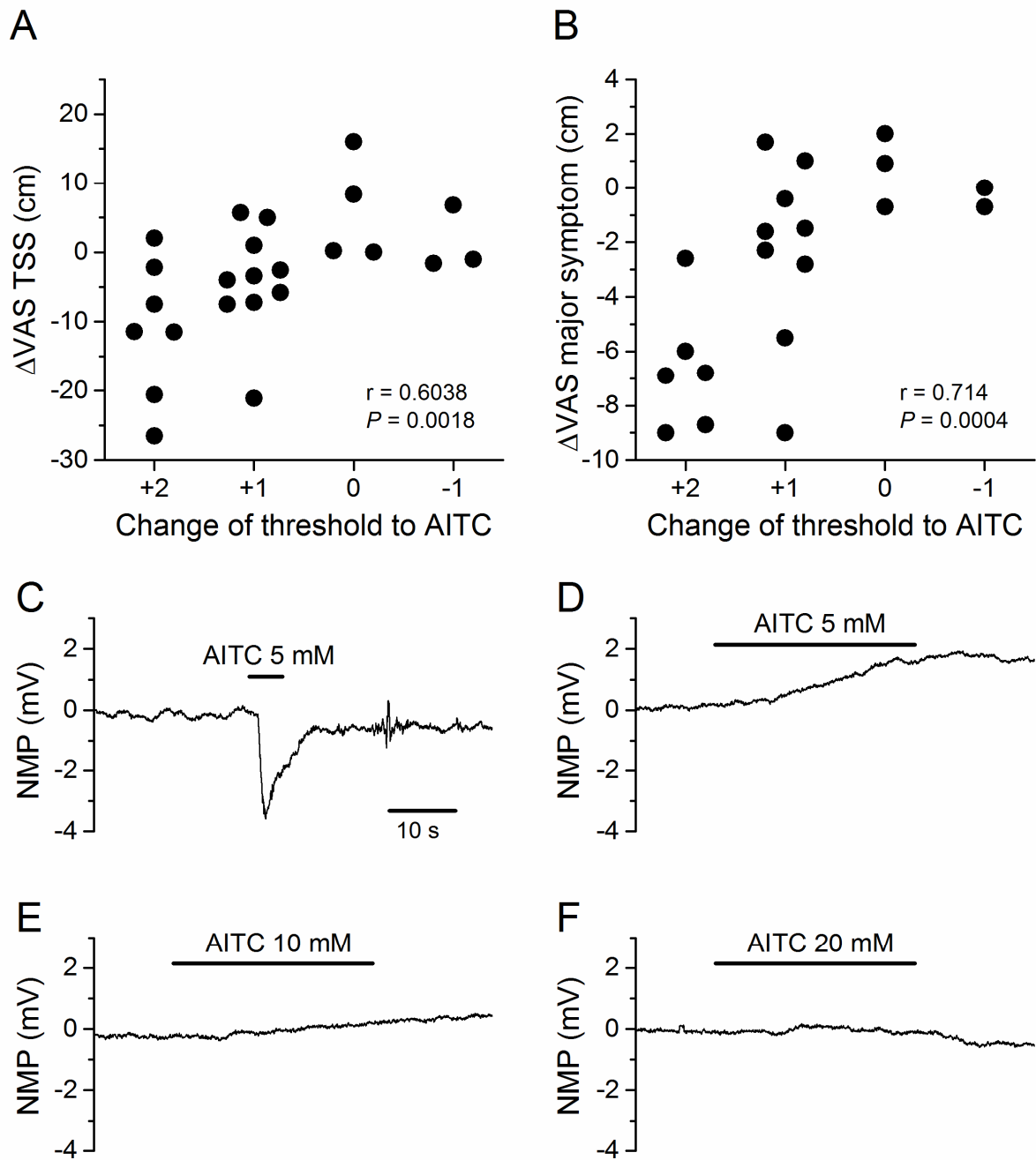
Patient Characteristics	Capsaicin group (N = 24)	Placebo group (N = 9)	Healthy controls (N = 12)
Average age (range)	40 y (19-62)	44 y (20-63)	29 y (19-57)
Gender (M/F)	10/14	5/4	6/6
Non-responsive to INS	100%	100%	-
Smokers	0%	0%	0%
Allergy (SPT for 18 allergens tested)	0%	0%	0%
Infection (NE)	0%	0%	0%
Concomitant nasal anatomical deformities	0%	0%	0%
Previous nasal surgery	46% (N = 11)	33% (N = 3)	0%
Subjective NHR	63% (N = 15)	44% (N = 4)	0%
Major symptom	58% nasal obstruction (N = 14) 38% rhinorrhea (N = 9) 4% sneezing (N = 1) 0% itch (N = 0)	78% nasal obstruction (N = 7) 11% rhinorrhea (N = 1) 0% sneezing (N = 0) 11% itch (N = 1)	-

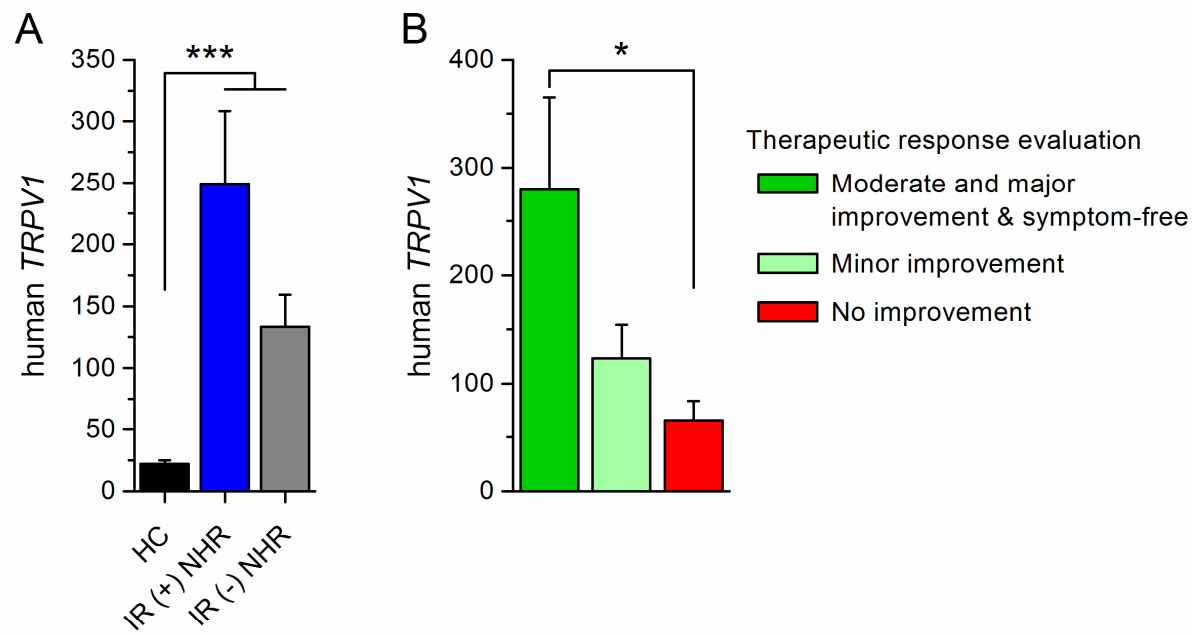


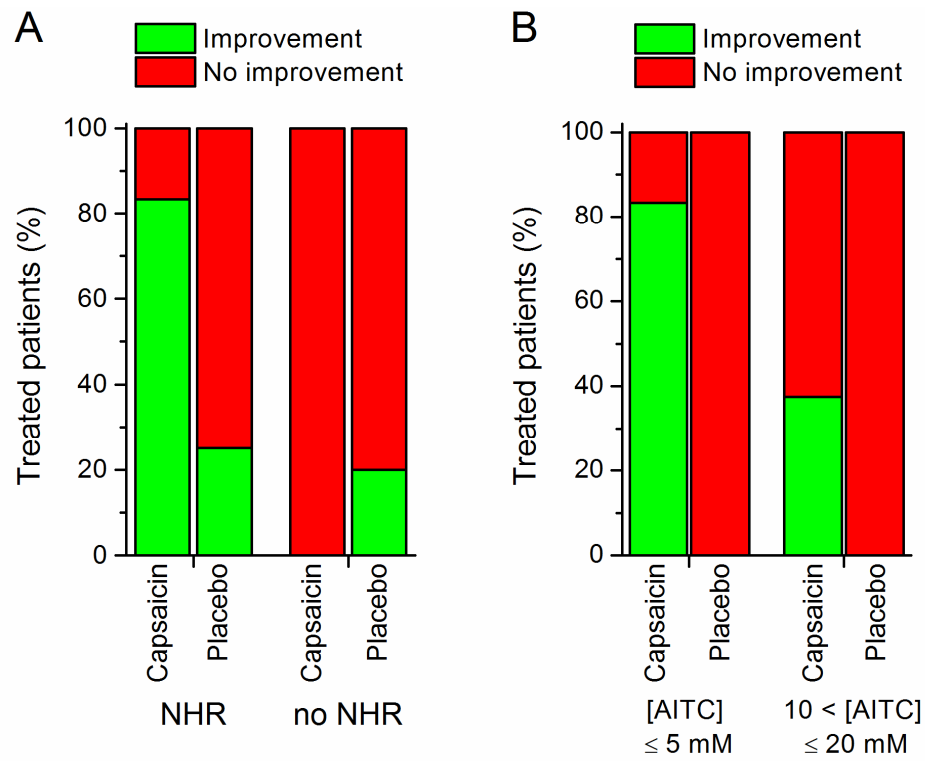












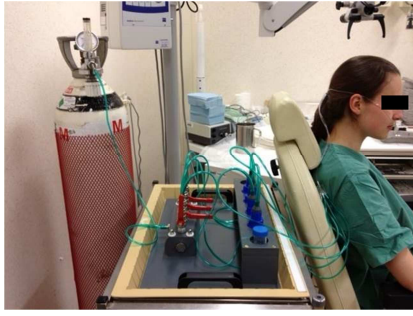


Table S1. List of registrations and sample collections that were performed at each visit in the HC and IR group.

	Screening visit (week -3)	Baseline visit (week -1)	Treatment visit (0)	Follow-up visit 1 (week 4)	Follow-up visit 2 (week 12)	Follow-up visit 3 (week 26)
Explanation of study rationale and protocol	+	-	-	-	-	-
Evaluation inclusion and exclusion criteria	+	+	-	-	-	-
Informed consent	+	-	-	-	-	-
Skin prick tests	+	-	-	-	-	-
Clinical ENT-examination	+ (NE)	+ (RA)	+ (RA)	+ (RA)	+ (RA)	+ (RA)
NMP-measurements	-	+	+	+	+	+
Symptom evaluation (VAS)	+	+	-	+	+	+
PNIF measurements	+	-	-	-	-	-
Nasal biopsy	+	-	-	+	-	+
Explanation treatment	-	-	+	-	-	-
Therapeutic response evaluation (TRE)	-	-	-	+	+	+